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Abstracts of the 5th Croatian Congress of Toxicology with International Participation

CROTOX 2016

Poreč, Croatia, 9 to 12 October 2016

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Cover page: *Sunset is still my favourite colour.*

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Croatian Society of Toxicology

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Abstracts of the 5th Croatian Congress of Toxicology with International Participation

CROTOX 2016

EDITORIAL

Dear reader,

In front of you is the Supplement to the *Archives of Industrial Hygiene and Toxicology* dedicated entirely to the abstracts of the presentations by invited speakers, as well as other oral and poster presentations that will be presented at the 5th Croatian Congress of Toxicology with international participation. The Congress will take place in Poreč, Croatia, from 9 to 12 October, 2016. In this Supplement, all participating authors are presenting the results of their scientific work, enabling thus communication with a broader scientific community and connecting young and senior scientists from different fields of toxicology.

It is a great honour to be able to gather so many scientists from different countries at our Congress. We are pleased to see it will act as a platform for our distinguished guests and participants to inform attendances of the latest achievements and state-of-the art knowledge in the various fields of toxicology. Communication and fruitful discussions, which we hope to accomplish during the Congress, are crucial for solving different problems arising in toxicology worldwide.

The presence of different chemicals and their mixtures, which are intentionally or unintentionally released into the environment, is a great problem in all countries. At certain point these compounds begin to interfere with the living organisms of an ecosystem, causing thus tremendous changes and irreversible events in the food chain. In this Supplement, you will find the recent results on the influence of nanoparticles, pesticides, endocrine disruptors, and metals on the members of a community or an ecosystem.

The toxicological approach in forensic science and drug abuse plays an important role in modern society – a society overwhelmed with hundreds of chemicals that can be misused or are newly synthesised as psychoactive drugs. Identifying such drugs, their bioavailability, and the mechanisms of action is vital in finding a cure, preventing abuse, defining new regulations, and raising public awareness on the problems associated with the introduction of novel psychoactive substances on the market, but also on the misuse of the regularly used substances. In this Supplement, you will find some interesting issues relative to the misuse of different chemicals, prevention of drug abuse, and implementation of safety measures aimed at reducing accidental situations.

The quality of life has nowadays improved considerably thanks to an increasing use of nanoparticles. Their implementation in medicine, cosmetics, and industry is unquestionable, but there is growing evidence of their side effects on cells, organisms, and communities. For many of these, there is not enough data on their mechanisms of toxicity. Similar to nanoparticles, there is a great concern about metals and metalloids. Even though they are an important component of various items of everyday use, once released into the environment they can cause severe health problems. It is therefore crucial to know how they impact biochemical and regulatory pathways in cells and organisms. Recent findings on this topic are provided in a number of abstracts published in this Supplement.

Food-borne or environmental contaminants found in food is a subject that has intrigued scientists for decades. The toxicity (or the protective effect) of compounds naturally occurring in food is an ongoing issue, as is the presence of metals in food, metabolic products of other species living on the foodstuffs, their origins and influence on the organism. Here you will have the opportunity to have a look at a variety of problems surrounding food toxicology.

Finally, we would like to thank all the participants who have recognised this Congress as a place to share their scientific results and ideas, despite the fact that this is a national conference organised within the realm of the European Toxicology Society and the International Toxicological Society.

We would like to thank all the people who have been involved in the organisation of this Congress; Editor-in-Chief, Assistant Editors, and Editorial Board of the *Archives of Industrial Hygiene and Toxicology* who gave us the opportunity to present the ongoing toxicological research in different fields of toxicology. Also, we are grateful to the diligent and dedicated members of the Organising and Scientific Board. We hope that this Congress will significantly contribute to the improvement of environmental issues and to an enhanced level of understanding as to how chemicals and their mixtures really affect our organisms. Furthermore, we hope that the contacts which will be established among young and senior scientists, as well as with working groups from different countries, will result in further collaboration schemes and networks of scientists at national, European, and global levels.

Guest Editor

Prof Ksenija Durgo, PhD

WELCOME ADDRESS

Dear Friends and Colleagues,

It is my great pleasure and honour to be able to welcome you on behalf of CROTOX 2016 and the Croatian Society of Toxicology at this 5th Croatian Congress of Toxicology in the charming town of Poreč.

As the Croatian Society of Toxicology celebrates its 25th anniversary this year, we can proudly say that our Society is very active and that our activities provide an adequate response to modern challenges in toxicology.

Our Society regularly organises and invites all members of our and other similar societies to lectures in various fields of toxicology. When we recently realised that our young scientists had no possibility to broaden their knowledge in toxicology due to the lack of systematic education in our country, this year in spring we organised the "Principles of Toxicology" under the auspices of EUROTOX. We were very surprised by the interest in this course; besides Croatia, our participants arrived from countries as far as Estonia, Ukraine, and Spain.

Nevertheless, the biggest challenge for our small Society is the regular organisation of the congress every four years. I am always delighted by the enthusiasm of the Organising Committee which plans in advance every detail of the Congress and this results in joyful, smooth and relaxed time at the congress.

The Scientific Committee of CROTOX 2016 has invited a number of eminent scientists to present you the latest results of research in a wide range of topics in toxicology. Besides the invited speakers, who have kindly accepted our invitation, you will also hear the presentations of six young scientists, the winners of the Congress Award for Young Scientists. At the poster section, you will have the opportunity to meet the authors of posters and discuss their work with them.

This Abstract Book is a Supplement to our scientific 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 2016 to be published in this journal. This Abstract Book contains the abstracts of the invited lecturers' presentations, as well as other oral presentations and presentations of the winners of the Congress Award for Young Scientists and poster presentations.

Congress will be held under the auspices of the Institute for Medical Research and Occupational Health and the Croatian Institute for Toxicology and Antidoping which is greatly acknowledged. We would like to thank the Croatian Academy of Sciences and Arts, the City of Zagreb and 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

CROTOX 2016, Poreč, Croatia, October 9-12

Programme

Sunday, 9 October 2016 (Day 1)		
13:00 – 17:00	Registration of participants	
18:15 – 19:00	**Opening ceremony**	
SESSION: DRUGS OF ABUSE AND ANTIDOTES **Chairs:** Maja Peraica, Davor Želježić, Davorka Sutlović		
19:00 – 19:45	**IL – 1**	**Elisabetta Bertol** (Florence, Italy) Advanced EWS: NPS challenge: our strategy and related analytical cases
19:45 – 20:30	**IL – 2**	**Zoran Radić** (La Jolla, USA) 50 years of allosteric hypothesis in cholinesterases, 25 years of its proof and its role in design of enhanced antidotes for organophosphate intoxication
21:00	**Welcome reception**	
Monday, 10 October 2016 (Day 2)		
8:00 – 8:30	**Posters will be put up by presenters**	
SESSION: EXPOSURE AND RISK ASSESSMENT **Chairs:** Maja Peraica, Corrado L. Galli		
8:30 – 9:15	**IL – 3**	**Marijana Ćurčić** (Belgrade, Serbia) Exposure to multiple chemicals – challenges
9:15 – 10:00	**IL – 4**	**Javier Esteban** (Alicante, Spain) Alterations of the retinoid system by brominated flame retardants
10:00 – 10:15	**OP – 1**	**Balázs Tóth** (Veszprém, Hungary) Testing technically challenging materials by inhalation
10:15 – 10:30	**OP – 2**	**Maurice Smith** (Neuchatel, Switzerland) Indoor air chemistry (IAC): Comparative study between conventional cigarette and heat-not-burn technology
10:30 – 11:00	**Poster viewing and coffee break** sponsored by **Kobis d.o.o.**	
SESSION: REGULATORY TOXICOLOGY **Chairs:** Irena Žuntar, Zdravko Lovrić		
11:00 – 11:45	**IL – 5**	**Corrado L. Galli** (Milan, Italy) Risk assessment in toxicology for plant protection products: much ado about nothing?
11:45 – 12:30	**IL – 6**	**Stefan Mandić-Rajčević** (Milan, Italy) From field exposure assessment to preventive biological exposure limits in agricultural pesticide use

12:30 – 12:45	**OP – 3**	**Judit Hargitai (**Veszprém, Hungary) Extended One-Generation Reproductive Toxicity Study – OECD 443
12:45 – 13:30	**Sponsor's presentation Alphachrom d.o.o.**	**Alphachrom users and applications team** (Zagreb, Croatia) The modern techniques in applications for toxicology analysis
13:30 – 14:30	**Lunch break**	
SESSION: FOOD TOXICOLOGY **Chairs:** Tomislav Klapec, Dušanka Milojković Opsenica		
14:30– 15:15	**IL – 7**	**Dušanka Milojković Opsenica** (Belgrade, Serbia) Urban honey – truths and misconceptions
15:15 – 16:00	**IL – 8**	**Bojan Šarkanj (**Osijek, Croatia) Mycotoxins in Croatian cereals: 101 reasons to worry?
16:00 – 16:15	**OP – 4 (YSL)**	**Ana Stanić** (Oslo, Norway) Presence of glutathione conjugates of mycotoxin deoxynivalenol and its breakdown products in naturally contaminated grain samples
16:15 – 16:30	**OP – 5**	**Zdenko Franić** (Zagreb, Croatia) Long term investigations of radioactivity in honey of north-west Croatia
16:30 – 16:45	**OP – 6 (YSL)**	**Antonija Sulimanec Grgec** (Zagreb, Croatia) Essential and toxic elements in three fish species typical for the dietary pattern in coastal Croatia
16:45 – 17:45	**Poster viewing and coffee break** sponsored by **Kobis d.o.o.**	
SESSION: GENOTOXICITY **Chairs:** Ksenija Durgo, Davor Želježić		
17:45 – 18:30	**IL – 9**	**Gonca Çakmak Demircigil** (Ankara, Turkey) *In vitro* particulate matter (PM) genotoxicity with regards to the season and region
18:30 – 18:45	**OP – 7**	**Tshepiso Jan Makhafola** (Florida/Pretoria, South Africa) *Combretum microphyllum* and *Leucospermum erubescens* inhibit the genotoxic effects of 4-NQO, MMC and EMS *in vitro*
18:45 – 19:00	**OP – 8**	**Mirta Milić** (Zagreb, Croatia) hCOMET COST action 15132 – an international network of researchers who are using comet assay in human biomonitoring studies
19:00 – 19:15	**OP – 9 (YSL)**	**Matjaž Novak** (Ljubljana/Maribor, Slovenia) Genotoxicity of selected cytostatic drugs in human hepatoma HepG2 cells
19:15 – 20:00	**Posters will be taken down by presenters**	

Tuesday, 11 October 2016 (Day 3)		
SESSION: TOXICOLOGY OF METALS AND NANOTOXICOLOGY **Chairs:** Jasna Jurasović, Vesna Matović		
8:30 – 9:15	**IL – 10**	**Vesna Matović** (Belgrade, Serbia) Overview of our results on cadmium toxicity
9:15 – 10:00	**IL – 11**	**Damjana Drobne** (Ljubljana, Slovenia) What makes nanoparticles toxic?
10:00 – 10:15	**OP – 10**	**Anita Jemec** (Ljubljana, Slovenia) From nanoparticles to microplastics: retrospective and prospective views
10:15 – 10:30	**OP – 11 (YSL)**	**Veno Kononenko** (Ljubljana, Slovenia) Phototoxicity of mesoporous TiO_2+Gd microbeads with the potential for cancer diagnosis and treatment
10:30 – 11:00	**Coffee break**	
SESSION: TOXICOLOGY OF METALS AND NANOTOXICOLOGY **Chairs:** Suzana Ćavar, Željko Jakšić		
11: 00 – 11:45	**IL – 12**	**Marijana Erk** (Zagreb, Croatia) Metals as contaminants in aquatic environment and their effects on aquatic organisms
11:45 – 12:30	**IL – 13**	**Željko Jakšić** (Rovinj, Croatia) Fate and effect of engineered (manufactured) nanomaterials on marine ecosystem
12:30 – 12:45	**OP – 12**	**Maja Levak** (Rovinj, Croatia) Impact of bio-corona on the toxicity of silver nanoparticles on the embryonal development of Mediterranean sea urchin *Paracentrotus lividus*
12:45 – 13:00	**OP – 13**	**Petra Burić** (Rovinj, Croatia) Sensitivity to different sizes of silver nanoparticles in the early life stages of two Mediterranean sea urchins *Arbacia lixula* (Linnaeus, 1758) and *Paracentrotus lividus* (Lamarck, 1816)
13:00 – 13:20	**Sponsor's presentation Shimadzu d.o.o.**	**Shimadzu d.o.o.** (Zagreb, Croatia)
13:20 – 14:20	**Lunch break**	
15:00 – 22:00	**Excursion with congress dinner**	

Wednesday, 12 October 2016 (Day 4)		
SESSION: ECOTOXICOLOGY **Chairs:** Emil Srebočan, Uwe Kierdorf		
9:00 – 9:45	**IL – 14**	**Uwe Kierdorf** (Hildesheim, Germany) From bats to kangaroos – research topics and approaches in wildlife toxicology
9:45 – 10:30	**IL – 15**	**Branimir K. Hackenberger** (Osijek, Croatia) Computing ecotoxicology – actuality and perspectives
10:30 – 11:00	**Coffee break**	
SESSION: ECOTOXICOLOGY **Chairs:** Damjana Drobne, Daniel Mark Lyons		
11:00 – 11:30	**IL – 16**	**Bojan Hamer** (Rovinj, Croatia) Interaction between climate change and biological monitoring of pollution
11:30 – 11:45	**OP – 14 (YSL)**	**Marija Dvoršćak** (Zagreb, Croatia) Determination of terbuthylazine in urine of rats exposed to low doses
11:45 – 12:00	**OP – 15 (YSL)**	**Mirna Velki** (Aachen, Germany/Osijek, Croatia) Assessment of pesticide effects on zebrafish embryos
12:00 – 12:45	**Closing lecture**	**Zdravko Lovrić** (Zagreb, Croatia) Implementation of chemicals legislation in Croatia
12:45 – 13:15	**Closing ceremony**	

ABSTRACTS

IL-1

ADVANCED EWS: NPS CHALLENGE: OUR STRATEGY AND RELATED ANALYTICAL CASES

Elisabetta BERTOL

Forensic Toxicology Division, Department of Health Science, University of Florence, Florence, Italy

New psychoactive substances (NPS) have increased in number and diffusion across the illegal drug market. Forensic toxicologists struggle with their identification and quantification. Developing a new analytical method could be the winning approach. The Forensic Toxicology Division (FTD) of the Florence University focused on the NPS issue, working also as the Coordinator of the European I-SEE Project (JUST/2013/ISEC/DRUGS/AG/6426). Its main goals are: to strengthen NPS information exchange between Italy and South East Europe (Slovenia and Croatia); to support the development and consolidation of national Early Warning System networks; to create a joint mechanism for information and good practice exchange and mutual learning; to increase information flow towards law enforcement and health professionals about NPS. FTD was involved in the foundation of the Unit of Research and Innovation in Forensic Toxicology and Neuroscience of Addiction (U.R.I.To.N.). The latter is the first Italian, and European as well, highly specialised Unit entirely focused on all aspects of drugs of abuse (especially NPS) by means of a multidisciplinary approach. Here we describe the NPS detection cases analysed by the FTD in seized material, *in vivo* and in post-mortem samples. A new screening method in liquid chromatography-tandem mass spectrometry (LC-MS/MS) was built and fully validated for a fast and sensitive detection of 69 compounds. Over the last few months there were several NPS detection cases where the seized material contained: 3-MMC, 4-FA, penthedrone, methoxethamine, AB-FUBINACA, 5-MAPB + bk-2C-B + 5-IT. The latter, requiring more analytical investigation (NMR), was analysed in collaboration with the Laboratory of the Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA) of the University of Florence, in the frame of U.R.I.To.N. *In vivo*: JWH-073, MDPV, AM-694, AB-FUBINACA, 3-MMC + 4-MEC, 3-MeO-PCP. Postmortem analysis: mephedrone, methylone + MDMA (and its metabolite, MDA) + ketamine (and its metabolite, nor-ketamine). Our experience in NPS detection in seized material, both *in vivo* and in postmortem samples, is a clear sign that their spread is real and worrisome. Analytical methods, as the one here described, are effective tools to face this phenomenon. Collaboration schemes, as those achieved with U.R.I.To.N, and the European I-SEE Project could be a starting point in planning an advanced Early Warning System against NPS.

KEY WORDS: *illegal market, postmortem analysis, psychoactive substances*

IL-2

50 YEARS OF ALLOSTERIC HYPOTHESIS IN CHOLINESTERASES, 25 YEARS OF ITS PROOF AND ITS ROLE IN DESIGN OF ENHANCED ANTIDOTES FOR ORGANOPHOSPHATE INTOXICATION

Zoran RADIĆ

Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego, La Jolla, CA, USA

Cholinesterases are a family of serine hydrolases comprised of two oligomeric globular enzymes: acetylcholinesterase (AChE, EC 3.1.1.7), a key enzyme in vertebrate cholinergic neurotransmission, and butyrylcholinesterase (BChE, EC 3.1.1.8), a structurally and functionally very close but physiologically much less important enzyme. Both enzymes have been implicated in several different therapeutic approaches including treatments of organophosphate (OP) intoxications. In that context allosteric properties of cholinesterases, first proposed in 1961 for AChE by Jean-Pierre Changeux, are particularly useful and we are currently developing several different approaches to OP intoxication treatments that rely on allosterism of both AChE and BChE. Based on the available X-ray 3D structures of the two enzymes, first solved for AChE in 1991, our goal is to develop enhanced small molecule antidotes of OP poisoning from the family of nucleophilic aldoximes. This has been a long lasting international multi institutional effort with particularly significant contribution of Dr. Zrinka Kovarik of the Institute for Medical Research and Occupational Health, Zagreb, Croatia, along with Drs. Barry K. Sharpless and Valery V. Fokin of the Scripps Research Institute in La Jolla, CA, USA. An overview of our most recent advances in this research will be presented.

KEY WORDS: *acetylcholinesterase, butyrylcholinesterase, organophosphate poisoning, oxime antidotes, structure-based drug discovery*

IL-3

EXPOSURE TO MULTIPLE CHEMICALS – CHALLENGES

Marijana ĆURČIĆ

Department of Toxicology "Akademik Danilo Soldatović", Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

Typically, exposure to chemicals is unintentional and often in everyday life it is low-dose exposure to multiple chemicals, either simultaneous or sequent. The objective of this literature overview of challenges is to indicate the most frequently proposed models for interpreting the effects caused by exposure to multiple chemicals, particularly to toxic metals in combination with polyhalogenated organic compounds. A key challenge in the risk assessment of exposure to multiple chemicals is whether the adverse effects of a mixture may occur when chemicals are combined at low doses which individually do not induce adverse effects. Even if chemicals individually cause observable effects, the challenge in data interpretation is selecting a mathematical model that can be used with high level of certainty to prove whether such interactions occur, and what type of interaction could be expected. Reviewing the literature on co-exposure, we found evidence demonstrating significant mixture effects with combinations of chemicals below their individual no observable adverse effect levels (NOAELs) or benchmark dose of 5 % (BMDL5) for both types of mixtures, i.e. composed of agents acting similarly or dissimilarly. If toxic metals and organohalogen compounds act dissimilarly, the effect additivity approach is proposed. Comparing the curves' shapes of the effects assessment is valuable; however this approach is two-dimensional. Response surface methodology described with a polynomial equation could be used for gaining insight into the type of interaction considering the significance and impact of quadratic polynomial subject, while central composite design is one step further. In conclusion, co-exposure to dissimilarly acting chemicals is a challenge in toxicological risk assessment and needs more models and experimental evidence supporting models for reliable data interpretation.

KEY WORDS: *metals, multiple chemicals exposure, polyhalogenated organic compounds, risk assessment*

IL-4

ALTERATIONS OF THE RETINOID SYSTEM BY BROMINATED FLAME RETARDANTS

Javier ESTEBAN[1], Ismael SANCHEZ-PEREZ[1], Sergio MARTÍNEZ[1], Xavier BARBER[2], Sabina LITENS KARLSSON[3], Helen HÅKANSSON[3], and Leo van der VEN[4]

Instituto de Bioingeniería[1], Centro de Investigación Operativa[2], Universidad Miguel Hernández, Elche (Alicante), Spain, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden[3], Centre for Health Protection, National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands[4]

Brominated flame retardants (BFRs) are commercial technical mixtures, which are mainly used in different fire-fighting and prevention applications and products, including electrical and electronic equipment, textiles, plastics, and thermal insulations. Polybromodiphenyl ethers (PBDE) and hexabromocyclododecane (HBCD) are among those BFR, which have been banned or restricted on the basis of their persistence in the environment, bioaccumulation in living organisms, and their similarity in toxicity profile to other banned persistent organic pollutants. These properties imply that humans and wildlife will continue to be exposed to BFRs in the future even when restrictions are in place. Thus regulatory agencies, such as EFSA, have recommended that the toxicological knowledge database on BFRs needs to be extended and improved. The objective of the current work was to extend the knowledge database for the effects of BFRs on the retinoid system. Wistar rats were exposed to pentaBDE, decaBDE, or HBCD technical mixtures for 28 days according to the OECD repeated-dose test-guideline (TG407). Retinoid levels and expressions of target genes involved in the retinoid system were measured in the livers. The tested BFRs induced changes in hepatic retinoid levels and gene expressions in chemical-and-gender-specific manners. Reductions of hepatic retinoid levels differed in potency and efficacy among the three tested BFRs and between genders. Likewise, there were compound and gender differences in gene expression modulations, which included inductions of phase I and II biotransformation enzymes, as well as enzymes and binding-proteins involved in the maintenance of cellular retinoid homeostasis. From this communication, there is substantial support for the recently initiated OECD-TG-project on analysing the possible need to incorporate the retinoid system in future toxicity testing methods.

KEY WORDS: *decaBDE, genes, HBCD, pentaBDE*

IL-5

RISK ASSESSMENT IN TOXICOLOGY FOR PLANT PROTECTION PRODUCTS: MUCH ADO ABOUT NOTHING?

Corrado L. GALLI

Section of Toxicology and Risk Assessment – UNISAFE, Department of Pharmacological and Biomolecular Sciences (DiSFeB), University of Milan, Milan, Italy

Before an active substance can be used within a plant protection product in the EU, it must be approved by the European Commission under the Regulation (EC) No 1107/2009. A dual system is in place, under which the European Food Safety Authority (EFSA) evaluates active substances used in plant protection products and Member States evaluate and authorise the products at national level. A very comprehensive and intensive risk assessment that evaluates the potential for harm to humans, wildlife, fish, and plants, including endangered species and non-target organisms, contamination of surface water or ground water from leaching, runoff, and spray drift has been developed. The EU-coordinated programme aims to provide statistically representative data regarding pesticide residues in food available to European consumers. Thus, the results obtained in the coordinated programme are considered an indicator for the Maximum Residue Level (MRL) compliance rate in food of plant and animal origin placed on the European common market and they allow an estimation of the actual consumer exposure. Usually, MRLs are derived from supervised field trials representative of the intended Good Agricultural Practice (GAP). The MRL is not a toxicological limit, but it is based on GAP. If control activities identify samples with pesticide concentrations which are of concern for consumer health, Member States have to inform other Member States and the European Commission via the Rapid Alert System for Food and Feed (RASFF). In 2013, in the EU Member States, a total of 80 967 samples of a wide variety of unprocessed raw agricultural commodities and processed food products were analysed for residues of 685 distinct pesticides. Overall, 97.4 % of the tested food samples fell within the legal limits and 54.6 % of the samples contained no quantifiable residues at all. The results of dietary exposure estimations support the conclusion that, in the light of current knowledge, the presence of residues found in the food products covered by the EU-coordinated monitoring programmes was unlikely to have a long-term effect on the health of consumers.

KEY WORDS: *European Food Safety Authority (EFSA), Good Agricultural Practice, long-term health effect residues*

IL-6

FROM FIELD EXPOSURE ASSESSMENT TO PREVENTIVE BIOLOGICAL EXPOSURE LIMITS IN AGRICULTURAL PESTICIDE USE

Stefan MANDIĆ-RAJČEVIĆ[1], Federico Maria RUBINO[1], Danilo COTTICA[2], Sara NERI[2], and Claudio COLOSIO[1]

Department of Health Sciences of the University of Milan and International Centre for Rural Health of the San Paolo Hospital, Milan[1], Centre for Environmental Research, Fondazione Salvatore Maugeri, Pavia[2], Italy

Risk assessment of agricultural workers should be performed in all scenarios of pesticide use, but they are rarely a priority for exposure and risk assessment activities. The aim of our study was to perform exposure and risk assessment in vineyards of North Italy, to analyse determinants of exposure to pesticides, and to explore the possibilities for further use of field study results to provide tools for a simple, cheap, but accurate risk assessment. A broad field study was done in 2011 in the Region of Lombardy to assess the exposure and risk of 29 agricultural workers applying Mancozeb for 38 work days using open tractors and closed tractors with air filters. Contamination on clothes and skin was done using the "pads" approach, and 24-hour post-exposure urine was collected for biological monitoring. All samples were analysed using LC-MS-MS. Most workers used closed and filtered (29), while only nine used open tractors. Between one and 20 hectares of vineyards were sprayed daily, and the most commonly used personal protective devices were coveralls, gloves, and masks. Median exposure on clothes was 255 mcg (more than 6000 mcg for open tractors, and just above 150 for closed and filtered ones, $p<0.01$), while the median exposure on skin was 1.62 mcg (3.9 versus 1.4 for open and closed tractors, $p>0.05$). Median absorbed dose, calculated utilising a custom algorithm, was 0.02 ng, which is several hundred times lower than the AOEL.

KEY WORDS: *agricultural workers, biological monitoring, LC-MS-MS, risk assessment, vineyards*

IL-7

URBAN HONEY – TRUTHS AND MISCONCEPTIONS

Dušanka MILOJKOVIĆ OPSENICA

Faculty of Chemistry, University of Belgrade, Belgrade, Serbia

Honey is defined as natural sweet substance produced by *Apis mellifera* L. bees from the nectar (nectar honey) and secretions of the living parts of plants, or from excretions of plant-sucking insects (honeydew honey). Chemically, honey is a concentrated aqueous solution of different carbohydrates (mainly fructose, glucose, and sucrose), which also contains amino and organic acids, proteins, minerals, polyphenolics, and other phytochemicals. The composition of this complex mixture containing a few hundred various substances depends on different factors such as bee species, nectar-providing plant species, geographic area, season, mode of storage, and even harvest conditions and technology. Being considered since ancient times a natural, healthy and clean foodstuff, honey must be free of any chemical contaminants and safe for consumers. However, honey is prone to various types of contamination either from beekeeping practices (acaricides, antibiotics, bee repellents) or from environment (heavy metals, pesticides, pathogenic bacteria, organic pollutants, radionuclides). Numerous studies have investigated the role of honeybees and their products, especially honey, as bioindicators for monitoring environmental pollution in rural, industrial, or urban areas. It seems that minerals are especially useful for these purposes due to their stability in honey. Some researchers reported a considerably lower content of heavy metals and other contaminants in honey than in honeybees and, therefore, a certain "filtering" of pollutants by bees can be assumed. Since honey reflects the contamination in the forage plants, air, and soil of the hive surrounding, the content of possible pollutants in honey produced in urban area has been discussed.

KEY WORDS: *bioindicators, heavy metals, organic pollutants, pesticides, quality of urban honey*

IL-8

MYCOTOXINS IN CROATIAN CEREALS: 101 REASONS TO WORRY?

Bojan ŠARKANJ

Department of Applied Chemistry and Ecology, Faculty of Food Technology, University of Josip Juraj Strossmayer, Osijek, Croatia

Mycotoxins are one of the unavoidable contaminants of crops, having the highest impact on cereals. The data on the occurrence of regulated mycotoxins in Croatian cereals is scarce, and the data on the unregulated ones is virtually inexistent. The EFSA has requested additional occurrence data for many of the unregulated mycotoxins, but there has been no published data from Croatia. The CroMycoScreen project was based on this premise and it launched a first systematic screening of both regulated and unregulated mycotoxins, together with some plant, bacterial, and yeast toxins in cereal crops from all Croatian regions. The result of the project was the first public map and database on the occurrence of mycotoxins in Croatia, both regulated and unregulated. The applied method analysed a total of 650 different microbial and plant metabolites (mainly mycotoxins), and detected over 100 different mycotoxins in Croatian cereals harvested in season 2015, including all those regulated in the EU. The information on the occurrence was reported to EFSA, and is a base for establishing an annual mycotoxin monitoring programme in Croatia to ensure food and feed quality. The database will also be applied as a starting point to monitor the effect of climate change on the occurrence of mycotoxins. By developing this kind of databases it should be possible to develop the prediction models which will enable the undertaking of timely and adequate measures to prevent mycotoxin contamination of cereals in Croatia.

KEY WORDS: *contaminants of crops, Croatia, LC-MS/MS, microbial metabolites occurrence*

IL-9

IN VITRO PARTICULATE MATTER (PM) GENOTOXICITY WITH REGARDS TO THE SEASON AND REGION

Gonca ÇAKMAK DEMIRCIGIL

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Genotoxicity as the intermediate step of cancer could be of use in disease prevention when assessed using short term assays. Genotoxicity assays can be used in the regulation of chemicals or in mechanistic *in vivo* and *in vitro* studies. Also, genotoxicity tests involve the effect of biomarkers of possible exposure scenarios in environmental and occupational molecular epidemiology studies. Particle toxicology has quite a long history when considering occupational exposure to coal dust, asbestos, and silica. In today's industrial world, engineered particles (especially nano-sized) are particularly under scrutiny because of their likely adverse health effects besides their advantages. At the same time, environmental particulate matter (PM) exposure and related toxicity are receiving attention, especially in urban and industrialised environments. The adverse health effects of PM are crucial for daily life, mostly for vulnerable populations like children. Therefore, studies on PM toxicity are based on the understanding of seasonal and regional characterisation of particles and subsequently on the mechanisms of toxicity such as genotoxicity. In this study, PM toxicity studies will be examined, from molecular epidemiology to *in vitro* approaches.

KEY WORDS: *air pollution, genotoxicity,* in vitro *toxicology, molecular epidemiology, particle toxicology*

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IL-10

OVERVIEW OF OUR RESULTS ON CADMIUM TOXICITY

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Discovered in 1817, cadmium (Cd) was recognised as an occupational agent in the first half of 20th century and is nowadays considered one of the most important environmental pollutants. Our first investigations carried out on rabbits showed significant effects of Cd on bioelement homeostasis in different organs confirming interactions with essential elements as an important mechanism of Cd toxicity. This fact was confirmed by our studies on mice and rats. Investigations on mice were extended to include the effect of both acute and subacute Cd intoxication on the oxidative status in the liver and kidneys. These investigations showed that Cd induced a more profound effect on reactive oxygen and nitrogen species in the liver in acute intoxication while this effect was predominantly observed in the kidneys after subacute intoxication. We also compared the effect of single *p.o.* and single *i.p.* Cd administration on the parameters of oxidative stress in rat blood and liver and concluded that the oxidative status was impaired more profoundly after *i.p.* treatment. Our recent studies were aimed to investigate the effects of different Cd *p.o.* doses on various organs in rats. The study demonstrated significant effects on body weight gain suggesting possible developmental toxicity, and also confirmed hematotoxic, hepatotoxic and nephrotoxic effects of this toxic agent. The obtained results also provided evidence of thyroid disrupting effects with a more pronounced decrease in T3 hormone levels indicating predominant disruption of extrathyroid processes. Special attention should be given to the findings that suggest that even low doses of Cd corresponding to human environmental exposure can result in a toxicological response.

KEY WORDS: *cadmium, experimental studies, mechanisms of toxicity*

IL-11

WHAT MAKES NANOPARTICLES TOXIC?

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Any application of nanoparticles requires information on their toxicity. In conventional toxicology, concentration and time are two key parameters in which a chemical compound may be considered either "safe" or "dangerous". However, in nanotoxicology the dose metric is not straightforward. It is known that NP-induced toxicity arises from a complex interplay between particle size, shape, surface chemistry, charge, dose, route of exposure, and host response. In addition, particles interact with biological matter, which changes their surface characteristics and determines their fate in the body. Scientific knowledge about nanoparticle–cell/organism interaction mechanisms has been accumulating dramatically in recent years, indicating that there may be no common mechanism of nanoparticle toxic action. Since nanoparticles have many characteristics which are not found in conventional chemicals, some unique (nano)particle-specific biological effects may be anticipated, such as the adsorption to the external surfaces of an organism, adsorption of biomolecules when entering biological fluids, and ability of nanoparticles to hijack a pre-existing transport mechanism through the body using endocytotic mechanisms. In this presentation, some recently discovered bio-nano interactions, which occur before oxidative stress, will be listed and discussed. Since a widespread translation of nanoparticles within the human body and their overall presence in the environment seems likely, we need to take any toxicological risks seriously.

KEY WORDS: *biomolecules, cell/organism interaction mechanism, environmental risk, nanoparticles*

IL-12

METALS AS CONTAMINANTS IN THE AQUATIC ENVIRONMENT AND THEIR EFFECTS ON AQUATIC ORGANISMS

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Next to natural sources, metals are present in the environment as the result of human activities which affect the state of aquatic ecosystems. Metals occur in aquatic environment in various concentration ranges (major, minor, and trace metals), and in a number of physico-chemical forms. According to their role in living organisms metals can be classified as essential and non-essential. Essential metals are necessary for normal development, growth, and functioning of all living organisms (e.g. Zn is a component of many enzymes; Cu and Fe are functional parts of respiratory proteins haemocyanin and haemoglobin, respectively). Non-essential metals (Cd, Pb, Hg, Ag) are usually toxic to an organism, as are essential metals when present internally at concentrations above the limits of the cellular regulatory process. Aquatic organisms are continuously exposed to variable concentrations of metals in water. Metal uptake by aquatic organisms depends on specific and various environmental conditions such as water hardness, salinity, temperature, irradiance, pH, and organic matter content, as well as on the species of an organism. High metal uptake occurs particularly in the areas which are influenced by anthropogenic heavy metal contamination. In this respect, molluscs, crustaceans, fish, and other aquatic organisms, which are known to accumulate high levels of heavy metals in their tissues and yet survive in these polluted environments, are suitable as bioindicator organisms. The ability of these animals to cope with elevated metal concentrations depends on their capacity to regulate the heavy metal concentration inside the cell and to accumulate excess metal in non-toxic forms. Case studies encompassing freshwater (rivers in Croatia: Sava, Sutla, and Krka, and Macedonian rivers Kriva, Zletovska, and Bregalnica) and marine ecosystems (the central part of the eastern Adriatic coast) and using various bioindicator organisms (bivalves, small crustaceans, fish, and fish parasites) will be presented.

KEY WORDS: *bioindicator organisms, biomarkers, differential pulse voltammetry, inductively coupled plasma mass spectrometry, metallothionein*

IL-13

FATE AND EFFECT OF ENGINEERED (MANUFACTURED) NANOMATERIALS ON MARINE ECOSYSTEM

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In the recent two decades Engineered (Manufactured) Nanomaterials (ENMs) have become one of the marine contaminants of emerging concern. Their versatile physicochemical characteristics influence their fate and effect in the marine ecosystem, and represent novel challenges to the established suit of hazard assessment tests. The presentation will describe the pathways of ENMs in the marine ecosystem (water column and sediment), their fate and behaviour as well as interactions with macromolecules and living organisms. Special attention will be devoted to the physico-chemical processes of ENMs, like adsorption, dissolution, transformations, and homo/hetero-aggregation in marine environment. A comprehensive state-of-the-art overview of marine nanotoxicology research (test systems and model organisms, uptake routes and mechanisms, body and/or intracellular trafficking/translocation/storage and bioaccumulation; trophic transfer; specific targets of effects, mode of actions and mechanisms of toxicity, sub-lethal effects and acute toxicity) will be outlined by presentation of case studies' results and discussion of ways forward. The important processes such as immune response, oxidation stress, and genotoxic effects will be explained and commented in details. Spatial emphasis will be provided to question how environmental conditions determine exposure to and effect of ENMs. Furthermore, identification and avoidance of potential artefacts and misinterpretations in nano(eco)toxicity measurements will be pointed out. Finally, the lecture will provide an overview of the ENMs regulatory assessment (REACH, OECD) and future research needs/challenges (integration of ENMs chemistry, experimental modelling, high throughput screening, systems toxicology approach, realistic exposure and chronic effects/responses across different levels of biological organisations, effects on sediment species).

KEY WORDS: *acute toxicology, engineered (manufactured) nanomaterial, marine nano(eco)toxicology, mode of action, physico-chemical processes, regulatory assessment, sub-lethal effects*

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IL-14

FROM BATS TO KANGAROOS – RESEARCH TOPICS AND APPROACHES IN WILDLIFE TOXICOLOGY

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Free-living wild mammals are suitable indicators of the presence of contaminants in ecosystems and the effects of these substances on organisms. Tissue concentrations can be used to study both geographical variation of contaminant levels in the environment and changes of these levels through time. Additionally, biochemical, physiological, or morphological alterations in organisms can be used as biomarkers, providing information on (potentially) harmful effects on organisms. Interpretation of contaminant-induced changes requires a detailed knowledge of the biology of the species under study. This lecture gives examples for the use of wild mammals as biological indicators in a wide range of species. Analysis of trace metal concentrations in hairs allows the assessment of exposure of wild mammals to these elements. This non-destructive and non-harmful method is particularly suited for protected species like bats. Determination of lead and fluoride concentrations in the periodically replaced antlers of deer has been applied to study regional variation in exposure, and for historical monitoring of changing environmental levels of these bone-seeking substances. Over the last decades, dental fluorosis has been successfully used as a biomarker of chronic fluoride toxicosis caused by emissions from natural or anthropogenic sources. Our studies were performed on various mammalian (placental and marsupial) species and led to a deeper understanding of the structural changes in the formation of dental hard tissues caused by excess exposure to fluoride. More recently, also the effects of excess fluoride on the skeleton of various marsupial species were studied, and the first results of this work will also be presented.

KEY WORDS: *environmental contamination, exposure monitoring, fluoride, tissue effects, trace metals*

IL-15

COMPUTING ECOTOXICOLOGY – ACTUALITY AND PERSPECTIVES

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Computational technology has been one of crucial drivers of science progress over the past half-century. Today, the development is unimaginable without disciplines such as bioinformatics, computational chemistry, computational biology, etc. Computers have ceased to be a tool whose main purpose is to accelerate calculation or store a large amount of data. Now they play a role in decision making and adaptation of algorithms based on the power of parallel computing methods and the opportunities of deep learning, neural networks, and artificial intelligence methods. Forward scientific march of toxicology has resulted in the blossoming of new areas ripe for further investigation. The major part of new disciplines such as –omics, high-throughput screening and, particularly, nanotoxicology rely on the creation of new computational capabilities and a new software. In addition to a significant role in predictive toxicology (mainly assessment of toxic effects of untested chemicals), computational methods are increasingly being used in ecotoxicology. They enable computational experiments which have a strong correlation with realistic conditions, often impossible to obtain in practice. Within DEFENSoil project, methods for computational toxicological and ecotoxicological experiments are being developed and tested against conventional toxicological tests. The preliminary results show a high degree of correlation at all experimental levels. The use of hardware based on GPU and CUDA based software enables the development of highly realistic simulations in the first phase of experiment and execution of experiment in the second phase.

KEY WORDS: *DEFENSoil, ecotoxicological experiments, GPU and CUDA software, predictive toxicology*

IL-16

INTERACTION BETWEEN CLIMATE CHANGE AND BIOLOGICAL MONITORING OF POLLUTION

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Increasing evidence shows that climate change (CC) is making seas and oceans around the world warmer and more acidic. In the Croatian Adriatic, we have identified temperature increase, and especially unusually warm summer periods, as one of the main threats. High seawater temperatures during summer push organisms over their tolerance limits with several adverse effects finally resulting in high mortality rate, which interferes with biological monitoring of pollution. The problem is already present in mussels in mariculture and wild populations, as sessile organisms are especially sensitive to "thermal bath" shock during high summer months. Winter and summer progressive acclimation of mussels *Mytilus galloprovincialis* to lower salinities proved salinity and season effect on several biochemical markers and physiological parameters investigated. Further, an estimation of freshwater influx along the eastern Adriatic coast by carbon and oxygen isotopic composition analyses of mussel shell carbonate layers indicated that salinity was relatively constant at the open sea, with strong variations in estuaries, locations close to under-sea freshwater springs and during rainy days in closed lagoons. Temperature values measured at two reference points for the open sea: Rovinj (northern Adriatic) and Stončica (southern Adriatic), in the 1961-1990 and 1998-2007 periods (average monthly values) showed a small general increase (0.1-1.5 °C) of surface seawater temperatures in the latest 1998-2007 period. Similar SW temperature increases are predicted for the lower emission scenario (B1) by IPCC. Mussels living in the infralitoral zone at the investigated locations of the Adriatic Sea coastal area were exposed to a wide range of temperatures that followed a clear annual cycle with seasonally averaged values ranging from approximately 11-14 °C in winter (March) to 19-26 °C in summer (August), with unusual low (<10 °C) and high (>27 °C) temperature and their duration. Legislation and practices have highlighted the importance of the biomarker integrated approach in environment quality assessment, and laboratory studies using model substances (contaminants) usually show potential of different biomarkers. The main difficulty in using biomarkers in monitoring programmes is the interference of natural environmental factors with biological responses. Additionally to the mentioned research, a general overview of climate change impacts on the ecosystem and biota, how these interact with contaminants, and their fate and effects will be summarised.

KEY WORDS: *biological monitoring of pollution, biomarkers, mussels, salinity tolerance limits, thermal acclimation limits*

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IL-17

IMPLEMENTATION OF CHEMICALS LEGISLATION IN CROATIA

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Serious improvements in EU chemicals legislation began in 2006 with REACH Regulation, and continued in 2008 with CLP Regulation. Even during the preparation of these two regulations Croatia enacted its own Chemicals Act that was fully in compliance with the EU legislation. Therefore, after joining the EU, the Croatian chemical legislation was completely harmonised with the European one. Special enforcement acts were laid down; the ordinance that regulates the production, marketing, and handling of chemicals; the ordinance that regulates the storing of dangerous gaseous chemicals; the ordinance that regulates mandatory training of persons dealing with chemicals; the ordinance on the registration of chemicals that are produced, imported, or in any other way marketed in Croatia. Besides the Chemicals Act, the main Croatian act regulating chemicals, there are several other acts related to this piece of legislation. Acts on the implementation of the PIC Regulation and the Biocidal Regulation are also very important in managing chemicals. This presentation will show how this legislation applies to the management of chemicals in Croatia. All these pieces of legislation with amendments can be found on the web page of the Croatian Institute for Toxicology and Antidoping www.hzt.hr.

KEY WORDS: *Chemicals Act, CLP, implementation, ordinance, REACH*

OP-1

TESTING TECHNICALLY CHALLENGING MATERIALS BY INHALATION

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The most challenging part of inhalation studies is to generate respirable atmosphere. In order to have a proper dosing of animals, a stable concentration of the test item with an appropriate particle size should be achieved. Usually, atmosphere generation from gases, vapours, and water solutions can be solved without major difficulties, but dusts, powders, and fibres can be technically challenging. In order to successfully perform inhalation studies using these types of materials, the critical point is to prevent heat generation, electrostatic charging, turbulence, and sudden pressure drop during aerosol generation and in the inhalation system, because these events might lead to clump formation, sedimentation, and clogging, and thus the stable aerosol for the study cannot be maintained. The other important parameter is the particle size. For acute studies the mass median aerodynamic diameter (MMAD) required in rats is four microns, while for long term studies this size is three microns, since only the particles under this size might reach the alveoli in the lung. In the presentation, different technical solutions will be shown as to how we can achieve the sufficient inhalation parameters and how we can handle technically challenging materials.

KEY WORDS: *aerosol, atmosphere generation, inhalation, MMAD, particle size*

OP-2

INDOOR AIR CHEMISTRY (IAC): COMPARATIVE STUDY BETWEEN CONVENTIONAL CIGARETTE AND HEAT-NOT-BURN TECHNOLOGY

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Philip Morris International (PMI) is developing products with a potential to reduce the risk associated with smoking. The Tobacco Heating System (THS 2.2) operates by heating tobacco rather than burning it and results in an aerosol with substantially lower levels of harmful or potentially harmful constituents when compared to combustible cigarette (CC) smoke. Additionally, THS 2.2 does not produce sidestream aerosol in the same manner as CC, since aerosol is only generated when puffs are taken. Thus, the impact on air quality of using THS 2.2 indoors is expected to be very different to CC. To verify this hypothesis, PMI built an environmentally controlled furnished room and developed analytical methods to measure air pollutants under diverse simulated indoor environments focusing on: (i) ISO measurement standards for Environmental Tobacco Smoke and, (ii) selected carbonyls and volatile organic compounds. A study was conducted with three simulated conditions (office, hospitality, residential) with conditions defined according to CEN standard EN 15251:2007. Three test items were compared: CC (Marlboro Gold 6 mg), THS 2.2 and background (measured with people in the room without product use). Each study was duplicated, resulting in 18 separate sessions in total, each with duration of five hours, with four hours of sample collection. For each analyte and condition, measured THS 2.2 and CC levels were compared to background levels. In case of statistical equivalence, no impact on air quality is reported. When levels are statistically above background, the levels are adjusted by subtraction of the background and reported (in mass per cubic meters). For CC, all analytes for the three conditions were above background. For THS 2.2, no difference was detected between background and THS 2.2 for fifteen of the eighteen analytes investigated, irrespective of the environmental conditions applied. For the three analytes that were demonstrated to be statistically increased between THS 2.2 and background (nicotine, acetaldehyde, and nitric oxides), the levels measured for THS 2.2 were only slightly increased compared to the background, one or two orders of magnitude lower than those measured for CC.

KEY WORDS: *heat-not-burn tobacco products, indoor air chemistry*

OP-3

EXTENDED ONE-GENERATION REPRODUCTIVE TOXICITY STUDY – OECD 443

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The main objective of the Extended One-Generation Reproductive Toxicity Study (EOGRTS, OECD No. 443) is to evaluate specific life stages not covered by other types of toxicity studies and provide an assessment of the pre- and postnatal effects of chemicals on development and systemic toxicity in pregnant and lactating females and young and adult offspring. The general study design is used to detect the effects on the reproductive organs of parental males and females, as well as to examine three cohorts of F1 animals. Cohort 1 is used for the assessment of reproductive/developmental endpoints; this cohort may be extended to include an F2 generation if needed. Cohort 2 is used for the assessment of a potential impact of chemical exposure on the developing nervous system, while Cohort 3 is used for the assessment of a potential impact of chemical exposure on the developing immune system. The test substance is administered continuously to several groups of sexually-mature males and females. This parental (P) generation is dosed for a defined pre-mating period and a two-week mating period. P males are further treated at least until weaning of the F1 generation; parental females are also treated during pregnancy and lactation until termination after the weaning of their litters. The F1 offspring receive further treatment with the test substance from weaning to adulthood (including until weaning of the F2 if needed). A range of special methods for the assessment of neurotoxicity and immunotoxicity have been validated for the conduct of this study type.

KEY WORDS: *EOGRTS, immunotoxicity, neurotoxicity, reproductive toxicology*

OP-4 (YSL)

PRESENCE OF GLUTATHIONE CONJUGATES OF MYCOTOXIN DEOXYNIVALENOL AND ITS BREAKDOWN PRODUCTS IN NATURALLY CONTAMINATED GRAIN SAMPLES

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Mycotoxin deoxynivalenol (DON) contains many structural features that may give rise to biotransformation reactions in living organisms. Such products are not commonly detected during routine analysis. Conjugation with sulphur compounds has been identified as a significant reaction pathway and putative DON–glutathione (DON–GSH) conjugates have been reported *in planta*. We prepared analytical standards of L-cysteine and glutathione conjugates of DON, which were linked to DON either irreversibly to the α,β-unsaturated carbonyl group or/and irreversibly to the epoxide ring. Cysteine conjugates of DON were tested *in vitro* for toxicity and proved to be much less toxic than DON at the same concentration.We also tentatively identified DON-γ-glutamyl-cysteine,-cysteinyl-glycineand-*N*-acetylcysteine ("mercapturic acid") conjugates using liquid chromatography coupled with high resolution mass spectrometry. Naturally contaminated cereal extracts(n=35, oats and spring wheat) contaminated with DON at concentrations of 1.1 to 11 mg kg^{-1} were screened for the presence of DON-GSH conjugates and its breakdown products. DON-GSH conjugates were detected in 63 %, DON-Cys-Gly in 6 %, and DON-Cys in 31 % of the samples and *N*-acetylcysteine conjugated DON isomers were detected in 26 % of the samples. The LC-MS data (comparison to NMR characterised standards, retention time, $HRMS^2$) showed that major conjugates were *S*-linked to DON via the opening of the epoxide ring. Our data confirms that GSH-conjugation to DON occurs in plant and suggests that a rare plant conjugate, DON-mercapturate, could be a breakdown product of such DON-GSH conjugates.

KEY WORDS: *biotransformation, conjugates, HRMS, mercapturate, trichothecene*

OP-5

LONG TERM INVESTIGATION OF RADIOACTIVITY IN HONEY OF NORTH-WEST CROATIA

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We present long-term investigation of radioactive contamination of honey of north-west Croatia by ^{137}Cs and ^{134}Cs and by naturally occurring ^{40}K. In Croatia, caesium radionuclides in honey were first investigated after the Chernobyl nuclear accident in 1986. The activity concentrations of both radionuclides peaked in May 1986, decreasing exponentially afterwards. The estimated mean residence time, corrected for radioactive decay, was found to be 1.2 y for ^{137}Cs and 0.9 y for ^{134}Cs. In the early 1990s, activity concentrations in honey for both radionuclides were under the detection limit, until Fukushima Daiichi accident in 2011. Radiocaesium activity concentrations in honey are correlated with their activity concentrations in fallout, with coefficient of correlation r=0.92. Effective radiation doses due to radiocaesium, received by the Croatian population by honey consumption, were estimated to be very small, annual *per caput* dose being <1 μSv. Investigation of activity concentrations of naturally occurring ^{40}K in honey can serve as a rapid screening method for honey adulteration. When foraging for food, honey bees are exposed to potential pollutants in a surface area of more than 7 km^2. Therefore, honey and other bee products could be useful as biomonitoring tools for collecting information regarding the environmental pollution within the bees' forage area. Based on the mobility of honey bees and their ability to integrate all exposure pathways, inclusion of honey and other bee-farming products in the routine radioecological monitoring programme for the Croatian environment could add another level of confidence to the present environmental monitoring programme.

KEY WORDS: *Croatian honey, ^{134}Cs, ^{137}Cs, effective dose, mean residence time*

OP-6 (YSL)

ESSENTIAL AND TOXIC ELEMENTS IN THREE FISH SPECIES TYPICAL FOR THE DIETARY PATTERN IN COASTAL CROATIA

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The study evaluated levels of essential (Fe, Cu, Zn, Se) and toxic (Pb, Cd, Hg) trace elements in the edible tissue of three commonly consumed fish species in coastal Croatia: the oily fish European anchovy (*Engraulis encrasicolus; n*=90) and sardine (*Sardina pilchardus; n*=59), and lean demersal fish red mullet (*Mullus barbatus; n*=64). Samples were collected in the coastal and open waters of the eastern Adriatic Sea at 11-13 locations for each of the species. Composite samples of muscles were freeze-dried, homogenised, and digested before analysis. Inductively coupled plasma mass spectrometry (Agilent 7500cx ICP-MS) was used for multielement analysis. Oily fish species had 3-4 times higher levels of essential elements Fe, Cu, and Zn in comparison to the lean fish. Levels of Se ranged as follows (mg kg^{-1} wet wt): anchovy 0.27-0.37; sardine 0.66-1.1; red mullet 0.35-0.73. Ranges of Hg concentrations (mg kg^{-1} wet wt) for these fish species were: anchovy 0.02-0.13; sardine 0.05-0.14; red mullet 0.18-4.1.Toxic metal levels in all three species were below the maximum permissible values set by the EU Commission Regulation, with the exception of one red mullet sample with an Hg concentration of 4.1 mg kg^{-1} wet wt. The presented results are valuable for the assessment of nutritional and toxic metal intake *via* seafood in the Croatian population, especially for women of reproductive age and children, vulnerable population groups for toxic effects of common environmental pollutants including metals.

KEY WORDS: *element analysis, mercury, metals, seafood, selenium*

OP-7

COMBRETUM MICROPHYLLUM AND *LEUCOSPERMUM ERUBESCENS* INHIBIT THE GENOTOXIC EFFECTS OF 4-NQO, MMC, AND EMS *IN VITRO*

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The possibility of moderating the response of cells to mutagens by phytomedicines opens new horizons in cancer prevention. On this basis, the search for antimutagens presents possibilities for the discovery of new anticarcinogenic substances. In this study, the antimutagenic effects of *Combretum microphyllum* and *Leucospermum erubescens* leaf extracts were investigated using the Ames test (*Salmonella typhimurium* TA98, TA100 and TA102), cytokinesis-block micronucleus-cytome assay, and comet assay using human hepatocarcinoma C3A cells at concentrations ranging from 5000 to 500 μg mL^{-1} for 48 hours. Extracts of these two plants had promising antimutagenic effects in an initial screening of 31 plants in the Ames test using *Salmonella typhimurium* TA98 and TA100. We then carried on investigating the prevention of DNA damage resulting in chromosomal breakages, chromosomal rearrangements, and gene amplification in the micronucleus/cytome assay and lastly the prevention of DNA strand breakage in the comet assay. The two species had antimutagenic effects ranging from 10 % to more than 30 % in the Ames test. They prevented micronuclei induction by up to 65.9 %, chromosomal rearrangements by 76.8 %, and gene amplification by 86.1 % in the micronucleus/cytome assay. In the comet assay, there was a dose dependent decrease in comet tail length; however, a decrease in tail moment and % DNA in tail was not dose dependent. Taking into account that genotoxicity involving gene mutations, chromosomal aberrations, and rearrangements and DNA strand breakages play a major role in cancer initiation, these two plant species have potential in cancer prevention as they inhibit these genotoxic end-points.

KEY WORDS: *Ames test, antimutagenicity,* Combretum microphyllum*, comet assay,* Leucospermum erubescens*, micronucleus/cytome assay*

OP-8

hCOMET COST ACTION 15132 – AN INTERNATIONAL NETWORK OF RESEARCHERS WHO ARE USING COMET ASSAY IN HUMAN BIOMONITORING STUDIES

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The single cell gel electrophoresis assay, also called Comet Assay is a method for measuring DNA strand breaks in single cells or nuclei. According to different setups and the use of specific enzymes and treatments, the assay can measure specific types of DNA damage and repair. Its *in vivo* alkaline version has been adopted as the OECD guideline TG 489 in 2014. Due to its potential as a predictive marker of disease and cancer risk, but also due to inter-laboratory variations in measuring DNA damage and repair, this network aims to create a unified database of comet assay data related to human health and disease. It will serve as a base for pooled analysis of DNA damage and repair in humans in order to give definitive information on the factors causing/preventing DNA damage, so as to establish standard protocols and guidelines that would help reduce inter-laboratory variations and through ring studies, training schools, workshops, publications, and creation of cohort of skilled molecular epidemiologists explore further the possibilities of use of this valuable technique in disease prevention and outcome. The network will continue the work of the previous network ComNet, and research and capacity building objectives of the network will be elaborated on through eight different Working Groups: creating a database, pooled analysis, ring studies, guidelines and SOPs, DNA repair, different cell types, planning for the future, and technical innovation and implementation. First results are expected at the beginning of 2017, and a new ICAW Workshop will be organised at the University of Navarra in August 2017.

KEY WORDS: *comet assay, hCOMET, DNA damage, DNA repair, networking*

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OP-9 (YSL)

GENOTOXICITY OF SELECTED CYTOSTATIC DRUGS IN HUMAN HEPATOMA HEPG2 CELLS

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Cytostatics are a group of pharmaceuticals used in chemotherapy. Most of these drugs interact directly with DNA processing and are not enough specific to target only the tumour cells. Consequently, exposure to cytostatics might represent a risk for undesirable delayed side effects in treated patients as well as in occupationally exposed population. We explored the genotoxic potential of four widely used cytostatics with different mechanisms of chemotherapeutic action: etoposide (ET), cisplatin (CDDP), 5-fluorouracil (5-FU) (these three directly interacting with DNA processing), and imatinibmesylate (IM), a specific inhibitor of mutated tyrosine kinases, in HepG2 cells after 24 h of exposure. Genotoxicity of the selected cytostatics was determined with the γH2AX assay, which measures the formation of DNA double-strand breaks (DSBs). In addition, changes in the expression of selected genes involved in DNA damage response (CDKN1A, GADD45A, MDM2), oncogenesis (MYC, JUN), and apoptosis (BAX, BCL2) were studied. All four cytostatics at non-cytotoxic concentrations induce formation of DSBs, with IM being the least genotoxic. In cells exposed to ET, CDDP and 5-FU genes involved in DNA damage response were upregulated, meanwhile the anti-apoptotic gene BCL2 and oncogene MYC were downregulated. Contrary, IM did not deregulate the expression of these genes, indicating that its mechanism of DNA damage induction is different from that of ET, CDDP, and 5-FU. The genotoxic effects of cytostatics were observed at therapeutic concentrations, indicating an increased risk for development of long-term or delayed adverse effects in patients and in indirectly exposed population. Among the tested cytostatics, IM represents a lower risk for delayed effects such as cancer, reproductive effects, and heritable diseases.

KEY WORDS: *apoptosis, cytostatics, gene expression, γH2AXassay, oncogenes*

OP-10

FROM NANOPARTICLES TO MICROPLASTICS: RETROSPECTIVE AND PROSPECTIVE VIEWS

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It has been roughly a decades since the beginning of the nowadays widely employed discipline of *nanotoxicology*. Donaldson and co-workers already in 2004 suggested that: "a new discipline should be built up to address the new potential threats posed by the widespread use of nanoparticles" (*Occup Environ Med*, 61: 727-728, 2004). Ever since, the field has proliferated considerably, which is evidenced by an enormous number of publications and funded projects. A number of questions regarding nanoparticle (NP) fate and effects have been resolved during the last years. Among these, the most important NP properties in terms of their fate and effect have been recognised: dissolution, formation of NP coronas in biological fluids and environments, size/shape aspect ratio and absorption of substances and absorption by organisms. In parallel to nanotoxicology, a new environmental threat has emerged – microplastics (MP). In particular, small microplastic particles (≤300 μm; "nanoplastic") share some similarities to nanoparticles. Similarly as NPs, their effect could be governed by dissolution of plastic ingredients, size/shape of plastic particles, absorption of pollutants and absorption by organisms, and the formation of corona. In this presentation, we will shed a light on the most important findings revealed in the field of nanotoxicology and link them to microplastic research. Based on the presenting author's personal involvement in three large-scale EU funded projects and a number of experiences gained we present a prospective view of the direction which the studies on microplastics should follow.

KEY WORDS: *corona, dissolution, fate, hazard, microplastic, nanotoxicology, shape, size*

OP-11 (YSL)

PHOTOTOXICITY OF MESOPOROUS TiO_2+Gd MICROBEADS WITH THE POTENTIAL FOR CANCER DIAGNOSIS AND TREATMENT

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Cancer is one of the major public health problems in modern world, therefore considerable effort has been invested in the development of various nanostructured TiO_2 particles that can be used in alternative cancer treatments. We synthesised mesoporous TiO_2 microbeads, which can be used as a photosensitizer in photodynamic therapy. Additionally, we doped TiO_2 microbeads with 5 or 10 % gadolinium (Gd) in order to obtain microbeads which have the potential to be used as a contrast agent in magnetic resonance imaging. We compared the phototoxicity of TiO_2 microbeads with different Gd content (0, 5, and 10 %) to determine if Gd doping can be used to design TiO_2 microbeads for diagnostic and therapeutic purposes. Human osteosarcoma MG-63 cells were used as an *in vitro* model for cancer cells. Cells were treated with a non-cytotoxic concentration of TiO_2 microbeads (50 μg mL^{-1}) for 1 h. After treatment, cells were exposed to UV-A radiation for up to 6 min. Viability of the cells was examined by resazurin assay, neutral red uptake assay and differential staining using propidium iodide and Hoechst 33342. All the tested TiO_2 microbeads (0, 5, and 10 % Gd) significantly reduced cell viability after 3 min UV-A radiation, while the viability of untreated cells was unaffected by 3 min radiation. Additionally, the differential staining and resazurin assay results showed that TiO_2 microbeads containing 5 and 10 % Gd induced higher phototoxicity than those without Gd. The outcomes suggest that Gd doped TiO_2 microbeads have a potential in theranostic medicine.

KEY WORDS: *cancer theranostics, gadolinium, photodynamic therapy, TiO_2 microbeads, viability assays*

OP-12

IMPACT OF BIO-CORONA ON SILVER NANOPARTICLE TOXICITY TO THE EMBRYONAL DEVELOPMENT OF MEDITERRANEAN SEA URCHIN *PARACENTROTUS LIVIDUS*

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Silver nanoparticles (AgNPs) have been found to be toxic to a range of organisms, in large part due to their release of silver ions. However, ion release kinetics and the effect of nanoparticle coating in brackish and marine waters have not been investigated in detail to date. In this direction we have investigated the impact of bovine serum albumin (BSA), as a model for bio-corona-forming macromolecules, on the behaviour of AgNPs in high strength electrolytes. BSA was found to stabilise AgNPs from agglomeration in artificial seawater for periods of up to one month, and was also found to reduce the release of ionic silver with up to 3.3 times lower concentrations of silver ions present in solution measured. This slower silver ion release compared to citrate-coated AgNPs influences the toxicity of these nanoparticles towards marine organisms. *Paracentrotus lividus* embryos were treated with citrate-coated and BSA-coated (0-1 μmol L^{-1}) 40 nm AgNP (50 and 100 μg L^{-1}) 2 hours post fertilisation. After 72 h the percentages of normally developed pluteus larvae, retarded and deformed plutei or undeveloped embryos (UND) were determined. Untreated embryos resulted in 90 % of normally developed plutei, while this decreased to 83 % for those treated with 50 μg L^{-1}AgNP and 55 % for a concentration of 100 μg L^{-1}. The corresponding values for BSA-coated AgNPs showed 80 % normal plutei for 50 μg L^{-1}AgNP, and 75 % for 100 μg L^{-1}AgNP. Thus silver nanoparticles have shown to be less embryotoxic when coated with a bio-corona.

KEY WORDS: *bio-corona, bovine serum albumin, pluteus larvae, toxicity of silver ions, sea urchin*

OP-13

SENSITIVITY TO DIFFERENT SIZES OF SILVER NANOPARTICLES IN THE EARLY LIFE STAGES OF TWO MEDITERRANEAN SEA URCHINS *ARBACIA LIXULA* (LINNAEUS, 1758) AND *PARACENTROTUS LIVIDUS* (LAMARCK, 1816)

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There is increasing evidence that engineered nanoparticles can reach brackish and coastal marine waters and pose a serious threat to biota living in those environmental compartments. The aim of this study was to determine the effect of different sizes of silver nanoparticles (diameters of 10, 20, 40, 60, and 100 nm) on the embryonal development of two Mediterranean sea urchin species (*Arbacia lixula* and *Paracentrotus lividus*) and compare the differences in sensitivity levels between the two species. Embryos were exposed two hours post fertilisation to a range of silver nanoparticle (AgNPs) sizes and concentrations (1-1000 μg L^{-1}) and after 48 hours the percentage of normally developed, retarded/deformed and undeveloped larvae were scored. Nanoparticle concentrations of 1 and 10 μg L^{-1} did not show a statistically significant effect, the smallest AgNP diameters (10 and 20 nm) showed a significant decrease in the percentage of normally developed *A. lixula* larvae at a concentration of 50 μg L^{-1} whilst *P. lividus* larvae showed a statistically significant decrease in normally developed larvae at 100 μg L^{-1}. AgNPs of 40 nm diameter showed a similar response in both species while AgNPs with the largest diameters (60 and 100 nm) showed a dose dependent response with more than 50 % retarded larvae at the highest tested concentrations (500 or 1000 μg L^{-1}). These findings show that *A. lixula* embryos are more sensitive to AgNPs than the *P. lividus* embryos, indicating that the former may be a more appropriate gatekeeper species for determining nanoparticle embryotoxicity.

KEY WORDS: Arbacia lixula*, embryo,* Paracentrotus lividus*, sea urchin, silver nanoparticles*

OP-14 (YSL)

DETERMINATION OF TERBUTHYLAZINE IN URINE OF RATS EXPOSED TO LOW DOSES

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Pesticides are a major group of chemicals produced to be intentionally released in the environment. Many pesticides are not easily degradable; they persist in soil, leach to groundwater and surface water and contaminate the wider environment. Depending on their chemical properties they can enter organisms, bioaccumulate in food chains and consequently influence human health. There is much data available concerning acute exposure of people or laboratory animals to pesticides. However, long-term exposure to low concentrations corresponding to real-life scenarios is much less investigated. Exposure to triazine herbicides such as terbuthylazine mainly results in urinary excretion of the parent compound and its metabolites formed by dealkylation. The aim of this study was to determine mass concentrations of terbuthylazine and its metabolites in rat urine. Animals were exposed to three different concentrations of terbuthylazineon each of 28 consecutive days. Terbuthylazine and its metabolites were determined in a 24-hour urine sample collected on the 1st, 14th and 28th day of treatment, with the results serving to define the markers and biomarkers of exposure. Different extraction procedures were developed for determining the accumulation of triazine herbicide terbuthylazine and dealkylated metabolites in rat urine: extraction with ethyl-acetate and solid-phase extraction on styrene-divinylbenzene sorbent. These procedures were optimised for final analysis by high performance liquid chromatography. Mass concentration of terbuthylazine recovered from urine indicates that unchanged terbuthylazine represents only a minor part of the adsorbed dose while the majority of terbuthylazine was excreted as dealkylated metabolites. This work has been supported in full by the Croatian Science Foundation under project no. 8366.

KEY WORDS: *biomarkers of exposure, high performance liquid chromatography, solid phase extraction, solvent extraction, urinary excretion*

OP-15 (YSL)

ASSESSMENT OF PESTICIDE EFFECTS ON ZEBRAFISH EMBRYOS

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Aquatic ecosystems are impacted by chemical pollution originating from various sources. Pesticides used in agriculture, forestry, households, etc. eventually enter aquatic ecosystems and can have hazardous effects on aquatic organisms. In aquatic ecotoxicological testing, the zebrafish (*Danio rerio*) is commonly used as a model organism. The early life-stage test using the zebrafish embryo is one of the most widely used tools for assessing the toxicity and mechanisms of action of environmental pollutants. In the present study the acute toxicity of the insecticide diazinon and the herbicide diuron were assessed by conducting the Fish Embryo Toxicity (FET) test. From the results obtained in the FET test, sublethal concentrations for exposure experiments were derived. Subsequently, zebrafish embryos were exposed to the pesticides (0.02, 0.1, 0.5, 1, 2 mg L^{-1}) for 48 and 96 h and enzymatic biomarkers (activities of AChE, CES, EROD, GST, CAT, and GPx), fluorescent detection of GSH and gene expression (ache, ces2, cyp1a, gstp1, gpx1a, cat, and gsr) were assessed. Both pesticides caused morphological aberrations such as pericardial and yolk sac edema, as well as a reduction in spontaneous movements. Toxicity of the investigated pesticides was similar, with 96 h LC_{50} and EC_{50} values of 6.72±0.16 and 4.16±0.07 mg L^{-1}, respectively, for diazinon, and 6.31±0.19 and 4.37±0.21 mg L^{-1}, respectively, for diuron. The results from the exposure experiments showed significant changes in the measured biomarkers and indicated occurrence of oxidative stress. The measurement of endpoints at different organisational levels provided some new insights into the toxicity mechanisms of these pesticides in the zebrafish.

KEY WORDS: *biomarkers, gene expression, oxidative stress, pesticide, zebrafish*

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P-1

ACCIDENTS WITH DANGEROUS GASEOUS CHEMICALS

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Natural phenomena (forest fires, earthquakes, volcano eruptions) and various human activities and lifestyle (emissions from industries, internal combustion engines, cleaning products, tobacco smoking) make way for chemicals to enter the human environment. Their impact on human health and the entire ecosystem depends on their intrinsic properties, quantity, and the mode of contact and duration of exposure. The emission of chemicals into the air represents the highest risk, and while there is little we can do to counteract natural occurrences, anthropogenic sources of environmental pollution could well be mitigated. Thus, it is very important to have knowledge about chemicals in various workplaces, to determine safety precautions, and to be ready to undertake preventive measures in case of chemical accidental release. European legislation establishes the requirements for minimising the possibility for chemical accidents and the preparedness in case these occur. In Croatia, additional national legislation on the storage and methods for use of gaseous chemicals exists. According to it it is mandatory to obtain an approval from the Croatian Ministry of Health for the storage of dangerous gaseous chemicals. Companies that need such approval must first ask for the expert opinion of the Croatian Institute for Toxicology and Antidoping (CITA). This includes conducting calculations and/or simulations of the release and movement of maximum allowed stored quantities during worst-case meteorological conditions (temperature, air humidity, wind speed etc.). So far, CITA advised 83 companies on the territory of Croatia, mostly regarding the requests on the handling of chlorine and ammonia.

KEY WORDS: *chemical accident preparedness, environment, legislation, preventive measures*

P-2

THE ROLE OF LIPID PEROXIDATION AND GLUTATHIONE LEVELS IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide after Alzheimer's disease. There is emerging evidence on the complex aetiology of PD that include non-genetic (age) and genetic factors (race) as well as potential exposure to environmental factors, such as certain chemicals. PD is histopathologically characterised by a loss of dopaminergic neurons from the pars compacta of subtantianigra to striatal motor loci that result in characteristic motor clinical disorders. It is possible that oxidative stress plays an important role in the death of these neurons. The brain is generally vulnerable to oxidative stress due to its low antioxidant capacity. Increased levels of reactive oxygen species that cause oxidative stress and lipid peroxidation (LP) play an important role in aging, homeostasis, and the onset of age-related diseases such as PD. Changes in glutathione (GSH) levels and LP may be important bioindicators of oxidative stress associated with PD. The study focused on the evaluation of GSH and LP levels in the brain and the liver in *C57Bl/6* and *Swiss albino* that have been established as animal models for PD by exposure to neurotoxin 1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine (MPTP).We observed various profiles of glutathione levels and lipid peroxidation in different mouse species.

KEY WORDS: C57Bl/6*, MPTP, oxidative stress,* Swiss albino

P-3

AGE-RELATED BEHAVIOURAL ALTERATIONS IN RATS EXPOSED TO PROPIONIC ACID

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The chemical models of autism spectrum disorders (ASD) are yet a new field. Experimental data are still limited and need further research. Recently it has been found that the short-chain fatty acid – propionic acid (PPA) is an environmental factor for several neurodevelopmental diseases like ASD. PPA's wide concentration range can induce various gene activities. PPA makes also other metabolite disturbances and central nervous system is one of the most vulnerable. To evaluate the neurotoxic effects of PPA on the cognition, motor and social activity in rats at different age, male Wistar rats aged 21 and 90 days were used. Neurotoxicity was induced through oral administration of a water solution of PPA 250 mg kg^{-1} daily and controls were given phosphate buffered saline for 3 days. Standard tests were used for the evaluation of neuro-muscular coordination (Rot-a-Rod) and learning and memory (Step-trough test). Social behavioral interactions were analysed using the open field test. Experimental data were processed by a t- of Student-Fisher test. Both in young and adult PPA-treated animals cognitive and motor tests' performance worsened. However, younger rats were more sensitive to the PPA neurotoxic effect. We have also found that PPA's impact on the social interaction test is age dependent i.e. younger treated rats showed more atypical and aggressive behavioural elements in their social interactions than the older ones. The neurotoxic action of PPA is stronger in young animals compared to adult rats. Young age animals are more suitable for creating a chemical experimental model of ASD.

KEY WORDS: *age-dependence, ASD, autistic model, neurotoxicity, short-chain fatty acid*

P-4

PROTECTIVE EFFECT OF ELLAGIC ACID ON SOCIAL BEHAVIOUR AND COGNITION OF RATS INTOXICATED WITH PROPIONIC ACID

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Short-chain fatty acids as propionic acid (PPA) are recognised as an environmental factor for several neurodevelopmental diseases like autism spectrum disorders (ASD). Limited data were reported on an improving effect of the Ellagic acid (EA) on verbal memory in humans and on cognition of animals (Tancheva *et al*. 2014) as well as on its preventive action in several pathologies (cancer, neurodegeneration, Alzheimer's disease etc.). To evaluate the neuroprotective effect of EA on the cognition, motor and social activities of rats with ASD model, an experimental model of ASD was produced on male Wistar rats at two different ages (21 days and 90 days old) by oral PPA administration (250 mg kg^{-1}, *i.p.*, 3 days). Controls were given phosphate buffered saline. EA-treatment (200 mg kg^{-1}, *i.p.*, 3 days) was initiated together with PPA. On day 4, after the last treatment, some standard tests were used for the evaluation of: learning and memory (step-trough test); motor coordination (rot-a-rod test) and social behavioural interactions (open field test). Experimental data were processed by a t- of Student-Fisher test. All cognitive and motor test performances worsened by PPA-treatment, especially in young animals. EA-treatment improved significantly learning and memory and motor coordination of treated animals as compared to PPA-treated controls. EA impacted best social interactions and reduced significantly the atypical and aggressive behavioural elements characteristic for ASD social interactions. EA has a promising age-dependent neuroprotective effect on the PPA-induced model of ASD. EA preventive effect is better in young animals as compared to adult rats.

KEY WORDS: *ASD model, autism, EA, neurotoxicity, short-chain fatty acid*

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P-5

WISTAR HANNOVER RAT REPRODUCTIVE PARAMETERS

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The aim of the study was to compare the results of own created historical control (HC) of reproductive toxicity and features of developmental defects with the HC of Charles River Laboratories (CRL). All studies were conducted according to the approaches described in OECD's guideline 415 and carried out in conformity with standard operational procedures (SOP) and the rules of Good Laboratory Practice. Preparation of historical control was made on the basis of own research carried out with the use of a segmented approach for the study of gonadotoxic influence of chemical substances. Only control animal group data were used for comparison for the 2013-2015 period. Research was carried out in the laboratory of experimental toxicology and mutagenesis in L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety in compliance with requirements of GLP, in conformity with the abovementioned OECD guidelines. Own results were compared with the results of CRL which were published in 2009. The results of the comparison confirmed the proximity of own HC data and CRL data which enables its further application in the evaluation and monitoring of own research.

KEY WORDS: *chemical substances, GLP, historical control, reproductive toxicity*

P-6

PARAMETERS OF OXIDATIVE STRESS IN THE ORGANS OF ADULT SHAM-OPERATED AND GONADECTOMISED MALE AND FEMALE RATS

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Aging is a physiological process characterised by a reduced ability to respond to environmental stressors. Despite many aging theories, this process is still poorly understood. As evidenced in recent studies, aging may be associated with increased oxidative stress (OS), which may affect an organ's functional and morphological characteristics. Reactive oxygen species can change the structure and function of membrane lipids, inhibit enzyme activities and oxidative phosphorylation, and oxidize nucleic acids and proteins. This study used gonadectomised rats (N=5) as an experimental model of aging and sham-operated rats as the controls to investigate if sex hormones affected OS parameters in major internal organs. Experiments were performed on three-month old Wistar rats of both sexes six weeks after the surgical procedures. Glutathione (GSH) was measured in the kidneys, liver, brain, and plasma. A significant difference in GSH concentration was observed only in the plasma of sham-operated females (3.62±0.34 nmol g^{-1} tissue) compared to sham-operated males (4.93±0.68 nmol g^{-1} tissue, mean±SD; $P<0.05$). Ovariectomy in females and castration in males had no effect on the liver or kidney GSH concentration. Malondialdehyde (MDA) concentration was measured in the kidneys, liver, brain, plasma, and urine. Significant sex-related changes ($P<0.05$) were noted only in sham-operated rats with levels (mean±SD) in the liver higher in females than in males (44.6±9.02 *vs*. 30.7±3.92 nmol g^{-1} tissue); and in urine higher in males than in females (6.67±1.58 *vs*. 4.98±1.46 μmol L^{-1}). These results indicate that sex hormones have a limited influence on the measured OS parameters in rat internal organs. This work was financially supported by Project No.1481 Aging-related Expression of Membrane Transporters in Rat (AGEMETAR), funded by the Croatian Science Foundation.

KEY WORDS: *aging, glutathione, malondialdehyde, sex hormones*

P-7

ROSUVASTATIN REDUCES KIDNEY MALONDIALDEHYDE CONCENTRATIONS IN RATS

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Statins are drugs widely used to treat hypercholesterolemia and reduce the risk of cardiovascular morbidity and mortality in patients with coronary heart disease. The mechanism of their action may be the inhibition of oxidant formation and the blocking of the effects of reactive oxygen species (ROS). As reported in recent studies, rosuvastatin has a beneficial effect on the plasma lipid profile and can reduce serum cholesterol and ROS formation in diabetic patients. The aim of this work was to determine the influence of rosuvastatin on the prooxidant-antioxidant balance in rat kidneys. This study was performed on male normolipidemic Wistar rats (N=36) divided into two control (treated with saline 5 mg kg^{-1} per day) and two experimental rosuvastatin-treated (5 and 10 mg kg^{-1} per day) groups. Animals were treated orally for 21 days. Malondialdehyde (MDA) – the parameter of lipid peroxidation - was measured in the kidney tissue homogenate of control and treated animals using the method by Drury et al. (1997). Data were analysed using a t-test. Results are expressed as Mean±STD. MDA concentration was significantly lower in both experimental groups compared to controls: 0.41±0.05 *vs.* 0.62±0.08 µmol g^{-1} tissue (5 mg kg^{-1} per day *vs*. control; $p<0.001$) and 0.58±0.05 *vs*. 0.68±0.09 µmol g^{-1} tissue (10 mg kg^{-1} per day *vs*. control; $p<0.05$). Our results have shown that rosuvastatin possesses significant antioxidative effectiveness that is not dose-dependent. This antioxidative action extends beyond the lipid-lowering effects of statins. This work was financially supported by Projects No. 108-0000000-0013 and 022-0222148-2142 funded by the Ministry of Science, Education and Sports of the Republic of Croatia.

KEY WORDS: *lipid peroxidation, oxidative stress, rats, statins*

P-8

TOBACCO HEATING SYSTEM (THS) 2.2, A CANDIDATE MODIFIED RISK TOBACCO PRODUCT: TOXICOLOGICAL ASSESSMENT

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The policy of tobacco harm reduction – making less harmful products available to smokers who would otherwise continue smoking – has the potential to be beneficial to public health, provided that (1) risk reduction is established by sound science and (2) smokers find these products satisfying as alternatives to smoking cigarettes. Philip Morris International (PMI) has developed the Tobacco Heating System (THS) 2.2, which has the potential to reduce the risk to smokers of developing smoking-related diseases in comparison to continued use of cigarettes. Because THS2.2 heats tobacco (at temperatures not exceeding 350 °C) instead of burning it, there is a substantial reduction in the formation of harmful or potentially harmful constituents (HPHCs), whilst many sensory attributes of cigarettes are retained. PMI's product characterisation studies show that THS2.2 does not combust tobacco and that the formation of a number of harmful and potentially harmful chemicals is reduced on average by over 90 % in comparison to a reference cigarette. Assessment of the THS2.2 aerosol *in vitro* and *in vivo* reveals reduced toxicity and no new hazards when compared to cigarette smoke. Additional mechanistic endpoints, measured as part of *in vivo* studies confirm reduced impact on smoking-related disease networks. The contribution of toxicological assessment as part of an overall integrated assessment approach necessary to substantiate reduced risk will be described.

KEY WORDS: *heat-not-burn, Tobacco Harm Reduction, toxicological assessment*

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P-9

NORM FOR BUILDING MATERIALS – NETWORK [NORM4BUILDING]

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The depletion of energy resources and raw materials has a huge impact on the building market. In the development of new synthetic building materials the reuse of various (waste) residue streams becomes a necessity. This COST initiative stimulates scientists, industries, and regulators to collaborate to gather knowledge, experiences, and technologies in order to stimulate research on the reuse of residues containing enhanced concentrations of natural radionuclides (NORM) in tailor-made building materials in the construction sector. In doing so, the impact on both external gamma exposure of building occupants and indoor air quality must be considered. By improving radiological impact assessment models for the reuse of NORM residues in building materials we hope to further stimulate justified uses of NORM residues in different types of newly developed building materials. Based on these models, we aim at investigating realistic legislative scenarios so that the authorities concerned can allow reuse pathways for NORM that can be accepted from a radioprotection point of view in concordance with the Lead Marked Initiative (LMI) and sustainable construction.

KEY WORDS: *impact assessment, indoor air quality, radiation protection, tailor-made sustainable building materials, validation of NORM residues*

P-10

RESIDUES OF MADURAMICIN IN MUSCLE TISSSUES OF BROILER CHICKENS AFTER DRUG ADMINISTRATION

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Maduramicin ammonium, a polyether carboxylic ionophore agent, is authorised for use in feedstuffs as an anticoccidial drug for fattening chickens at a maximum content of 5-6 mg kg^{-1} pursuant to Regulation (EU) No. 388/2011. The aim of the present study was to investigate maduramicin distribution in the muscle tissue of broilers following treatment with doses exceeding the authorised dose, i.e. a dose about twice the maximum authorised level. Broiler chickens (*n*=25) were treated with feed medicated with maduramicin at concentrations of 10 mg kg^{-1} for 21 days. The broilers were sacrificed and muscle tissue was collected over a period of 15 days after the end of administration. Maduramicin concentrations were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with electrospray ionisation. The limit of quantification of the method for maduramicin was 1.29 µg kg^{-1} and recovery 95.8 %. The maximum concentrations of maduramicin (44.1 µg kg^{-1}) were measured on the first day post-treatment. Concentrations gradually decreased from the third to the fifteenth day following drug administration (µg kg^{-1}) as follows: 3rd day 34.0; 5th day 15.7; 7th day 10.7; 9th day 8.44; 11th day 6.57; 13th day 4.66; 15th day 2.37. On the 15th day after the end of treatment, maduramicin levels were still above the limit of quantification. However, maduramicin levels dropped below the permitted maximum residue limits of 30 µg kg^{-1} in muscles on the 5th day after the end of treatment. The results indicated rapid excretion of maduramicin from muscles, even when administered at doses exceeding the authorised maximum content.

KEY WORDS: *anticoccidial drugs, broiler chickens, LC-MS/MS, maduramicin, muscle tissue*

P-11

TRANS FATTY ACIDS IN FOOD – HAH RESEARCH 2015-2016

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Trans fatty acids (TFA) occur in partly hydrogenated vegetable oils, and in milk and meat of ruminants where they form under the influence of bacteria in their stomach. They have no vital function in the body. TFA have a harmful influence on health and are closely associated with the development of cardiovascular diseases if consumed in an amount of 5 g day^{-1}. The European Commission is still looking for a solution to regulate their content in food. The World Health Organisation recommends a maximum of 1 % of daily energy intake by TFA which is a maximum of 2 g day^{-1}. In the period of 2015-2016, 114 samples were analysed in 10 food categories collected from the market of Croatia. Results are expressed by the content of TFA per 100 g of fat/oil and content of TFA per 100 g of product. Only eight samples, of which four were imported, contained more than 2 g per 100 g TFA fats/oil. The highest content of TFA was found in confectionery products with fatty fillings. In conclusion, the situation regarding the content of TFA in the food market of Croatia is satisfactory. Recommendation of the European Food Safety Authority is to keep the content of TFA in food as low as possible.

KEY WORDS: *confectionery products, Croatia, daily intake, health impact, trans fatty acid*

P-12

QUICK ESTIMATION OF DIETARY EXPOSURE TO HETEROCYCLIC AROMATIC AMINES AND ACRYLAMIDE IN A CROATIAN FEMALE POPULATION

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Acrylamide (AA) and heterocyclic aromatic amines (HAA) are formed in food in Maillard-type reactions. These products are common in the daily diet worldwide and are suspected to contribute to a higher risk of cancer. This study used a quick interview and calculation for a preliminary estimation of dietary exposure to heterocyclic aromatic amines MeIQx (2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline) and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine), as well as to AA in 94 adult women from eastern Croatia. Subjects were given a questionnaire to obtain information on their anthropometric and socio-economic characteristics, and dietary habits. The intake of main sources of HAA and AA was determined and combined with literature data on their levels in food. Both high and low estimates were calculated using the maximum and minimum published levels. The low estimate approach produced mean daily exposures to MeIQx (0.93±0.77 ng kg^{-1} body weight, bw) and PhIP (2.34±2.49 ng kg^{-1} bw), as well as a provisional total HAA intake of 4.43 ng kg^{-1} bw per day, which were within the range of results reported by other authors. Similarly, the low estimate of AA daily exposure (122.66±60.00 ng kg^{-1} bw) was below the lower end of the range determined by the European Food Safety Authority but was more in line with other published levels.

KEY WORDS: *dietary exposure, Maillard-type reactions products, questionnaire, women*

P-13

ACCUMULATED HEAVY METALS IN TISSUES OF DOMESTIC PIGS

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Accumulation of heavy metals such as arsenic (As), cadmium (Cd), and lead (Pb) in the edible tissue of domestic pig represents one of the major risk factors for human health. The aim of this paper was to examine the amount of heavy metals and to determine the extent of their accumulation in edible tissues such as the liver, kidney, spleen, heart and lung of domestic pigs (15 samples). All samples were taken from fattening domestic pigs at the slaughter line, randomly selected from the territory of the South Bačka district in Serbia. The concentration of heavy metals in the samples was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES ICAP 6500 Duo, Thermo Scientific) using the EPA 6010C method. The detection limit was 0.005 mg kg^{-1} for all tested samples. According to national regulations (RS Official Gazette, No 41/09) the maximum allowable concentration (MAC) of As, Pb, and Cd in pigs' internal organs, except for 1.0 mg kg^{-1} for Cd in kidneys, is 0.50 mg kg^{-1}. In all tested samples, at the detection limit 0.005 mg kg^{-1}, the accumulated concentration of heavy metals (As, Cd, and Pb) was under the MAC value. Based on these results it can be concluded that the concentration of accumulated heavy metals in edible tissues of domestic pigs does not represent a danger to human health.

KEY WORDS: *arsenic, cadmium, lead, liver, kidney*

P-14

TOXICITY OF MYCOTOXIN FUMONISIN B1 TO DUCKWEED (*LEMNA MINOR* L.)

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Mycotoxin fumonisin B_1 (FB_1) is produced by moulds from *Fusarium* genera and is found as a contaminant on cereals all around the world. FB_1 is toxic to domestic and experimental animals and development of some human diseases is associated with exposure to this mycotoxin. The International Agency for Research on Cancer classified FB_1 as a group 2B carcinogen. Although FB_1 is found in environment, its impact on plants is not studied thoroughly. The aim of this study was to test toxicity and oxidative stress as a mechanism of toxicity of mycotoxin FB_1 on duckweed (*Lemna minor* L.). Duckweed was treated with FB_1 (0.5 and 1 mg L^{-1}) for 3 days. Toxicity was assessed in terms of growth rate (based on either frond number or fresh weight) and content of photosynthetic pigments (chlorophyll *a*, chlorophyll *b,* and carotenoids). Malondialdehyde (MDA), non-protein thiols, antioxidant enzymes activities, level of glutathione and reactive oxygen species *in situ* were evaluated as parameters of oxidative stress. FB_1 reduced grow rate by 16 % and 21 %, at 0.5 and 1 mg L^{-1} treatment, respectively ($p<0.05$), but had no effect on the level of photosynthetic pigments. Ascorbate peroxidase activity increased (by 30 % after 0.5 mg L^{-1} treatment and by 46 % after 1 mg L^{-1} treatment; $p<0.05$) while the activity of catalase decreased (by 18 % after 0.5 mg L^{-1} treatment and by 12 % after 1 mg L^{-1} treatment; $p<0.05$). The obtained results indicate that FB_1 is toxic to duckweed and that oxidative stress is involved in the mechanism of its toxicity.

KEY WORDS: *environment, experimental model, mycotoxin, oxidative stress, photosynthetic pigments*

P-15

FLAVONOIDS AS CYTOCHROME CYP3A4 INHIBITORS: HOW SIGNIFICANT IS THEIR INHIBITION POTENTIAL?

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Flavonoids are a big class of polyphenol compounds characterised with a phenyl chromane core. Their diversity comes from hydroxylation and subsequent methylation and glycosylation of the basic skeleton. These compounds are widely distributed in plant kingdom, mainly as the secondary metabolites of higher plants, and antioxidant activity is their major pharmacological effect. However, the list of biological effects of individual flavonoids is much more extensive and includes antibacterial, virustatic, fungistatic, and hepatoprotective effects among others. CYP3A4 is the most significant enzyme for the metabolism of xenobiotics, namely medicinal drugs, and potential interactions of flavonoids with drugs whose metabolism is mediated by this enzyme are of our interest. Direct inhibition of CYP3A4 can be avoided by simple discontinuation of one drug that interacts with another, whereas irreversible inhibition leads to inactivation of the enzyme. To evaluate the potential of flavonoid inhibition of CYP3A4 we have conducted a screening of 30 flavonoid aglycones. Enzyme activity was tested on bicistronic membranes with CYP3A4 coexpressed with NADPH reductase for electron transfer from NADPH. The RP-HPLC-DAD method was used to monitor marker reaction of CYP3A4 (6β-hydroxylation of testosterone) and determine residual enzyme activity. Out of 30 flavonoids analysed, 7 have shown the potential to inhibit CYP3A4 atmicromolar concentrations. Chrysin, apigenin, and acacetin showed direct inhibition. Irreversible inhibiton of CYP3A4 was observed with pinocembrin, isorhamnetin, tangeretin, and chrysin-dimethylether. Although antioxidant activity is desirable, preliminary results suggest caution when high amounts of vegetables and fruits rich in aforementioned flavonoids are consumed with drugs that are mainly metabolised by CYP3A4 (e.g. erythromycin, cyclosporine, simvastatin) as this can result in high concentrations of drugs leading to undesirable, even toxic effects.

KEY WORDS: *cytochrome CYP3A4, flavonoids, metabolic interactions*

P-16

BIOLOGICAL ACTIVITY OF ISOLATED COMPOUNDS FROM *CENTAUREA RAGUSINA* L.

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Antioxidant, antibacterial, and cytotoxic activity of three flavonoids (chrysin, oroxylin A, and hispidulin) and sesquiterpene lactones (hemistepsin A, deacylcynaropicrin and (3aR,4S,6aR,8S,9aR,9bR)-[Dodecahydro-8-dihydroxy-3,6,9-tris(methylene)-2oxo-2(3H)-azuleno[4,5-b]furanyl]-3-methyl-butanoate) isolated from ethanolic extract of the Croatian endemic plant species *Centaurea ragusina* L. were studied. The interactions of isolated compounds with double stranded polynucleotides ctDNA and poly A – poly U (possible biological targets) were studied with circular dichroism spectroscopy (CD spectroscopy) at pH=5.0 and pH=7.0 to provide a better understanding of the biological activity of the tested compounds. Flavonoid hispidulin showed moderate antioxidant activity measured by the ABTS method while other isolated compounds from ethanolic extract showed weak antioxidant activity. Two sesquiterpene lactones (3aR,4S,6aR,8S,9aR,9bR)-[Dodecahydro-8-dihydroxy-3,6,9-tris(methylene)-2oxo-2(3H)-azuleno[4,5-b]furanyl]-3-methyl-butanoate) and hemistepsin A showed significant antibacterial activity against *Staphylococcus aureus* ATCC25923 (MIC 31.3 g mL^{-1}). All tested compounds showed weak antibacterial activity against *Acinetobacter baumannii* DURN Among the isolated compounds, only (3aR,4S,6aR,8S,9aR,9bR)-[Dodecahydro-8-dihydroxy-3,6,9-tris(methylene)-2oxo-2(3H)-azuleno[4,5-b]furanyl]-3-methyl-butanoate) exerted selective and prominent tumour cell-growth inhibitory activity towards SCCVII cell line (IC50 = 2.55μM). Simultaneously, (3aR,4S,6aR,8S,9aR,9bR)-[Dodecahydro-8-dihydroxy-3,6,9-tris(methylene)-2oxo-2(3H)-azuleno[4,5-b]furanyl]-3-methyl-butanoate) showed a weak interaction with DNA indicating that some other target/mechanism is responsible for its prominent cytotoxic activity towards SCCVII cell line.

KEY WORDS: *biological activity, flavonoid, sesquiterpene lactones interactions with polynucleotides*

P-17

ASSESSMENT OF THE INFLUENCE OF *LISTERINE COOL MINT* MOUTHWASH ON BUCCAL CELLS MEASURED BY BUCCAL MICRONUCLEUS CYTOME ASSAY

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Listerine is worldwide the most used brand of mouthwash for oral hygiene maintenance. *Listerine Cool Mint* mouthwash is made from four herbal extracts: thymol, menthol, eucalyptol, and methyl salicylate in a solution of 21.6 % of ethanol. All these individual ingredients can cause cell damage. Buccal epithelial cells are in direct contact with the mouthwash and their analysis using the buccal micronucleus cytome assay (BMCyt) can show the influence of mouthwash on the differentiation (frequency of basal, differentiated, binuclear cells, and apoptosis phases: condensed chromatin, karriorhectic, pyknotic, and karyolitic cells) or genomic stability of buccal cells (frequency of micronucleus (MN), nuclear buds or structures called *broken eggs*). Ten healthy individuals used mouthwash twice per day during a two-week treatment, with sample collection before and after the treatment. The treatment did not significantly influence cell differentiation or genomic instability, although MN frequency was higher after the treatment (1 *vs.* 1.5). Individual differences were found and consumers of strong alcoholic drinks had a higher MN frequency when compared to other individuals. Following experiments should be performed on a greater number of individuals and specific groups, such as individuals who regularly consume alcohol, to analyse whether a synergistic influence of mouthwash with an additional source of ethanol exists and to discover if this population runs a higher risk of genomic instability. BMCyt has demonstrated its sensitivity in evaluating changes before and after the treatment, and it has been used for the first time in Croatia as well as on *Listerine Cool Mint* mouthwash.

KEY WORDS: *buccal cells, buccal micronucleus cytome assay, genome damage,* Listerine Cool Mint *mouthwash*

P-18

GENOTOXICITY TESTING OF GENERIC PESTICIDES IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES STUDIED *IN VITRO* WITH COMET ASSAY

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Approval and registration of generic pesticides requires a comprehensive assessment of their potential genotoxic properties. Genotoxicity testing is an integral component of regulatory toxicity evaluation in Ukraine. Determination of potential genotoxic effects within generic pesticide studies is an obligatory requirement for justifying their safe usage and evaluating potential risks. In our research centre, we successfully conduct the recommended standard test battery, which includes gene mutation *in vitro* tests in bacteria (fluctuation Ames assay OECD 471) and both *in vivo* and *in vitro* gene mutation tests in mammalian cells (micronucleus assay OECD 475, 487 and metaphase chromosomal aberration assay OECD 474). Nowadays, comet assay is a logical continuation of initial techniques and is recognised as one of the most sensitive and reproducible assays. In general, the assay offers a wide range of possibilities, i.e. it can determine the single- and double-strand breaks of DNA, alkali labile sites (apurinic/apyrimidinic sites), DNA cross-links, base-pair damages, and apoptotic nuclei in cells. We studied DNA damage in cells (human peripheral blood lymphocytes) using alkaline comet assay with exogenous metabolic activation. It allows the detection of single- and double-strand DNA breaks and genotoxic properties of test substances. The main result of our group work was creating a standard operating procedure for the successful implementation under GLP laboratory conditions. All steps in our protocol are in compliance with the JaCVAM/OECD *In Vitro* Pre-Validation Study recommendation.

KEY WORDS: *comet assay, generic pesticide, genotoxicity, gel electrophoresis, GLP*

P-19

COMBINED GENOTOXIC EFFECTS OF TWO CYANOBACTERIAL TOXINS, MICROCYSTIN-LR AND CYLINDROSPERMOPSIN

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Cyanobacterial blooms are associated with the presence of hazardous cyanotoxins in surface waters. Many cyanotoxins are simultaneously present in freshwaters. As it is known that complex mixtures can evoke more pronounced adverse effects than individual compounds, we studied the genotoxic potential of a binary mixture of microcystin-LR (MCLR), which is classified as possible human carcinogen, and cylindrospermopsin (CYN) that has only recently been recognised as of concern to human health. It is known that both cyanotoxins are genotoxic; however, the mechanisms of their toxicity differ. In our study human hepatoma HepG2 cells were exposed to non-cytotoxic graded doses of CYN (0.01-0.5 μg mL^{-1}), single dose of MCLR (1 μg mL^{-1}) and their combinations for 4 and 24 h. Subsequently DNA damage was assessed with the comet and cytokinesis-block micronucleus cytome assay. Furthermore, the influence on transcription of genes involved in xenobiotic metabolism, immediate-early response, and DNA-damage response was examined by qPCR. CYN (0.5 μg mL^{-1}) significantly increased the formation of single-strand breaks and micronuclei after 24 h, while MCLR (1 μg mL^{-1}) had no effect on DNA. The effects of binary mixtures were similar to those obtained with the corresponding concentrations of CYN alone. The trend of gene deregulation induced by the binary mixture was also comparable to that induced by CYN alone. We can conclude that binary mixtures of CYN and MCLR at non-cytotoxic concentrations do not exhibit synergistic or potentiating genotoxic effects in HepG2 cells. Slovenian Research Agency and Spanish Ministry of Economy and Competitiveness for financial support.

KEY WORDS: *combined effects, cylindrospermopsin, genotoxicity, microcystin-LR, risk assessment*

P-20

GENOTOXIC EFFECTS OF GLYPHOSATE IN HUMAN DERIVED HEPATOMA (HEPG2) CELLS ASSESSED BY CYTOME ASSAY

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Glyphosate is the most widely used nonselective herbicide. Until recently, it was considered environmentally safe and minimally toxic to humans. Recent studies suggest that glyphosate and its metabolites could affect normal human cell development. In this study, glyphosate was tested at concentrations of 0.5, 2.91, and 3.5 μg mL^{-1}, which corresponded to the values of acceptable daily intake (ADI), residential exposure level (REL), and occupational exposure level (OEL). Analyses of micronuclei, nuclear buds, nucleoplasmic bridges, apoptosis/necrosis, and nuclear division index in HepG2 cells were performed following 4 and 24 hours of *in vitro* treatment. The results demonstrated that 4-hour exposure to glyphosate slightly increased cytogenetic damage in terms of micronuclei, statistically significant only at OEL. Despite a nonsignificant increase in micronuclei frequencies at ADI and REL, a significant increase in nuclear bud frequency was found. Since nuclear budding represents a micronuclei formation mechanism, our results indicate that even a low dose such as ADI can influence the DNA/cytogenetic damage level. After 24h of exposure, a lower number of binucleated cells was found at all of the three tested concentrations compared to control. Nuclear bud frequency changed only in the ADI sample, while in the OEL- and REL-treated samples, it was significantly lower. The control and treated cells did not significantly differ in the number of nucleoplasmic bridges. It is possible that the two exposure times selected in this study were too short for a reliable estimation of glyphosate genotoxicity, which has to be further clarified through other *in vitro*/*in vivo* models. This work was financially supported by Project No.8366 Organic Pollutants in Environment – Markers and Biomarkers of Toxicity (OPENTOX), funded by the Croatian Science Foundation.

KEY WORDS: *cytome assay, genotoxic effect, glyphosate, human derived hepatoma cells*

P-21

CHARACTERISATION OF HUMAN HEPATIC CELL LINES FOR THE APPLICATION IN GENOTOXICITY AND CARCINOGENICITY STUDIES

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The current legislation requires genotoxicity testing of nearly all chemical products prior to the marketing. Genotoxicity assessment follows a step-wise approach, beginning with a battery of *in vitro* tests with bacteria and mammalian cells and, in case of positive results, *in vivo* tests with rodents are performed. However, currently used *in vitro* tests are associated with a high rate of false-positive results due to inadequate expression of metabolic enzymes or tumour suppressor p53 protein in the cell lines used. The aim of the study was to characterise different hepatic cell lines and select the most suitable candidate for the application in genotoxicity studies and routine testing. Eleven lines were collected (HepG2, HCC1.2, SNU398, SNU449, Huh6, Huh7, JHH6, Hep3B, SK-Hep1, PCL/PRF, and WRL68) and characterised according to the basic criteria: growth behaviour, karyotype stability, and p53 status. Their sensitivity to detect genotoxins was evaluated with the comet assay after 24-h exposure to model indirect-acting genotoxic carcinogens (AFB_1, IQ, PhIP, NDMA, and B(a)P). All cell lines are of hepatoma origin and none of them have a normal karyotype. Q-PCR analyses revealed the absence of *p53* expression at transcriptional level in Hep3B line. Mutated p53 protein was determined in HCC1.2 and SNU398 cells by Western blot. Only HepG2, HCC1.2, and Huh6 lines detected all model compounds but with different sensitivity. We conclude that the most promising candidates are HepG2 and Huh6 cells. Detailed determination of metabolic enzymes' expression, including their transcription factors, and the expression of DNA damage response proteins in these cells is in progress.

KEY WORDS: *cell characterisation, comet assay, genotoxic carcinogens,* in vitro *genotoxicity testing, risk assessment*

P-22

PARTICULATE MATTER GENOTOXICITY AND CYTOTOXICITY DUE TO SPATIAL AND TEMPORAL CHANGES

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The attempt to understand the human health effects of air pollutants, especially for Particulate Matter (PM), has increasing in both industrial and urban areas. Accordingly, the daily $PM_{2.5}$ samples were collected on the nucleophore filters for one year from two stations, Kütahya (urban) and Göbel (rural, thermal power plant region). For each station, parallel days in summer (eight days) and winter (seven days) were chosen. The aim was to determine *in vitro* genotoxicity (Comet Assay) and cytotoxicity [trypan blue (TB), WST-1 and Lactate Dehydrogenase (LDH) tests] using A549 cells (human lung cancer epithelial cell line). 50 μg mL^{-1} PM concentration and 24 h incubation duration were used for Comet and TB assays for each 30 PM sample. Genotoxicity was higher in each of the samples ($p>0.05$) compared to the control group and they had cell viability over 70 %. The daily PM sample genotoxicity was statistically significantly higher only for one winter day in Kütahya *vs*. Göbel ($p<0.05$). There was no seasonal difference between the stations ($p>0.05$). PM genotoxicity in Kütahya was higher than that of Göbel when the summer and winter data were combined ($p>0.05$). The cell viability of pooled summer and winter samples of the two stations decreased below 70-80 % for LDH and WST-1 for concentrations higher than 100 μg mL^{-1} in both seasons and at both stations, in which Kütahya had the lowest viability in winter. Since PM has a complex composition showing spatial and temporal changes, our study could contribute to PM *in vitro* genotoxicity and cytotoxicity. *This study was supported by TUBITAK-112Y305 and Anadolu University-BAP-1306F272 grants.*

KEY WORDS: *Comet Assay, human health, summer, thermal power plant, winter*

P-23

DIFFERENTIAL PULSE ANODIC STRIPPING VOLTAMMETRIC (DPASV) AND HIGH RESOLUTION INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRIC (HR-ICPMS) ANALYSIS OF Zn^{2+} MASS CONCENTRATION IN TREATED LYMPHOCYTES

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In the present study we used extended-term human lymphocyte cultures to assess a possible genotoxic effect of ZnO nanoparticles (NPs) after subacute *in vitro* exposure which lasted for 14 days. Using the alkaline comet assay we wanted to explore whether lymphocyte subpopulations differed according to their ability to accumulate ZnO after exposure to the same concentration of ZnO NPs. Using comet-FISH we studied specific effects on the structural integrity and copy number of the *TP 53* gene and the corresponding centromere on chromosome 17. Further, we measured effective levels of ZnO that penetrated the cellular membrane for a more relevant understanding of the comet assay results and interpretation of measured primary lesions. Mass concentrations of Zn^{2+} in the treated cells were measured by DPASV and HR-ICPMS. We applied DPASV and HR-ICPMS as the referent methods for determining Zn^{2+} mass concentrations in lymphocytes. Regression analysis was conducted to determine if primary DNA damage was correlated with measured intracellular Zn^{2+} mass concentrations. Both methods used for the determination of intracellular Zn^{2+} levels showed very consistent and reproductive results regarding the measured concentrations within the entire range of tested ZnO NPs concentrations. Regression analysis indicated significant correlation between the applied ZnO NPs concentration and DPASV detected intracellular Zn^{2+}. Both treatments and intracellular mass concentrations of the corresponding Zn^{2+} entities significantly affected comet tail length and impaired the structural integrity of *TP 53* gene.

KEY WORDS: *comet-FISH, human lymphocyte cultures, primary DNA damage, regression analysis, ZnO NPs*

P-24

ASSESSMENT OF EXPOSURE AND RISK OF TOXIC EFFECTS OF CADMIUM IN METAL PROCESSING INDUSTRY

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Cadmium is a common occupational and environmental pollutant and a highly toxic metal. It is used in the process of corrosion protection due to its anticorrosive properties. Occupational exposure to cadmium occurs during the autogenous cutting and heat treatment of metal structures. The effects of occupational poisoning occur after long-term exposure. Given that, biological markers of chronic low-level exposure are important biological indicators of health risk. An epidemiological cohort study was performed during ten years in exposed workers employed in metal processing industry "NISSAL" ad. In this study, cadmium concentrations in blood and urine (24-h urine sample) were measured using atomic absorption spectrometry. For statistical analysis, Excel, Matlab, and SPSS19.0 software packages were used. The average concentration of blood cadmium was 0.053 μmol L^{-1}, maximum value was 0.196 μmol L^{-1}, while the most frequent value was 0.023 μmol L^{-1}. The mean value of urine cadmium concentration was 0.015 μmol L^{-1}, compared to the maximum value of 0.058 μmol L^{-1}. The level of cadmium in blood and urine of exposed groups (N=60) was positively correlated with age ($r=0.722$, $p<0.01$ and $r=0.656$, $p<0.01$). Positive correlation was also determined between the concentration of cadmium in blood and urine and the length of occupational exposure ($r=0.806$, $p<0.01$ and $r=0.705$, $p<0.01$). The results showed that chronic occupational cadmium exposure increased metal concentrations with a potential for a high risk of toxic effects that positively correlated with age and exposure duration. These data confirm that occupational exposure to cadmium should be regularly controlled to prevent adverse health effects in exposed persons.

KEY WORDS: *metal industry, occupational exposure, risk of occupational poisoning*

P-25

THE POTENTIAL ROLE OF METAL-INDUCED OXIDATIVE STRESS IN HUMAN PANCREATIC CANCER: PRELIMINARY RESULTS

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Pancreatic cancer (PC) is one of the most aggressive types of cancer and a worldwide health treat. However, it is rather unclear which environmental pollutants can be linked to PC development. Exposure to toxic metals through various sources can be one of the risk factors, especially having in mind that some toxic metals can induce oxidative stress, which has already been associated with the pathogenesis of PC. The aim of this study was to investigate the levels of cadmium (Cd) and lead (Pb), toxic metals of great environmental concern known to induce oxidative stress, in the blood of PC patients and healthy control subjects, as well as to examine the following biomarkers of oxidative stress: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione S-transferase (GST), glutathione (GSH), sulfhydrylgroups (SH), and lipid peroxides (TBARS) in blood. Blood samples were obtained from 15 PC patients and 7 healthy subjects. The present study demonstrated a significant increase in Pb concentrations in patients with carcinoma when compared to healthy subjects ($p<0.05$), while no significant differences were observed in Cd levels. The activities of SOD and CAT, as well as the concentration of SH groups, were significantly higher in people with PC compared with controls ($p<0.05$) proving oxidative stress induction in the blood of PC patients. This work contributes to a better understanding of the potential role of metal-induced oxidative stress in PC aetiology. However, confirmation of these pilot findings in a larger study is needed.

KEY WORDS: *antioxidative enzymes, environmental polutants, lipid peroxidation, pancreatic cancer*

P-26

CYTOTOXICITY OF HEAVY METALS AND ORGANOCHALOGENATED COMPOUNDS – *IN VITRO* STUDY

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In the course of their life, humans are exposed to various xenobiotics through food consumption, including toxic metals and organochalogenated compounds. The aim of this study was to compare the cytotoxic effects of individual contaminants – cadmium (Cd), lead (Pb), mercury (Hg), 1,1,1-trihlor-2,2-bis(4-hlorfenil)ethan (DDT), non-dioxin-like polychlorinated biphenyls (ndl-PCB), and polybrominated diphenylethers (PBDE), as well as their mixtures, through simultaneous exposure to these toxic substances. The investigated concentrations in mixtures were in the range of 1:1000 and the relationships between contaminants were as follows: Cd:Pb:Hg:DDT:PCB:PBDE=10:5:50:10:15:1. A human colon carcinoma cell line (SW 480) and the *Neutral Red* test were used for cytotoxicity examination. The dose–response relationship was demonstrated for both individual contaminants and their mixtures. High cytotoxicity induced by Cd at a concentration of 10 μg L^{-1} killed more than 20 % of cells. Organohalogenated compounds showed a lower cytotoxic potential. The *in vitro* investigations generally showed a higher cytotoxic potential in contaminants' mixtures compared to the sum of effects of individual contaminants. In the mixture where the concentrations of contaminants were designed to simulate the concentrations commonly found in food, the cytotoxic effect was three times higher than the theoretical values based on the individual potential of contaminants. At higher concentrations, this effect decreased in mixtures. Taking into account various mechanisms of action, further investigations are necessary in order to assess the risk from harmful effects of investigated mixtures with significant confidence.

KEY WORDS: *cell lines, effect assessment, food contaminants, mixture of environmental pollutants*

P-27

HOW DECABROMINATED DIPHENYL ETHER INFLUENCES THE ANTIOXIDATIVE DEFENCE SYSTEM IN KIDNEYS EXPOSED TO CADMIUM

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The objective of this study was to assess the influence of decabrominated diphenyl ether (BDE-209) on the antioxidative defence system in rat kidneys previousy exposed to cadmium (Cd). Male Wistar rats, weighing 200 g, were used as an experimental model. Animals were exposed to three doses of Cd of 2.5, 7.5, and 15 mg kg^{-1} day^{-1}, to one dose of 1000 mg kg^{-1} BDE-209 kg^{-1} day^{-1}, and all three combinations of Cd and BDE-209, by gavages, during 28 days. The study was approved by the Ethics Committee of the Military Medical Academy, Republic of Serbia, No 9667-1/2011. Evaluation of the antioxidative defence system in kidneys was based on the level of -SH groups in kidney homogenates measured by the Ellman method. Interactions were interpreted using multiple factorial regression analysis in the Statistica 7.0 software while benchmark doses of 5 % (BMD_5) were calculated using the PROAST software. BDE-209 given alone caused a dose dependent increase in the level of –SH groups and the calculated BMD_5 was 87.7 mg kg^{-1} day^{-1}. Contrary to BDE-209, Cd induced a decrease in the total content of –SH groups. Addition of 1000 mg BDE-209 to the whole dose range of Cd induced a dose dependent decrease in the –SH groups' concentration, and this effect was significantly more intensive compared to the effect caused by Cd alone and significantly lower than the effect caused by BDE-209 in a dose of 1000 mg kg^{-1} day^{-1}.The results of the present work add up to the issue of BDE-209 and Cd mixture toxicity profile with a focus on the relationship between the doses and the oxidative stress defence system in the kidney. Multiple factorial regressions confirmed the obtained results and imply no synergism or antagonism when Cd and BDE-209 were given as a mixture (Project III 46009).

KEY WORDS: *BDE-209, Cd, kidney, mixture, oxidative stress*

P-28

ASSESSMENT OF MERCURY INTAKE VIA FISH AND FISH PRODUCTS IN PREGNANT WOMEN IN BELGRADE, SERBIA: PRELIMINARY RESULTS

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Increased intake of methyl-mercury through diet during pregnancy can lead to neurodevelopmental disorders in newborns. This study was designed to assess mercury intake via fish and fish products in pregnant women in Belgrade, Serbia. Data on mercury concentrations in fish and fish products available on the market have been obtained from the Institute of Meat Hygiene and Technology, Belgrade. Data on body weights of pregnant women, species of consumed fish and fish products, and frequency and amount of consumption were obtained through questionnaires completed by 107 pregnant women. Levels of exposure were determined using a deterministic approach for the worst-case scenario, and the @RISK software (Monte Carlo simulation with 100 iterations) was used for probabilistic modeling data integration. A deterministic estimated weekly intake for canned tuna, which contained the highest levels of mercury (worst case scenario) was 2.38 μg kg^{-1} bw, which is higher than the provisional tolerable weekly intake (PTWI) for methyl-mercury recommended by FAO/WHO in 2003 (1.6 μg kg^{-1} bw per week). Mercury weekly intake through different fish and fish products obtained by probabilistic modeling was 0.0063, 0.0714, and 0.6048 μg kg^{-1} bw for 5^{th}, 50^{th}, and 95^{th} percentiles, respectively, while the estimated maximum mercury weekly intake of 3.1451 μg kg^{-1} bw exceeded the recommended PTWI level. Although these results show the intake of total mercury through fish and fish products in pregnant women, this subpopulation should be under controlled optimal diet and consumption of certain types of fish and fish products. (Projects: III 46009, 451-03-3095/2014-09/03)

KEY WORDS: *diet, exposure, neurotoxic substances, newborns, pregnancy*

P-29

HEAVY METALS IN HONEY – THE ROLE OF GOOD BEEKEEPING PRACTICE

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Honey is generally considered a natural and healthy foodstuff. However, it can be contaminated by different agents such as heavy metals, pesticides, radioactive materials, etc. Heavy metals in honey originate mainly from soil and air, which are polluted by industry, traffic, etc. They can also occur in honey during processing and packaging if inappropriate equipment and materials are used. Toxic metals (lead and cadmium) are a huge risk for human health. The goal of good beekeeping practice is to analyse and reduce all potential risks in order to obtain honey that is safe for consumption. This, among other things, includes the selection of an adequate location for the hives, in order to reduce the possibility of environmental contamination, as well as the usage of safe materials for the processing equipment. Nineteen samples of linden honey from Vojvodina, 2014 harvesting season, were analysed for Pb, Cd, As, Cu, Zn, and Fe by atomic absorption spectrometry (AAS). For the determination of Pb and Cd, the graphite furnace technique was employed, As was determined by hydride generation AAS and Cu, Zn, and Fe were determined by flame AAS. Samples were prepared using microwave digestion. Sixteen samples met the requirements of Serbian official regulations (RS Official Gazette No. 29/14, with amendments). In three samples the content of Pb (0.90, 0.92, and 0.93 mg kg^{-1}) and Zn (15.4, 15.0, and 15.9 mg kg^{-1}) was above the permitted threshold. Very similar concentrations of Pb and Zn in these three samples indicate that they were processed using the same equipment and that equipment, or some parts of it, were old, possibly made of Zn-Pb alloy.

KEY WORDS: *contamination of honey, good beekeeping practice, processing equipment, toxic metals*

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P-30

MACRO- AND MICROELEMENTS IN RAT LIVER, KIDNEY, AND BRAIN TISSUES: SEX DIFFERENCES AND EFFECT OF BLOOD REMOVAL BY PERFUSION *IN VIVO*

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The concentrations of macro- and microelements in tissues could be sex-related and affected by blood retained in dissected organs. To test these hypotheses, in 3-mo old female and male rats, the concentrations of various elements were measured in the blood-containing (nonperfused) and blood-free (perfused *in vivo* with an elements-free buffer) liver, kidney, and brain (N=5 in each group). In these organs, 6 macroelements and 15 microelements were determined by ICP-MS. In non-perfused organs, female-dominant differences were observed for Fe, Se, and I in the liver; Fe, Co, Zn, Cd, and Li in the kidney; and As and Li in the brain. In blood-free organs, similar differences were detected for: Mg, K, Fe, Mn, Cu, Se, I, and Cd in the liver; Fe, Co, and Li in the kidney; and none in the brain. In females, the perfused liver, kidney, and brain had significantly lower concentrations of 4 (Na, Ca, As, B), 5 (Na, K, I, As, Li), and 2 (Fe, As) elements, and higher concentrations of 10 (Mg, P, S, K, Mn, Cu, Zn, Cd, Hg, Li),4 (Co, Pb, Cd, Hg), and 7 (Mg, S, K, Zn, Se, I, Li) elements. In males, perfusion caused lower concentrations of 4 (Na, Ca, As, B), 7 (Na, Ca, Fe, I, As, Hg, Li), and 2 (Fe, As), and higher concentrations of 1 (Li), 1 (Zn), and 7 (Mg, P, S, K, Zn, I, Li) elements in the liver, kidney, and brain, respectively. Therefore, residual blood in organs can significantly influence the tissue concentrations of various elements and their sex-dependency. This work was financially supported by Project No. 1481 Aging-related Expression of Membrane Transporters in Rat (AGEMETAR), funded by the Croatian Science Foundation.

KEY WORDS: *gender differences, ICP-MS multielement analysis, perfusion, rat organs*

P-31

MERCURY CONCENTRATIONS IN THE LIVER OF BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) STRANDED ON THE CROATIAN ADRIATIC COAST

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Mercury (Hg) pollution in the Mediterranean Sea is particularly relevant, as this is a semi-enclosed body of water with high natural and anthropogenic inputs of this metal. Natural sources include the weathering of large cinnabar ore deposits found along the seabed bottom. Anthropogenic emissions include intensive current and past mining, fossil fuel combustion, cement production and specific industries such as chlor-alkali plants. The aim of this study was to determine Hg concentrations in stranded bottlenose dolphins collected along the Croatian Adriatic coast, in order to increase the sparse database of contaminant data available for this species and to compare the concentrations found to those in other species from the study area. Between 2010 and 2013, 42 stranded bottlenose dolphins (*Tursiops truncatus*) were sampled along the Croatian Adriatic coast. Mercury concentrations in liver tissues were determined by a flow injection mercury system. Concentrations in the liver ranged from 0.449 to 933 mg kg^{-1}. A high correlation was observed between total Hg concentration and age. Also, adult dolphins contained statistically significant higher Hg concentrations compared to juvenile dolphins, suggesting a major impact of age on the accumulation of Hg. Using benchmarks relevant to marine mammals, this study indicated that the maximum Hg threshold of 400 mg kg^{-1}, defined to indicate hepatic damage, was exceeded in six individuals.

KEY WORDS: *Adriatic coast, bottlenose dolphin, contamination, liver, mercury*

P-32

TRACE ELEMENT CONCENTRATION IN MEN WITH NEWLY DIAGNOSED PROSTATE CANCER

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Prostate cancer is among the most common cancers diagnosed in men in developed countries. Imbalance between toxic and essential elements and the mechanisms of antioxidant defence in an organism can lead to increased sensitivity and contribute to the development of this disease. Data concerning the levels of trace elements and antioxidant system parameters in men with prostate cancer are limited as their results are conflicting. In this study we determined the concentration of several toxic and/or essential trace elements and the activity of antioxidative enzymes copper, zinc-superoxide dismutase (Cu,Zn-SOD) and glutathione peroxidase (GSH-Px) in blood obtained from 53 patients with newly diagnosed prostate cancer and from 53 healthy volunteers. In subjects with newly diagnosed prostate cancer we found a significantly higher concentration of lead, mercury and molybdenum in the blood and serum, arsenic and chromium in the serum and copper and iron in the blood, as well as a significantly higher activity of Cu, Zn-SOD and GSH-Px. Subjects with prostate cancer had a significantly lower concentration of serum zinc, blood manganese, and cobalt in both serum and whole blood compared to healthy volunteers. Elevated levels of toxic elements (such as lead, mercury and arsenic) measured in patients with prostate cancer suggest that even low exposure to these elements may present a risk for the development of cancer, probably via the formation of reactive oxygen species and/or reduction of bioavailability of zinc. The increased enzymatic activity is most likely the result of cell response to oxidative mechanisms involved in the development and progression of prostate cancer.

KEY WORDS: *essential elements, oxidative stress, prostate cancer, toxic elements*

P-33

ANTIAFLATOXIGENIC EFFECT OF FULLERENE C_{60} NANOPARTICLES

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This study examined the antifungal and antiaflatoxigenic effect of environmentally plausible concentrations of fullerene C_{60} nanoparticles (nC_{60}) on aflatoxicogenic fungi *Aspergillus flavus* NRRL 3251. Fungi were grown in YES medium for 168 h at 29 °C in the presence of 0, 10, 50, and 100 ng mL^{-1} of nC_{60}, and dry mycelia weight determination was used for antifungal activity estimation, while aflatoxin content in yeast extract sucrose (YES) media was used to determine the antiaflatoxigenic effect. Aflatoxin B1 content in YES media was determined by a dilute and shoot LC/MS-MS method validated and fitted for this purpose. In addition, oxidative status markers (TBARS, Cu, Zn- SOD, Mn-SOD, CAT, GPX, GR and GSH/GSSG ratio) in *A. flavus* mycelia were determined by standard biochemical assays. Fullerene C_{60} nanoparticles neither exhibited the antifungal activity against *A. flavus* NRRL 3251 at any examined concentration, nor did they cause any significant change in oxidative status markers. However, exposure of the fungus to 10 and 100 ng mL^{-1} nC_{60} significantly reduced aflatoxin B1 production. The present data clearly indicate the antiaflatoxigenic effect of nC_{60}, but only a detailed examination of the transcription factors which regulate cellular response to oxidative stress and activate secondary metabolism in aspergilli could shed light on the exact antiaflatoxigenic mechanism.

KEY WORDS: *aflatoxin B1,* Aspergillus flavus *NRRL 3251, fullerene C_{60}, nanoparticles, oxidative stress*

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BIOLOGICAL SURFACE COATING WITH TiO_2 NANOPARTICLES IN ACUTE TEST WITH DAPHNIDS RESULTS IN THEIR INCREASED IMMOBILITY IN POST-EXPOSURE PERIOD

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Extensive use of TiO_2 nanoparticles (NPs) represents a risk to human health and environment. The contributing factors in NPs' mediated toxicity to daphnids are exposure duration and TiO_2NP aggregate adhesion which may cause physical effects and loss of mobility. We studied the impact of TiO_2 NPs on the immobility of *D.magna* in a standard acute test (48 h). We observed daphnids' immobility in the post-exposure period in ISO dilution water for additional 24 h and evaluated the level of adsorption of TiO_2NPs on the body surface with scanning electron microscopy in order to study the impact of biological coating on *D. magna* mobility and moulting. The results showed that tested TiO_2NPs did not cause daphnids' loss of mobility after 48 h exposure in more than 30 % when the highest TiO_2 NPs concentration (100 mg L^{-1}) was tested. We noticed a strong adoption potential of TiO_2NPs on the body surface. However, after prolonging the test with transferring daphnids to ISO dilution water without NPs for another 24h, increased immobility (up to 75 %) was recorded when previously the 10 and 100 mg L^{-1} TiO_2 NPs concentrations had been used. All daphnids undergo moulting in the first 48 h of the test, but the second moulting did not occur in the prolonged period. We concluded that irreversible adsorption of TiO_2 NPs on the body surface interrupted both the daphnids' swimming ability and the second moulting in the post-exposure period. Even if acute immobility test with these NPs did not show any toxicity, the observation of daphnids in the post-exposure period revealed that TiO_2 NPs cannot be considered biologically inert.

KEY WORDS: Daphnia magna, *TiO_2 nanoparticles aggregate adhesion, scanning electron microscopy*

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EFFECT OF MAGHEMITE NANOPARTICLES ON NON-NEURONAL CHOLINERGIC SYSTEM

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The cholinergic system plays an important role in neurotransmission in the human nervous system. Moreover, in non-neuronal cells, it also takes an important part in the regulation of basic cell functions, such as proliferation, differentiation, cytoskeletal organisation, cell-cell contact, migration, and immune functions. Recent advances in the synthesis of nanoparticles (NPs) have brought about a significant increase in their biomedical application and magnetic iron oxide NPs seem to be the most promising ones among these. Their applicability is largely dependent on their biocompatibility and interaction with cells. Due to its very extensive role in the human body, it is of great importance to understand to what extent they can affect the non-neuronal cholinergic system. NPs for biomedical application are usually administered intravenously and therefore blood cells and vascular endothelial cells are the first to be exposed. In our study we investigated the influence of pristine and silica coated maghemite NPs (γ-Fe2O3) in human monocytes (THP-1) and human umbilical vein endothelial cells (HUVEC). Cells were exposed to nanoparticles at different concentrations from zero to 100 μg mL^{-1} for 24 h. After exposure, resazurin viability test was done for the whole concentration range and for the concentration of 100 μg mL^{-1} acetylcholinesterase (AChE) specific activity was determined using the Ellman method. No alterations in cell viability were observed and the acetylcholinesterase activity was not changed in comparison to untreated control cells. Even though AChE activity was not changed, NPs exposure may result in altered expression of different AChE isoforms. Research is currently underway.

KEY WORDS: *acetylcholinesterase, endothelial cells, maghemite nanoparticles, monocytes, non-neuronal cholinergic system*

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HAEMATOTOXIC EFFECTS AND IRON LEVELS IN OFFSPRING AFTER MATERNAL EXPOSURE TO CADMIUM IN RATS

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Cadmium (Cd) is a pervasive toxic metal in the human environment that interacts with iron (Fe) during its gastrointestinal absorption and accumulation in internal organs. The aim of this study was to evaluate Fe levels in organs and haematotoxic effects in Wistar rat offspring following maternal oral exposure to 50 mg Cd L^{-1} (as $CdCl_2$ in drinking water) that began before and continued during gestation and lactation until weaning (on postnatal day 21). At delivery, one part of the litter was cross-fostered between control and exposed dams to discern gestational, lactational and gestational plus lactational Cd exposure and effects. Cd and Fe in organs were analysed by atomic absorption spectrometry. Haematological parameters in peripheral blood were analysed manually and organ samples prepared for standard histopathological examination by light microscopy. Cd increased and Fe decreased in the exposed mother rats and their offspring. In 21-day offspring, Fe levels decreased in the liver and brain after gestational plus lactational and after only lactational exposure. Red blood cell (RBC) count, haemoglobin (Hb) and haematocrit decreased, reticulocyte (immature RBC) count increased, and islands of extramedullary haematopoiesis were detected in their liver. After 4-week exposure cessation, at puberty, levels of Fe in the kidney and brain, and RBC and Hb in blood remained low. Cd-induced decrease in organ Fe levels and haematotoxic effects in the offspring were most pronounced by continuous exposure during gestation and lactation, the critical windows for growth and development. These changes may have postnatal health consequences during adult life.

KEY WORDS: *cross-fostering, gestation, haematological parameters, lactation, postnatal health consequences*

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COOPERATION OF PUBLIC HEALTH INSTITUTES AND HOSPITALS IN CROATIA IN THE IMPLEMENTATION OF A HUMAN BIOMONITORING SURVEY ON EXPOSURE TO MERCURY

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Mercury as a highly toxic heavy metal can cause adverse health effects in humans. Fish consumption represents the primary human exposure route. The aim of this survey was to determine Hg exposure in women of generative age and their newborn babies by determining a possible link between fish consumption and concentrations of mercury in women's hair and urine samples and babies' cord blood, posing as the indicators of mercury body burden. Through the network of public health institutes and with cooperation of staff at maternity wards in hospitals, samples were collected from September 2015 to February 2016. Data on dietary and living habits and frequency of fish consumption were collected. The research sample consisted of 302 women aged 19 to 43 with their newborn babies. The survey was conducted in 14 selected maternity wards in two regions in Croatia: coastal and continental region. The measured levels of total Hg in hair samples in the coastal region ranged from 0.01249-5.8258 μg g^{-1} and 0.00577-1.569 μg g^{-1} in the continental region. The concentrations of Hg in the cord blood ranged from 0.01-18.83 μg L^{-1} in the coastal region and 0.04-12.29 μg L^{-1} in the continental region. The concentrations of Hg in urine adjusted to creatinine ranged from 0.06 to 4.05 μg g^{-1} in the coastal region and 0.01-4.17 μg g^{-1} in the continental region. Results suggest that women in the coastal region have higher Hg concentrations in hair and cord blood than women from the continental region. The conducted survey is an excellent example of cooperation of all included institutions in Croatia; the Croatian Institute of Public Health, county institutes of public health, and hospitals.

KEY WORDS: *fish consumption, human samples, institutional cooperation, newborn babies, woman*

P-38

DERMAL EXPOSURE TO BISPHENOL A INDUCES CELLULAR, HISTOPATHOLOGICAL AND BEHAVIOURAL ALTERATIONS IN *EISENIA FETIDA*

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Bisphenol A (BPA) is an industrial synthetic chemical widely used in polycarbonate plastics and epoxy resin production. Due to its increasing usage, BPA has become one of the most manufactured volume chemicals with over 6 billion pounds produced per year. Presence of BPA in a wide range of products leads to its continual release into the environment by leakage from polycarbonate plastic. Despite the ubiquity, BPA effects on the soil-inhabiting organisms are mostly unexplored. *Eisenia fetida* earth worms were cutaneously exposed to environmentally realistic BPA concentrations, 100 nM and 10 μM BPA, up to 10 days (10-d). Next, a battery of biomarkers was used for ecotoxicological evaluation on the cellular, tissue and behavioural levels. HPLC analysis showed that after 10-d exposure BPA accumulation reached a maximum of 2.50 μg BPA per g of wet tissue weight. On the cellular level, up to 3-d BPA exposure caused increased lipid oxidation indicating oxidative stress. Histopathological assessment of the cell wall and ovaries after 7- and 10-d BPA exposure showed multiple abnormalities, *i.e.* hyperplasia of epidermis, increased body wall thickness and ovarian atrophy. Detection of these changes was facilitated by a newly proposed semi-quantitative scoring system. Finally, behavioural changes were detected after only 3 days of exposure to 100 nM BPA. Altogether, the presented multilevel toxicity evaluation indicates high sensitivity of earthworms to low BPA doses.

KEY WORDS: *bisphenol A,* Eisenia fetida*, filter paper contact test, histopathology, oxidative stress*

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OXIDATIVE STRESS, METALLOTHIONEINS AND ENVIRONMENTAL EXPOSURE TO METALS IN TWO EUROPEAN BROWN BEAR POPULATIONS

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Element levels in brown bear (*Ursus arctos*) blood are unknown. Previously, we discovered that brown bears in Croatia had the highest tissue levels of toxic metals among all hunted terrestrial wild mammals. However, associations with other related biomarkers of exposure and/or effect have not been studied. Considering that oxidative stress was suggested as one of the pathways of toxic damage caused by metals, its biomarkers could be the indicators of metal-related health effects. Our aim was to study the biomarkers of oxidative stress (lipid peroxidation, SOD activity) and metal exposure, (metallothionein, MT), in association with toxic metal levels in the blood of Croatian (N=8) and Polish (N=11) free-living brown bears. Metal content was measured using ICP-MS, products of lipid peroxidation (malondialdehyde, MDA) using HPLC, the activity of antioxidative enzyme using spectrophotometry, and MT using differential pulse voltammetry. Median (range) mass fractions of cadmium (Cd) and mercury (Hg) in brown bear blood were 0.356 (0.110-1.34) and 7.23 (1.09-13.5) μg kg^{-1}, and showed no differences between the two populations, nor were there differences in either MDA or MT. On the contrary, lead (Pb) was higher ($p=0.041$) in Polish (median 145; range 104-192 μg kg^{-1}) compared to Croatian (101; 58.9-184 μg kg^{-1}) bears, while SOD activity was lower ($p=0.007$) in Polish (738; 21.6-2328 UL^{-1}) than in Croatian (1428; 1008-2326 UL^{-1}) bears. Age, gender and season of collection had no significant influence on the measured parameters. These results represent a valuable contribution to toxicological studies of European brown bear and the first data describing metals and biomarkers in the blood of this species.

KEY WORDS: *biomarkers, blood, lipid peroxidation,* Ursus arctos

P-40

QUANTIFICATION OF DIOXINS AND FURANS IN SEDIMENTS AND WATER

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The group of Polychlorinated Dibenzo-p-dioxins (PCDDs, Dioxins) and Polychlorinated Dibenzofurans (PCDFs, Furans) consists of 210 organic compounds (congeners), which differ widely in their chlorine content and respective toxicities. In particular, 17 of these are extremely toxic. The most well-known compound is 2,3,7,8 tetrachloro-dibenzo-p-dioxin (2,3,7,8 –TCDD). The toxicity of dioxins and furans is calculated using the toxicity equivalence factor (TEF) specified by the World Health Organization (WHO). According to WHO, the TEF value for 2,3,7,8 TCDD is one. PCDDs and PCDFs have shown toxic effects in animal studies; they damage reproductive and immune systems and are also carcinogenic. Dioxins are mainly formed as an unwanted by-product in combustion processes in case of which traces of chlorine can be found as by-products of the combustion of contaminated chemical waste, chemical and pesticide manufacturing, pulp and paper bleaching processes, and other sources. These compounds are extremely stable, both in terms of their chemical and thermal qualities, and persist in the environment for many years. In the Teaching Institute of Public Health ''Dr. Andrija Štampar'', the gas chromatography coupled to triple quadrupole mass spectrometry (GC-MS/MS) method for determination of PCDDs and PCDFs is used. Until now, 135 water and 28 sediment samples were analysed. For all samples the results were below the limit of quantitation. Due to the environmental stability of PCDDs and PCDFs and their harmful effects on human health it is necessary to carry out continuous control for public health purposes.

KEY WORDS: *GC-MS/MS, PCDDs, PCDFs, Teaching Institute, TEF value*

P-41

PHYSICOCHEMICAL AND ECOTOXICOLOGICAL EVALUATION OF ROVINJ COASTAL AREA SEDIMENTS, NORTHERN ADRIATIC, CROATIA

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The aim of the study was to characterize physicochemical and ecotoxicological properties of marine sediments for the following testing of PCB accumulation by passive samplers. Further data on the sediment quality in the Eastern Adriatic regarding heavy metal, PAH and PCB concentrations are urgently needed, because the environmental authorities have to define the limits for contaminants in sediments for a different use of marine resources. We collected sediments at 5 locations in Rovinj costal area according to the degree/extent/intensity of human activities – pollution, and applied French and German regulations. Sediment chemical and leachates toxicity analyses classified our sites S5 and S4 as pristine areas. These also ranked our sites as per the degrees of contamination (S5<S4<S3<S2<S1) and decreasing phytotoxic effects (S5>S4>S1>S2>S3).

KEY WORDS: *chemical analyses, ecotoxicological analyses, marine sediment, Northern Adriatic Sea, passive samplers*

P-42

MERCURY CONCENTRATIONS IN COWS FROM THE VICINITY OF A NATURAL GAS TREATMENT PLANT IN CROATIA

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During a period of eleven years (2004-2015), as part of comprehensive monitoring of mercury levels in the vicinity of the natural gas production and treatment plant CPS Molve, biological samples from cows from households in the village of Molve were collected. The samples of milk, blood, urine, faeces, and hair from three cows were taken on ten occasions and analysed for total mercury concentration. Due to the implementation of a new analytical method in 2009, the results are presented separately for the samples collected 2004-2008 (analysed by CV-AAS) and for the samples collected 2010-2015 (analysed by AMA254). Median mercury concentrations in milk, blood, urine (μg ml^{-1}), faeces (μg g^{-1} on wet weight basis), and hair (μg g^{-1}) in 2004-2008 monitoring were 0.001, 0.001-0.009, 0.001-0.002, 0.001-0.041, 0.001-0.049 and in 2010-2015 0.00001-0.0031, 0.00002-0.0002, 0.00002-0.0019, 0.0027-0.0075, 0.0024-0.015, respectively. The highest concentrations of mercury were found in hair and faeces, and the lowest in the blood of tested animals, which corresponds to the toxicokinetics of inorganic mercury after long term exposure to normal ambient air concentrations. Generally, the measured concentrations are very low and below the formerly prescribed maximum permitted concentration of mercury in milk. Also, they do not represent a danger to the health of the people and cows that live in the area potentially contaminated with mercury due to natural gas production.

KEY WORDS: *biological samples, contamination, environmental monitoring, natural gas*

P-43

SOME PBDE CONGENERS IN THE SERUM OF DOGS

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BFRs (brominated flame retardants) have been used as flame retardants for the last 60 years, but their negative effects on animals and possibly humans have been observed only in the last 20 years, as this is when they began to be the subject of more intense studies. They can migrate out of products to which they are added and enter the environment; consequently they have been detected in a variety of samples, including the atmosphere, lake and sea sediments, human and pet (dogs, cats) serum and food, cow fat and milk, moos liver, arctic fox and polar bear adipose tissue, bald eagles and herring gull eggs. The widespread presence of flame retardants in the indoor environment makes domestic pets, who share human environment and even food, a perfect indicator for biomonitoring studies. Among BFRs, the polybrominated diphenyl ethers (PBDEs) have received the most attention. We measured the concentrations of some PBDE congeners (BDE 28, BDE 47, BDE 66, BDE 100, BDE 99, BDE 85, BDE 154, BDE 153, and BDE 183) in the serum of 20 obese and 20 normal house dogs. We found no statistical difference between the concentrations of all PBDE congeners in both groups of dogs. Average values were 0.0190±0.0302 ng g^{-1} and 0.0112±0.0091 ng g^{-1}, respectively. BDE 47 and BDE 99 were the predominant congeners in both groups of dogs.

KEY WORDS: *BFRs, contamination, indoor environment, POPs*

P-44

ARE SILICON BANDS USED FOR BRACELET WEAVING SAFE FOR CHILDREN REGARDING THE LEVEL OF PHTHALATES?

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A toy that helps to create brightly colored silicone bracelets has become really popular among children worldwide. Certain substances hazardous to children's health may be found in toys, including toxic metals, bisphenol A, phthalates, etc. Phthalates are used as plasticizers to provide softness and flexibility to polymer materials, but public concern has been raised about their potential harmful effects (reproductive and development toxicity). The aim of this study was to assess the level of phthalates in silicon bands used for bracelet weaving. Eight samples available in the local market of Belgrade, Serbia were analysed for six phthalates: benzylbutyl phthalate (BBP), dibutyl phthalate (DBP), di(2-ethylhexyl) phthalate (DEHP), di-n-octylphthalate (DNOP), di-isononylphthalate (DINP), and di-isodecylphthalate (DIDP). Extraction was performed using a mixture of tetrahydrofuran and hexane under such conditions that allow identification and level determination of the investigated phthalates by the HPLC-DAD method. Among the investigated phthalates only DEHP and DBP were found in band samples. DEHP was found in all examined samples in a concentration range of 0.001 %-0.05 %, while DBP was present in 6 samples in a concentration range of 0.001 %-0.013 %. These results indicate that all measured phthalate levels were lower than the European Union (EU) Directive 2005/84 limits and the allowed range according to the Official Gazette of the Republic of Serbia, no. 90/2013, 25/2015, and 2/2016 (0.1 %, w/w). In future work, the content of phthalates should be determined in plastic loom and crochet hooks that are sold in kits with silicone bands. (Projects: III 46009)

KEY WORDS: *child health, HPLC-DAD method, plasticizers, safe toys*

P-45

ASSESSMENT OF PROOXIDATIVE *IN VITRO* EFFECT OF DECABROMINATED DIPHENYL ETHER

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Fully brominated diphenyl ether (BDE-209) is one of commercial mixtures of polybrominated diphenyl ethers (PBDEs) widely used as flame retardants. Its areas of use are household products like electrical and electronic equipment but also the transport and construction sectors. The main route of human exposure to BDE-209 is by inhalation and it exerts toxicity mainly via the Ah receptor. The objective of this study was to assess the prooxidative *in vitro* effect *vs.* cell culture concentration of BDE-209 in order to identify whether its toxicity is mediated by the prooxidative activity. A human colon carcinoma cell line (SW 480) was used as a model for the intestinal system. Cells were grown as monolayer cultures in Dulbecco's Modified Eagle Medium, supplemented with 10 % foetal bovine serum, and 1 % penicillin/streptomycin solution. The concentration range used in the experiment included 2.5, 5, and 10 μg BDE-209 mL^{-1} (corresponding to 2.61, 5.21, or 10.42 μM, respectively). The formation of reactive oxygen species (ROS) in cells treated with BDE-209 was determined by the 2′,7′-dichlorofluorescein diacetate (DCF-DA) assay. The dose–response relationship was assessed using the PROAST software (RIVM, the Netherlands). The derived lower confidence limit of benchmark dose – 5 % (BMD_5) for ROS production was 2.57 while the calculated IC_{50} was 10.8 μM. The calculated IC_{50} value was lower than the values from several similar studies where ROS production caused by PBDEs was examined. We confirmed the dose dependent increase in ROS production in the SW 480 cell line caused by BDE-209 *in vitro*, and for the first time we derived a lower confidence limit of BMD_5 for ROS production.

KEY WORDS: *BDE-209, cytotoxicity, cell lines, effect assessment, free radical induction*

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THE *IN VIVO* ASSESSMENT OF GONADOTOXIC ACTIVITY OF THIAMETHOXAM PESTICIDE IN WISTAR HAN MALE RATS

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The purpose of this study was to assess the influence of Thiamethoxam TG (technical grade) pesticide (insecticide-neonicotinoid) produced by two different manufactures (test substances T1 and T2, each of them containing 95.5 % and 95.4 % of active ingredient respectively) on fertility parameters of Wistar Han male rats. The study was performed exposing 40 Wistar Han male rats to each test substance in two different doses (a low dose of 1.5 mg kg^{-1} and a high dose of 15 mg kg^{-1}). The detection of the functional state of gonads and the evaluation of the reproductive ability for males were performed at the end of the exposure period. The reproductive parameters for intact females mated with the exposed males were assessed on the 20th day of pregnancy. The test substance T1, in the maximum dose of 15 mg kg^{-1}, revealed signs of reproductive toxicity in exposed males only. The test substance T2, in the maximum dose, revealed signs of reproductive toxicity in the exposed males and intact females. No effect on the reproductive function of the exposed males and intact females were found at the minimal dose of both test substances. Based on the results we can conclude that the obtained effects are dose-dependent and no effect levels are identical for both test substances (1.5 mg kg^{-1}). The observed additional embryotoxic effect at the maximum dose of the test substance T2 is presumably the result of the origin of the test substance.

KEY WORDS: *gonadotoxic activity, pesticides, reproductive, thiamethoxam, Wistar Han rat*

P-47

TOXICOLOGICAL STUDY OF GENERIC PESTICIDES QUIZALOPHOP-P-ETHYL ON THE BLOOD SYSTEM OF WISTAR HAN RATS

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The aim of the study was to research the exposure effects of generic pesticides quizalophop-P-ethyl, 98.2 % (QpE 1) and quizalophop-P-ethyl, 95 % (QpE 2) on the hematological parameters of males Wistar Han rats in a subchronic 90-days oral toxicity study (according to OECD 408 guideline in compliance with GLP). Doses (0; 2; 10; 100 mg kg^{-1}) were defined. Blood for hematological measurements was studied at 4, 9 and 13 weeks after QpE exposure in the same groups of animals throughout the experiment. Hematological parameters: RBC, HGB, HCT, erythrocyte indices (MCV, MCH, MCHC), total amount of leukocytes (WBC) and platelets were measured. As a result: QpE 1 in a 100 mg kg^{-1} dose induced a significant decrease in MCH and MCHC after 9 weeks of exposure and a significant decrease in RBC and HGB concentration after 13 weeks of exposure. QpE 2 in a 100 mg kg^{-1} dose after 4 weeks of exposure caused a significantly increased total WBC value. A significant increase in segmented neutrophils was observed. Changes in leukocytes morphology were found. MCHC was significantly decreased at the highest dose of QpE 2 in all experimental periods compared to the control group. The study revealed that QpE 1 and QpE 2 affected the peripheral blood of males Wistar Han rats and could promote hematological alterations with high doses. According to EFSA documents and others regulatory reviews, the original molecule of QpE has no adverse effects on the blood system. We think that in case of generic pesticide studies, impurities can exhibit a different toxicological action.

KEY WORDS: *blood, generic pesticide, hematological parameters, quizalophop-p-ethyl, Wistar Han rats*

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STUDY OF SUBCHRONIC ORAL TOXICITY OF IMAZAMOX ON WISTAR HANNOVER MALE RATS

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The purpose of investigation was to assess the toxic effects of imazamox following subchronic oral exposure. Studies of short-term oral toxicity of two samples of imazamox from different manufacturers in a 90-day experiment on Wistar Hannover male rats were conducted using the following doses: 80 mg kg^{-1}, 800 mg kg^{-1}, and 1600 mg kg^{-1} b.w. day^{-1}. In both experiments noisy breathing was observed in certain animals at the dose of 1600 mg kg^{-1} b.w. day^{-1}. The study of the first sample revealed that the reintroduction of imazamox at the dose of 1600 mg kg^{-1} b.w. day^{-1} caused a body weight decrease from week 3 to week 13 and a decrease in body weight gain in week 4 and week 13 of the experiment. An increase in haematocrit at the dose of 1600 mg kg^{-1} b.w. day^{-1} was recorded in week 13 of the study. The study of the second sample revealed that the reintroduction of imazamox at the dose of 1600 mg kg^{-1} b.w. day^{-1} caused a decrease in body weight and body weight gain from week 2 to week 13 of the experiment. A decrease in urine pH at doses of 800 and 1600 mg kg^{-1} b.w. day^{-1} and diuresis at 1600 mg kg^{-1} b.w. day^{-1} was recorded in weeks 4, 9, and 13 of the experiment. Also, an increase in urea content in urine at the dose of 800 mg kg^{-1} b.w. day^{-1} was observed in week 9 and at the dose of 1600 mg kg^{-1} b.w. day^{-1} in weeks 4, 9, and 13. Due to the afore cited data, it could be considered that imazamox has a low general toxic effect at the dose of 1600 mg kg^{-1} b.w. day^{-1}.

KEY WORDS: *body weight, general toxic effect, rats, subchronic oral exposure, Wistar Hannover*

P-49

AN ESTIMATION OF URINARY BENZENE, TOLUENE, ETHYLBENZENE, AND ISOMERIC XYLENES CUT-OFF VALUES TO DISTINGUISH NONSMOKERS AND SMOKERS IN THE GENERAL POPULATION

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Volatile organic compounds benzene, toluene, ethylbenzene, *o*-, *m*-, and *p*-xylene (BTEX) are ubiquitous pollutants of the human environment since their main sources are vehicular exhausts and tobacco smoke. Due to the toxicological effects (carcinogenic, neurologic, immunologic, respiratory, etc.) of these airborne chemicals, exposure to low levels could also increase the health risk in the general population. The aim of this study was to estimate the urinary cut-off values to discriminate nonsmokers from smokers in the general population not occupationally exposed to BTEX. Nonsmokers (N=120; 65 females) aged 18-65 years (median 44 years) and smokers (N=120; 60 females) aged 20-63 years (median 43 years), smoking 16±9 cigarettes daily, were selected for the study. Urinary BTEX concentrations were measured by headspace solid phase microextraction (HS-SPME) followed by gas chromatography–mass spectrometry (GC-MS). The optimal urinary cut-off values for distinguishing current smokers from nonsmokers were 139 ng L^{-1} (95.0 % sensitivity, 80.0 % specificity) for benzene, 200 ng L^{-1} (80.8 % sensitivity, 85.0 % specificity) for toluene, 29 ng L^{-1} (90.8 % sensitivity, 50.0 % specificity) for ethylbenzene, 197 ng L^{-1} (67.5 % sensitivity, 74.2 % specificity) for *m/p*-xylene, and 86 ng L^{-1} (45.8 % sensitivity, 86.7 % specificity) for *o*-xylene. The data presented provides valuable information on the background exposure of the general population to BTEX and represents a baseline for future studies. Our results could provide guidelines for differentiating current smokers from nonsmokers in the Croatian population.

KEY WORDS: *BTEX, human biomonitoring, SPME-GC/MS, urine*

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P-50

POISONINGS DUE TO NEW PSYCHOACTIVE SUBSTANCE ABUSE REPORTED TO THE CROATIAN POISON CONTROL CENTRE IN 2008-2015

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To identify information required by healthcare professionals regarding symptomatology and treatment of new psychoactive substance (NPS) poisonings, 8-year records of the Croatian Poison Control Centre (PCC) were examined and cases were analysed for patient characteristics and poisoning circumstances. There were 70 incidents involving NPS abuse (synthetic cannabinoids, GHB, ketamine, ecstasy). The numbers gradually increased, from only 1 case recorded in 2008 to 39 cases in 2015. Overall, the majority of NPS poisonings happened in summertime (29 cases, 41 %). Synthetic cannabinoids were the most prevalent NPS (44 cases, 63 %). In 20 cases (29 %) two or more NPS were consumed, or NPS was combined with other drugs of abuse, pharmaceuticals or alcohol. Men (76 % of cases) were three times more often involved than women (24 %). The median age was 17 years (range 10-42 years) in 83 % of patients with a known exact age. Most common routes of exposure were inhalation (56 % of cases) and ingestion (36 %). Among the cases with known clinical presentation (68 cases), only mild symptoms were reported in most instances (45 cases, 66 %). However, in 21 cases (31 %) symptoms were serious (coma, severe aggressiveness and abnormal vital signs). In 2014, one 31-year old man died after smoking "air freshener" Galaxy which probably contained synthetic cannabinoids. The noted increased incidenceof NPS poisonings and the possibility for serious health effects stress the need for preventive strategies, which should focus on adolescents due to the worrisome finding that they account for almost half of all PCC cases.

KEY WORDS: *Croatia, Poison Control Center, recreational drugs, report, substance abuse*

P-51

CAN A HOUSEHOLD CHEMICAL MASK GC-MS TESTING OF URINE SAMPLES OBTAINED FROM CANNABIS USERS?

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Cannabis is the most abused illicit drug globally; hence drug screenings need to be conducted in health care, workplace, post-accident etc. Apart from substituting and diluting urine samples, adulteration is a common practice used to avoid the detection of drug use, as false negative results are obtained by interfering with the immunochromatographic test and/or by altering the chemical structure of a drug. The aim of this study was to investigate the influence of household chemicals, which can mask the positive result of immunochromatographic testing, on the GC-MS analysis of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (9-carboxy-THC). Adulterants, ethanol (70 %), "Visine" eye drops, sodium-chloride, hydrogen-peroxide (3 %), vinegar (9 % acetic acid), "Varikina" (bleach – 5 % sodium-hypochlorite), "WC Sanitar" (9 % hydrochloric acid), "Cevtok" (30 % sodium-hydroxide) were added to urine, previously confirmed by the GC-MS method to be positive for THC metabolites. False negative immunochromatographic test outcomes were obtained in the case of vinegar and "Varikina". In order to investigate if molecule degradation occurred during adulteration, the specimens were submitted to the GC-MS analysis. Samples were prepared using solid-phase extraction, while the GC-MS analysis was performed using a Shimadzu Gas Chromatograph Mass Spectrometer QP2010 Ultra. A DB-5 (30 m, 0.25 mm i.d., 0.25 µm film thickness) fused silica capillary column was used for chromatographic separation, where 371, 473, and 488 *m/z* ions (TMS derivatives) were monitored for mass detection. Results showed the presence of 9-carboxy-THC in adulterated analysed samples. "Varikina" and vinegar are successful adulterants when it comes to immunochromatographic tests, but they cannot affect the qualitative GC-MS analysis.

KEY WORDS: *cannabis abuse, GC-MS analysis, immunochromatographic tests, 9-carboxy-THC, urine adulteration*

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APPLICATION OF LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION FOR IDENTIFICATION OF UNKNOWN SUBSTANCES

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Nowadays, herbal products are considered a desirable supplement for weight reduction by many people. Because of this, they are in high demand. However, these supplements are not classified as drugs; therefore they are only tested for declared content according to the Regulation on Health of Dietary Products. The aim of this paper is to present the application of the HPLC-PDA method for identifying unknown substances in herbal products for weight reduction. Suspicion of the presence of substances with pharmacological action was raised in this case on the basis of the adverse effects (tachycardia, sweating, disturbed vision, tinnitus and panic) observed in the person using the product. The extract obtained from a capsule was analysed by liquid chromatography with a UV scanning detector. The unknown compound was identified in the sample on the basis of an analysis of the spectrum peak obtained after separation on a C8 column with mobile phases phosphate buffer pH 3.6 and acetonitrile using the gradient method. Comparing the UV spectrum of peak from the sample with UV spectra from the library, the presence of sibutramine was proven. Sibutramine is a substance whose chemical structure is similar to amphetamines and it was found to be the cause of these symptoms. The obtained results showed that the HPLC-PDA method can be applied in the identification of unknown compounds. Also, it is necessary to introduce more stringent criteria when testing products that are not classified as medicines but that could contain unpermitted and potentially toxic compounds.

KEY WORDS: *herbal products, HPLC-PDA, sibutramine, UV spectrum*

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ADMET PROPERTIES AND CORRELATION STUDIES IN A SERIES OF STIMULANTS OF THE WADA LIST OF PROHIBITED SUBSTANCES

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Structural features of stimulants (World Anti-Doping Agency List of Prohibited Substances, WADA 2016) and the impact of different substituents on their physico-chemical, pharmacological, and toxicological properties were analysed in correlation studies using molecular descriptors (MlogP, M_r, TPSA and V), topological indices (F, χ, J, H, W, WW, Wp and Sz), drug-likeness scores (dls) computed for GPCR ligand (GPCR l-dls), ion channel modulator (ICM-dls), kinase inhibitor (KI-dls), nuclear receptor ligand (NRL-dls), protease inhibitor (PI-dls) and enzyme inhibitor (EI-dls), as well as the ADMET parameters including the predicted toxicological properties. Molecular descriptors were calculated using Molinspiration property engine v2014.11 and Molinspiration bioactivity score v2014.03 (www.molinspiration.com) while topological indices were computed by means of Chem Axon software (www.chemicalize.org). The ADMET properties were predicted by MedChem Studio™ 4.0 and ADMET Predictor™ 8.0 (Simulations Plus, Inc., USA). All analyses were performed using OriginPro 8.0 software (Origin Laboratories, USA). Insignificant drug-likeness scores were computed for most investigated molecules, except for ICM-dls (fenbutrazate, *N*-methylephedrine, methylphenidate, oxilofrine, sibutramine and strichnine, 0,21 – 0,53), KI-dls and PI-dls for strichnine (0,56 and 0, 21, respectively), and EI-dls (pemoline, 0.26). The results of QSAR studies revealed the following significant correlations: M_r *vs.* Platt index (r = 0.9536, y = 0,2381x - 9.104) and M_r *vs.* V (r = 0.9498, y = 0,9022x +2165). According to the ADMET Predictor analysis, these molecules are mostly either CYP substrates and/or CYP inhibitors. For the investigated compounds, the following toxicological parameters were also predicted: ADMET risk between 0.0-4.446, CYP risk 0.0–1.956 and TOX risk 0.0-3.446.

KEY WORDS: *ADMET, drug-likeness, molecular descriptors, QSAR, stimulants*

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QSAR STUDIES AND PREDICTED TOXICITY OF N-ARYLHYDROXAMIC ACIDS

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The use of biological property predictions has increased in recent years due to computer technology improvements, the rising costs of drug discovery and a desire of regulatory agencies to better understand, predict, and improve drug safety. Recently, compounds with hydroxamic moiety, *i.e.*, hydroxamic acids ($R^1CON(OH)R^2$, R^1=alkyl/aryl and R^2=alkyl/aryl or H) have attracted researchers' interest since they show a wide range of biological activities and acceptable toxicities. The aim of this study was to investigate *N*-arylhydroxamic acids in order to explore their biological activity and potential toxicity in relation to their chemical structures. For this purpose different molecular descriptors including topological indices (MDs, *i.e.* n_{atoms}, M_r, *V*, Mlog*P* and TIs), drug-likeness (DLs), *i.e.*, GPCR ligand (GPCR l), ion channel modulator (ICM), kinase inhibitor (KI), nuclear receptor ligand (NRL), protease inhibitor (PI) and enzyme inhibitor (EI), and ADMET parameters in comparison with vorinostat* [ADMET Risk (1 – 5,272; 3.197*), CYP Risk (0 – 1,055; 0,697*), TOX Risk (1 – 2; 2*), TOX MUT Risk (1 – 4; 2*), TOX hERG Risk (4,541 – 5,579; 4,855*)] have been computed. The relationships between the chemical structure and either the antitumor activity or the predicted toxicity of the investigated compounds were analysed by QSAR methods. Metabolic pathways of *N*-arylhydroxamic acids with CYP enzymes have also been predicted by ADMET Predictor™ 8.0 (Simulations Plus Inc., USA) and analysed. The obtained results suggest that some of the investigated *N*-arylhydroxamic acids exhibit a better toxicological profile compared to hydroxamic acids registered for clinical use as antitumor agents (*e.g.*, vorinostat and belinostat).

KEY WORDS: *ADMET properties, drug-likeness, molecular descriptors, N-arylhydroxamic acids, QSAR*

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CLINICAL-TOXICOLOGY NETWORK IN SUSPECTED NEW PSYCHOACTIVE SUBSTANCE (NPS) INTOXICATIONS AS A PART OF "I-SEE" PROJECT

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The spread of New Psychoactive Substances (NPS) is increasing, as is the care for consumer health. The results of a research conducted on the Internet have shown increased NPS consumption in Croatia. Medical doctors from the Emergency Departments often treat patients with a typical clinical pictures of unknown intoxications. Furthermore, there are information on several death cases surely connected to NPS consumption available. Therefore, there is a need for a new joint form of intervention, better monitoring, information exchange on NPS intoxications, as well as detection of NPS in biological samples. One of the tools is the European project I-SEE for the strengthening of information exchange between Italy and South East Europe neighbouring countries on New Psychoactive Substances. The partners in this project are: Croatia (the University of Split, School of Medicine), Slovenia and Italy (the University of Florence) as the coordinator. In Croatia, it was proposed to build up a clinical-toxicology network, which would include all institutions that are in some way in contact with NPS. These are: health facilities (hospitals, ERs, primary health care out patient clinics, institutes for public health), chemical-toxicology laboratories (classified as per their equipment), non-governmental organisations (NGO's), the Poison Control Centre and law enforcement. All NPS related information is to be exchanged in accordance with the proposed algorithm; mutually, with the Office for Combating Drug Abuse of the Government of the Republic of Croatia, and with European centers and databases within the Early Warning System. In conclusion, this network could accelerate information exchange based on clinical-toxicology evidence and assist health professionals in better management of NPS intoxications.

KEY WORDS: *case of events, clinical-toxicology network, early warning system, information exchange, I-SEE project, new psychoactive substances*

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THE EFFECT OF HOUSEHOLD PRODUCTS ADDED TO URINE SAMPLES ON DETECTION OF PSYCHOSTIMULANTS USING COMMERCIAL TEST STRIPS

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Higher global prevalence of psychostimulant abuse increased the need for wider testing when consumption of these substances is suspected, even at home. Nowadays, commercial urine screening tests, intended for use at home, are available in pharmacies. However, illicit drug abusers try to beat these tests by adding household products to urine samples, as described on the internet. The aim of this study was to detect the possibility of falsifying results of detection of amphetamines, 3,4-methylenedioxymethamphetamine (MDMA), and cocaine using commercial urine test strips by intentionally adding household products. The selected chemicals were widely available on the Serbian market and could not have altered the organoleptic characteristics of urine: citric acid, "Varikina" (NaOCl), "Cevtok" (30 % NaOH), alcoholic vinegar (9 % CH_3COOH), "WC sanitar" (9 % HCl), "Asepsol" (5% benzalkonium chloride), and lemon juice. The analysed urine specimens were previously confirmed for the presence of amphetamines, MDMA, and cocaine, using the same techniques. The chemicals were regularly added to the aliquots of diluted urine and the presence of drugs was analyzed using single-component One Step Rapid Ameritek USA test strips. False-negative results of amphetamine and MDMA test strips were obtained by adding lemon juice in urine, and furthermore, "Varikina" and alcoholic vinegar altered the results, but only for amphetamine test strips. None of the chemicals added to urine altered the results of cocaine test strips. Household products, such as strong base or acid, altered test results to invalid for all three psychostimulants' detection. Our results undoubtedly confirmed the importance of fortifying urine specimen integrity for validated results in drug testing.

KEY WORDS: *adulterants, amphetamine, cocaine, MDMA, psychoactive controlled substances*

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LONG TERM STABILITY OF METHADONE AND EDDP IN CLINICAL BLOOD SAMPLES

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Methadone is a synthetic opiate, widely used to help heroin addicts undergoing maintenance programs. Determination of methadone and its metabolites represents a routine procedure in forensic toxicology. Biological samples, according to legislation, should be stored at least six months after the first analysis. Storage conditions, combined with the stability of drugs, can significantly affect the interpretation of results. Due to these reasons, it was necessary to examine the time effect on the stability of methadone and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in biological blood samples stored for more than six months. The effect of long-term stability of methadone and EDDP was studied by a repeated quantitative analysis of the same samples after long-term storage at -20 °C. A total of 50 clinical blood samples were analysed, and 20 samples, in which the presence of methadone and EDDP was confirmed, were reanalyzed after long-term storage period. The first analysis was performed 1-3 days after sampling, and the repeated analysis was performed after 293-369 days of storage for each sample. GC/MS analysis of clinical blood samples preceded the LLE sample preparation, and results were compared. In the repeated analysis, all blood samples showed lower methadone concentrations (from 2.04% to 80.74%). Contrary to expectations, reanalysis of all blood samples showed absence of EDDP. Further experiments on methadone and EDDP stability in stored biological samples, at different storage conditions and in combination with other factors, should be undertaken to ensure the proper interpretation, reliability and reproducibility of the analysis results.

KEY WORDS: *methadone, EDDP, blood samples, stability, GC/MS, toxicology*

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Due to the popularity and high visitation rate of the web page www.otrovno.com, we have decided to familiarise a wider, expert circle of people with it, and to present the most frequently asked questions. The creator of this web page is Professor Franjo Plavšić, PhD who has, after recognising the interest of common citizens, decided to share his long-time expertise in toxicology by answering many of their questions and confusions. Today, this kind of communication with the public has become one of the integral affairs of the Croatian Institute for Toxicology and Antidoping. The number of chemicals used in day-to-day life and human exposure to these through various sources continues to increase and, accordingly, the amount of information that people are exposed to through various media also keeps growing. Thus, it is understandable that recognition of quality sources of information has become a real problem for common citizens. Asbestos is all around us, "deadly" mercury from thermometers, disinfectants and fatal chlorine, wooden furniture and unfortunate formaldehyde, inebriation by organic solvents... These are only some of the topics in the minds of our fellow citizens. The web page www.otrovno.com is the only concrete and expert source of information that deals with questions from the field of toxicology that are part of everyday lives of Croatian citizens, so it is no wonder that the web page has become popular even in our neighbouring countries from which we are receiving more and more questions by the day.

KEY WORDS: *answers, questions, toxicology, web page*

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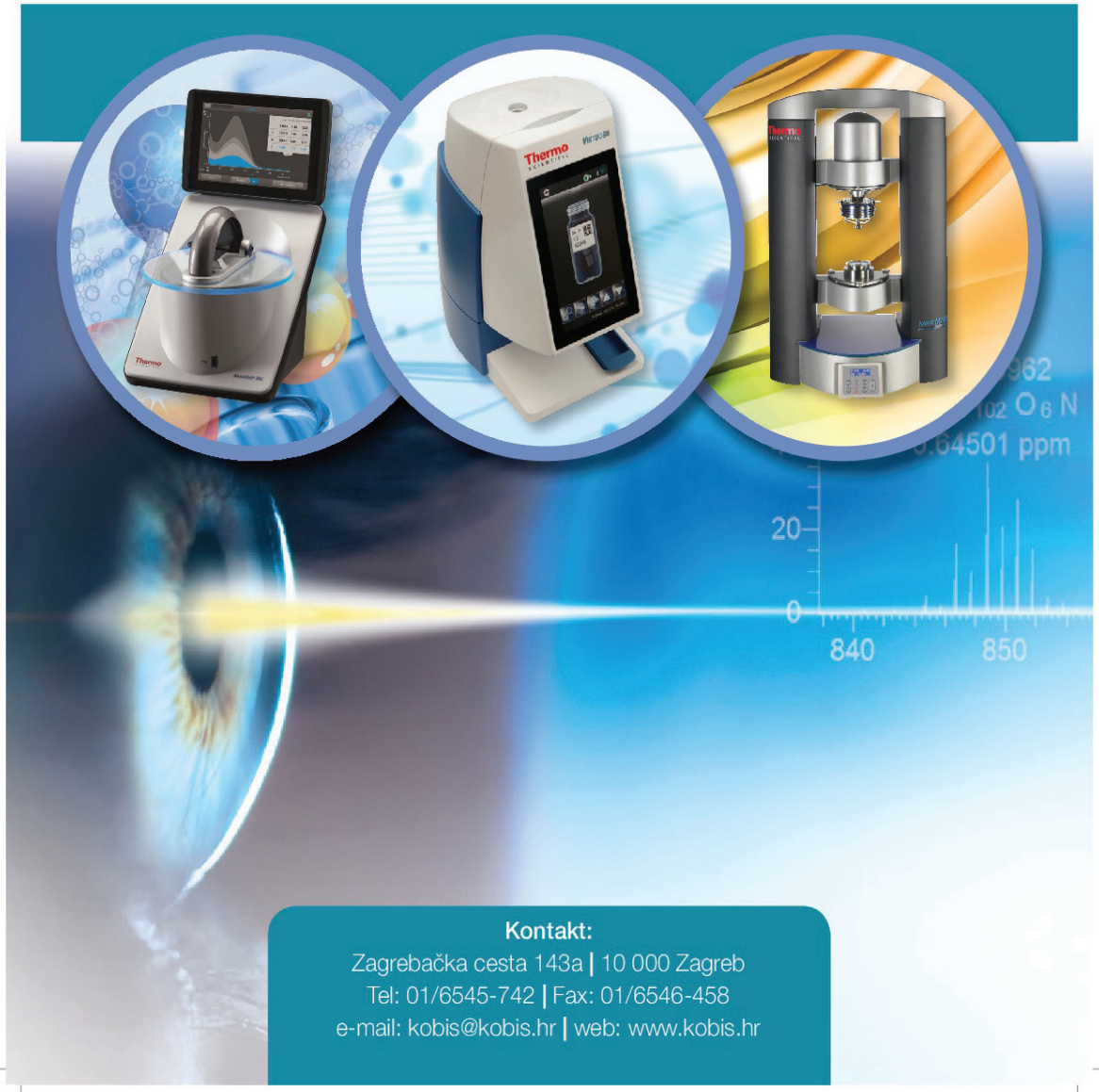

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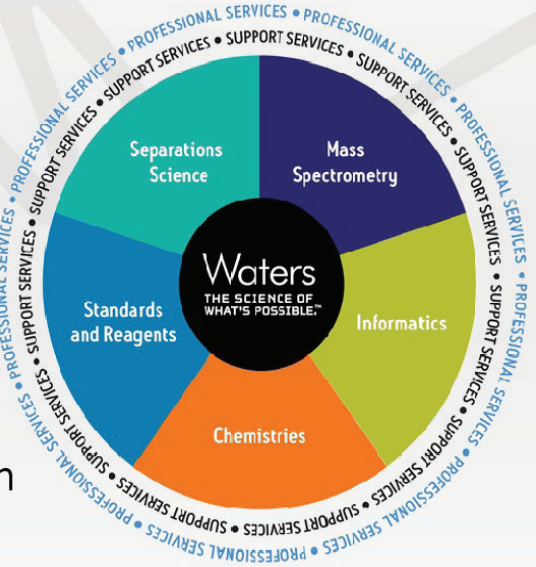

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