ARHIV ZA HIGIJENU RADA I TOKSIKOLOGIJU

ARCHIVES OF INDUSTRIAL HYGIENE AND TOXICOLOGY

2012;63(Suppl. 2)

Abstracts of the 4th Croatian Congress of Toxicology (CROTOX 2012)

Primošten, Croatia 2nd to 5th October 2012

GUEST EDITOR
Davor Želježić

MANUSCRIPT AND COPYEDITOR
Željana Pavlaković

TECHNICAL EDITOR
Marin Mladinić

Cover page: The cover shows a boat of local fisherman anchored near Primošten.
Photographed by Dubravka Rašić.
Disclaimer: This illustration is intended to evoke the content of this issue of the journal. It is not intended for instructional or scientific purposes.

Contents

HRVATSKO TOKSIKOLOŠKO DRUŠTVO
CROATIAN TOXICOLOGICAL SOCIETY

CROTOX 2012

4th Croatian Congress of Toxicology
with international participation
October 2-5, Primošten, Croatia

Abstracts of the 4th Croatian Congress of Toxicology (CROTOX 2012)

Under the Auspices of:

Institute for Medical Research and Occupational Health

Croatian Institute for Toxicology and Antidoping

Supported by:

ministarstvo znanosti obrazovanja i sporta

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

EDITORIAL

The present Supplement to *The Archives of Industrial Hygiene and Toxicology* brings the abstracts* of the invited, oral and poster presentations delivered at the 4th Croatian Congress of Toxicology with the international participation, held on 2nd to 5th October 2012 in Primošten, Croatia. We are proud to be able to give an equal opportunity to all the participating authors to present results of their scientific and professional work on equal footing, and thus make it available to a broad community of experts, but also to all enthusiasts in the field of toxicology.

We are honoured to be in the position to bring to the readers abstracts presenting the most recent scientific achievements of recognized experts in various fields of toxicology and the state-of-the art knowledge in the field of their expertise. Their willingness to contribute to the 4th Croatian Congress of Toxicology is highly appreciated and it represents an important recognition of the toxicological research in Croatia and the international scientific community. At the same time, it represents a strong motivation for efficiently answering the needs of current trends in toxicology worldwide.

Since the very beginning of the organised toxicological research in Croatia in the 1960s, toxicologists have been continuously confronted with new challenges. Rapidly growing trends in the synthesis of novel substances and their introduction into the environment require continuous work on improving methods able to quantify their presence in the environment and human organism, finding novel biomarkers of exposure to them as well as their effect, but also evaluating and defining biomarkers of susceptibility toward chemical and physical agents, most of which have a heritable nature. Beside factors such as gender and life-style, in the last two decades gene polymorphism has been recognized as a biomarker of extreme importance in the assessment of health risks and susceptibility to specific malignancies, as well as fertility disorders due to the exposure to environmental toxicants.

Starting in 2000, nanotechnology became unavoidable in every-day modern life. Most concerning aspect of its application is presence of artificially synthesised nanoparticles in significant number of medical healthcare and consumer products without reaching univocal decision at regulatory level regarding the potential hazard their use might represent. The issue has been addressed by authors of several abstracts presented in this very Supplement to *Archives of Industrial Hygiene and Toxicology*. Being in accordance with globally issued Nanomaterial Research Strategy they represent valuable contribution to still young area of toxicology - nanotoxicology.

Recent knowledge on the ability of metals and metalloids to interfere with the crucial biochemical and homeostasis regulatory pathways within the organism indicates the extreme significance of research aiming to deduce exact mechanisms of their toxicity. Up-to-date knowledge regarding their ability to act as endocrine disruptors and impose severe health risks was presented in several abstracts of the *Metals and metalloids toxicology* sections of the Congress.

Besides a rapid growth in number of potentially fatal anthropogenic toxicants, the introduction of novel psychoactive substances to the black market pose challenges to forensic, clinical, and regulatory toxicologists in the attempt to efficiently suppress their distribution among the most vulnerable social groups. Recent data on their presence and impact on the society are addressed in the presented abstracts and might be useful to clinicians dealing with drugs abuse issues, as well as to family medicine specialists as a guide for recognizing symptoms of addiction.

The official national poisoning control centres in the region in their abstract express concern regarding the management of poisoning data basis at the state level in accordance with the up-to-date requirements. The maintenance of detailed poisoning data bases is extremely important for indentifying hot-spots in the national policies for raising public awareness regarding the implementation of safety measures and safety procedures, and alerting protocols in accidental situations. It also helps in identifying the need for amending existing regulations regarding the placement of chemical products on the market and chemical safety in general.

Together with some other countries, Croatia is a country oriented toward tourism, proud of its food-producing capacities and representing one of the exquisite European gastronomic destinations, aiming to fulfil expectations of the most demanding gourmands, visitors willing to enjoy local gastronomic specialties as well as gastronomade. Respective number of scientific and professional reports presented in sections *Food toxicology* testifies about concerns and efforts

* All abstracts have been peer-reviewed by members of Scientific Committee and external reviewers.

EDITORIAL
Arh Hig Rada Toksikol 2012;63(Suppl. 2):5-6

of Croatian toxicologists to ensure the highest quality of food stuff and other consumer products present on the Croatian market in accordance with the national, but also EU regulations concerning quality requirements.

Exposure to such a broad spectrum of potentially hazardous agents of chemical, biological and physical nature, demands a multidisciplinary approach for revealing exact mechanisms and effects of their mutual interaction with the biological systems. We are proud that issues regarding the evaluation of levels and effects of mixed exposure, as one of the most important and up-to-date topics in the modern toxicology have also been addressed in the presented abstracts.

The present Croatian Congress of Toxicology with the international participation makes a small but significant part of a series of national toxicological conferences organised within the family of the European Toxicological Society and, more globally, the International Toxicological Society.

Finally, I would like to give a special thanks to the Editor in Chief, Assistant Editors, and Editorial Boards of the *Archives of Industrial Hygiene and Toxicology* for giving us the opportunity to present a broad spectrum of regionally ongoing toxicological research and professional work in this very Supplement. This generous act enabled fulfilment of broader goal of the Congress, which is to share knowledge and appreciation of toxicology from its basic scientific principles to a directly applicable knowledge as one of the leading scientific fields in ensuring a healthy living environment. Furthermore, in this way the principle of communicating results of the scientific and professional work to a broader public incorporated in the national and EU scientific strategies has been met.

I hope this event will significantly contribute to the further development of the toxicological research in the Republic of Croatia and the surrounding region.

Guest editor

Davor Želježić

WELCOME ADDRESS

Dear Friends and Colleagues,

On behalf of CROTOX 2012 and Croatian Toxicological Society, it is my privilege and honour to welcome you at the 4th Croatian Congress of Toxicology, in the charming town Primošten.

The Organising Committee has made every effort to make this congress innovative and exciting, with scientific presentations covering a wide range of topics that represent the latest scientific and regulatory developments in the field of toxicology.

At this Congress you will have the opportunity to hear lectures of invited speakers and oral presentations of our colleagues who kindly accepted our invitation to present you their work. The Organising Committee has decided that the presentations of five young scientists, the winners of the Congress Award for Young Scientists will also be included in the programme. We have also organised the poster section where you will have the possibility to meet the authors of the posters and gain a better insight into their work. This will certainly open up new ideas for your future work.

We are sure that you will find some time to see the exhibitors who will introduce you to the products that may be useful in your work.

This Abstract Book includes invited lectures, short lectures, presentations of the winners of Young Scientists' Award, and the poster section.

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 2012, Primošten, Croatia, October 2-5

Programme

Tuesday, 2 October 2012 (Day 1)		
13:00 – 17:15	Registration of participants	
17:30 – 18:00	**Opening Ceremony**	
18:00 – 18:45	**Plenary lecture**	**K. Savolainen** (Helsinki, Finland) Safety of engineered nanoparticles - knowledge gaps and research priorities
19:00	**Welcome Reception**	

Wednesday, 3 October 2012 (Day 2)		
8:00 – 8:45	**Posters will be put up by presenters**	
SESSION: ECOTOXICOLOGY **Chaired by:** E. Srebočan, N. Bihari		
9:00 – 9:45	**IL – 1**	**C. Bolognesi** (Genoa, Italy) Mussel micronucleus cytome assay: an approach to evaluate cytotoxic and genotoxic polutants in aquatic environment
9:45 – 10:30	**IL – 2**	**U. Kierdorf** (Hildesheim, Germany) Wild large mammals as pollution indicators
10:30 – 10:45	**OP – 1**	**M. Fafanđel** (Rovinj, Croatia) Ecotoxicological analysis of Rječina River and Bakar Bay sediment
10:45 – 11:00	**OP – 2**	**H. Lutnicka** (Krakow, Poland) Ultrastructural changes in fish gills as a symptom of water pollution by low concentrations of fungicides
11:00 – 11:30	**Coffee break**	
SESSION: TOXICOLOGY OF METALS AND NANOTOXICIOLOGY **Chaired by:** J. Jurasović, S. Ćavar		
11:30 – 12:15	**IL – 3**	**W. Goessler** (Graz, Austria) The usefulness of ICPMS in metal toxicology research
12:15 – 12:30	**OP – 3**	**P. Burić** (Rovinj, Croatia) Uptake and impact of engineered nanoparticles on embrional development and stress response in selected marine organisms
12:30 – 12:45	**YSL – 1**	**G. Kiliç** (A Coruña, Spain) Cytotoxic and genotoxic effect of titanium dioxide nanoparticles on human neuronal cells
12:45 – 13:00	**YSL – 2**	**B. Tariba** (Zagreb, Croatia) Influence of platinum and zinc on activity of superoxide dismutase in human erythrocytes *in vitro*
13:05 – 14:30	**Lunch break**	
SESSION: TOXICOLOGY OF METALS AND NANOTOXICIOLOGY **Chaired by:** J. Franekić, D. Želježić		
14:30– 15:15	**IL – 4**	**I. Sabolić** (Zagreb, Croatia) Membrane transporters of organic compounds in experimental cadmium nephrotoxicity
15:15 – 15:30	**YSL – 3**	**A. Buha** (Belgrade, Serbia) Prooxidative-antioxidant balance as a new parameter in investigation of cadmium - induced oxidative stress

15:30 – 15:45	**YSL – 4**	**N. Rajević** (Zagreb, Croatia) The effect of aluminium and silicon on the planarian *Polycelis felina* (Daly.)
15:45 – 17:00	**Poster Viewing**	
SESSION: METHODS IN TOXICOLOGY **Chaired by:** V. Garaj Vrhovac, D. Sutlović		
17:00 – 17:45	**IL – 5**	**A. Collins** (Oslo, Norway) Applications of the comet assay to measure DNA damage and repair
17:45 – 18:30	**IL – 6**	**M. Sollner** Dolenc (Ljubljana, Slovenia) Bisphenol A and its analogs - new aspects of their biological activity
18:30 – 18:45	**OP – 4**	**E. Coskun** (Ankara, Turkey) Androgen receptor (CAG)n repeat lengths and sperm chromatin integrity: possible risk factors for male infertility?
18:45 – 19:00	**OP – 5**	**S. H. Afifi** (Assiut, Egypt) Light and transmission electron microscopical observations on rat sciatic nerve induced by electrocution

Thursday, 4 October 2012 (Day 3)		
SESSION: FOOD TOXICOLOGY **Chaired by:** M. Peraica, D. Puntarić		
8:30 – 9:15	**IL – 7**	**M. F. Dutton** (Johannesburg, South Africa) Mycotoxins in South African foods: a case study on aflatoxin M1 in milk
9:15 – 10:00	**IL – 8**	**A. Ritieni** (Naples, Italy) Toxicity of fumonisin family and related risks for consumers
10:00 – 10:15	**OP – 6**	**A.-M. Domijan** (Zagreb, Croatia) Deregulation of calcium signalling in fumonisin B1 neurotoxiciy
10:15 – 10:30	**YSL – 5**	**M. Sertić** (Zagreb, Croatia) A new HPLC/DAD/FLD/MS^n method for quantification of lovastatin and citrinin in food and various red yeast rice products
10:30 – 11:00	**Coffee break**	
SESSION: SUBSTANCES OF ABUSE AND ANTIDOTES **Chaired by:** M. Čoklo, Z. Kovarik		
11: 00 – 11:45	**IL – 9**	**A. G. Verstaete** (Ghent, Belgium) Driving under the influence of drugs, the lessons of the DRUID project
11:45 – 12:30	**IL – 10**	**V. Matović** (Belgrade, Serbia) Psychoactive controlled substances - situation in Serbia
12:30 – 12:45	**OP – 7**	**Z. Kovarik** (Zagreb, Croatia) New scarfolds of oxime-assisted acetylcholinesterase reactivators for treatment in tabun exposure
12:45 – 13:15	**Sponsor's presentation**	**John Hopkins** (Manchester, United Kingdom) Mass spectrometric methods in forensic toxicology
13:15 – 14:30	**Lunch break**	
15:00 – 22:00	**Excursion with congress dinner**	

Friday, 5 October 2012 (Day 4)		
8:00 – 9:00	Posters will be taken down by presenters	
SESSION: REGULATORY TOXICOLOGY		
Chaired by: F. Plavšić, R. Turk		
9:00 – 9:45	**IL – 11**	**Z. Lovrić** (Zagreb, Croatia) Hazard and risk assessment in chemical laboratory
9:45 – 10:30	**IL – 12**	**R. Turk** (Zagreb, Croatia) Exposure assessment and human health risk assessment in the process of registration of plant protection products and biocides
10:30 – 10:45	**OP – 8**	**Z. Franić** (Zagreb, Croatia) Accrediation of medical, biomedical, animal diagnostic laboratories and animal production units
10:45 – 11:00	**OP – 9**	**I. Prlić** (Zagreb, Croatia) Radiation protection in a mixed contaminant context, risk assessment methodologies
11:00 – 11:30	**Closing lecture**	**F. Plavšić** (Zagreb, Croatia) Croatian toxicology
11:30	**Closing Ceremony**	

Abstracts

Pl-1

SAFETY OF ENGINEERED NANOPARTICLES - KNOWLEDGE GAPS AND RESEARCH PRIORITIES

Kai SAVOLAINEN

Finnish Institute of Occupational Health, Helsinki, Finland

Exposure to engineered nanomaterials (ENM) increases rapidly. Knowledge of health effects of, and exposure to, ENM in workplaces is though minimal. Hence, reliable assessment of health risks of ENM is hardly possible. Risk assessment using animal models is laborious. Remarkable challenges related to the safe use of ENM and nanotechnologies are associated with our ability to detect ENM from ubiquitous nanosized particles and distinguish between hazardous ENM or nanotechnology applications and safe ones. Omics methodologies, bioinformatics, and systems biology approaches are all tempting, provided that one can correlate the results with health-related endpoints. They may be a powerful tool for occupational hazard assessment, and enhance the assessment of workplace ENM risks when exposure data are available.
Data presented at the Congress are obtained within NANODEVICE Project (Grant CP-IP-211464-2) supported by the European Union 7th Framework Program.

KEY WORDS: *nanomaterials, occupational hazard, risk assessment*

I-1

MUSSEL MICRONUCLEUS CYTOME ASSAY: AN APPROACH TO EVALUATE CYTOTOXIC AND GENOTOXIC POLLUTANTS IN AQUATIC ENVIRONMENT

Claudia BOLOGNESI

National Cancer Research Institute, Genoa, Italy

Micronucleus (MN) test as an index of accumulated genetic damage during the lifespan of the cells is one of the most suitable techniques to identify integrated response to the complex mixture of contaminants. MN assay is today widely applied in a large number of wild and transplanted aquatic species. Bivalves are considered the ideal bioindicators for monitoring aquatic contaminants in coastal waters due to their wide geographic distribution, easy sampling, bioconcentration of a wide range of chemicals and resistance to stress. Mussels can be easily caged allowing the study of areas where they are not naturally present and reducing the influence of genetic and adaptive phenomena impairing the comparison among the animals from different stations. The development of a standardized protocol in haemocytes and gill cells of mussels started when the MN assay was proposed as a core genotoxicity biomarker in large-scale marine pollution programs. This protocol was further validated in biomonitoring studies in coastal areas using wild and caged mussels and in monitoring the long term impact of an oil spill accident, using mussels and oysters as bioindicators with different feeding habitats. The experimental protocol for MN assay was further refined and updated to include also the evaluation of other nuclear alterations, such as nuclear buds, apoptotic and necrotic cells, following the cytome approach already applied in mammalian cells. The use of a standardized protocol, allowing proper intra- and inter-laboratory comparison, is essential in field biomonitoring studies within and across the countries.

KEY WORDS: *accumulated genetic damage, biomonitoring studies, bivalves, ecogenotoxicology*

I-2

WILD LARGE MAMMALS AS POLLUTION INDICATORS

Uwe KIERDORF

University of Hildesheim, Hildesheim, Germany

Wild mammals and other wildlife species are widely used to study the occurrence of contaminants in ecosystems and the movements of these contaminants through food chains. In surveys and monitoring studies, tissue concentrations of contaminants provide information on their geographical distribution and changes in environmental levels through time. In addition, contaminant-induced physiological and/or morphological changes in organisms can be used as biomarkers of exposure. For a meaningful interpretation of tissue concentrations and the effects of contaminants on wildlife, a thorough knowledge of the biology of the studied species is required. This presentation focuses on the use of large wild mammals as biological indicators of lead and fluoride pollution. The concentrations of the two contaminants in mineralized tissues of different deer species and other mammalian taxa have been utilized to study regional differences in pollution and for establishing time trends of environmental contamination in a given area. Because they are periodically replaced and form during a species-specific seasonal growth period, the antlers of deer constitute naturally standardized monitoring units that offer a special opportunity for a historical monitoring of environmental levels of bone-seeking contaminants. Over the last decades, dental fluorosis has been established as a biomarker of fluoride toxicosis from either natural or anthropogenic sources in various mammalian species. The studies span the range from a detailed analysis of the structural effects of excess fluoride exposure at the dental tissue level to the monitoring of temporal changes in the frequency of dental fluorosis in exposed populations.

KEY WORDS: *biomarkers of exposure, fluoride toxicosis, lead, mammalian tissue*

I-3

THE USEFULNESS OF *ICPMS* IN METAL TOXICOLOGY RESEARCH

Walter GOESSLER

Institute for Analytical Chemistry, Karl Franzens University Graz, Graz, Austria

Since its introduction around 25 years ago inducitively coupled plasma mass spectrometry (ICPMS) became a valuable tool for trace and ultra-trace element analysis. Nowadays the instruments are improved with respect to stability so that ICPMS is a routine technique in many laboratories and replaced already atomic absorption spectrometry. The reason for its success are certainly the multielement capabilities, the wide dynamic range, and the excellent detection limits. Additionally, ICPMS offers the possibility to determine the isotopic composition of a sample which offers the possibility for tracer studies. The inductively coupled plasma is currently the most efficient ionization source. Especially when accurate quantitative results are needed a robust ionization source is superior to molecule-selective detection with electrospray ionization. Compound independent quantification should be mentioned as another benefit of ICPMS. Besides its use for the determination of trace and major elements in various samples, ICPMS is one of the most popular element-selective detectors for any chromatography as it is easily coupled to LC, GC or CE. The presentation discusses the importance of modern instrumental techniques for metabolism studies and focusses on arsenic which occurs in many different compounds of varying toxicity in our environment.

KEY WORDS: *arsenic, inductively coupled plasma mass spectrometry, metabolism, trace and ultra trace element analysis*

I-4

MEMBRANE TRANSPORTERS OF ORGANIC COMPOUNDS IN EXPERIMENTAL CADMIUM NEPHROTOXICITY

Ivan SABOLIĆ[1], Davorka BRELJAK[1], Marija LJUBOJEVIĆ[1], Carol M. HERAK-KRAMBERGER[1], Naohiko ANZAI[2], and Hermann KOEPSELL[3]

Institute for Medical Research and Occupational Health, Zagreb, Croatia[1], Dokkyo Medical University, Mibu, Tochigi, Japan[2], University of Würzburg, Würzburg, Germany[3]

Cadmium nephrotoxicity (Cd-NTX) is manifested by impaired reabsorptive and secretory functions of proximal tubules (PT). The symptoms, that include phosphaturia, proteinuria, aminoaciduria, glucosuria, increased excretion of inorganic and organic anions and cations, and polyuria, indicate that Cd targets specific transporters in the PT brush-border (BBM) and basolateral (BLM) membrane. Using subchronic (treatment with $CdCl_2$ for 14 days) and acute (treatment with Cd-metallothionein 6-12 h before sacrifice) experimental models of Cd-NTX in rats, we have studied the expression of representative transporters in the PT cell membranes. Various methods (immunocytochemistry, Western blotting, transmission and immunogold microscopy, end-point RT-PCR) have been applied to characterise the expression of transporters that reside in the PT BBM (NaPi2, V-ATPase, NHE3, SGLT1, SGLT2), BLM (Na/K-ATPase, OAT1, OAT3, OCT1, OCT2), or in both membranes (AQP1). In both models, PT exhibited the loss of BBM and BLM. In the subchronic model, expression of transporters was downregulated at the level of protein and mRNA. In the acute model, we observed a) time-dependent loss of the BBM and BLM transporters, and their accumulation in intracellular vesicles, and b) translocation of NHE3 from BBM to BLM. The data indicate that functional defects of PT in Cd-NTX result from a) loss of absorptive and secretory surface, b) loss of transporting proteins in BBM and BLM, and c) loss of cell polarity. In subchronic Cd-NTX, the loss of membrane transporters seems to be mRNA-related, whereas in acute Cd-NTX, the loss of membrane transporters may result from the disrupted intracellular vesicle sorting and trafficking.

KEY WORDS: *end-point RT-PCR, heavy metal, immunocytochemistry, kidney, rats, transmission and immunogold microscopy, Western blotting*

I-5

APPLICATIONS OF THE COMET ASSAY TO MEASURE DNA DAMAGE AND REPAIR

Andrew R. COLLINS[1], Amaya AZQUETA[1,2], Sabine LANGIE[1,3], and Jana SLYSKOVA[1,4]

University of Oslo, Oslo, Norway[1], University of Navarra, Pamplona, Spain[2], Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne, United Kingdom[3], Institute of Experimental Medicine, Prague, Czech Republic[4]

The comet assay is popular because of its simplicity and sensitivity, and is widely applied in genotoxicity testing, human biomonitoring, ecogenotoxicology, and basic research into DNA damage and repair. In its standard form, it has limitations: it detects strand breaks, while altered bases are probably more important lesions; the number of samples per experiment is limited by the number of slides that will fit in an electrophoresis tank; and inter-laboratory variation is substantial. High throughput versions of the assay permitting analysis of hundreds of samples in one experiment were developed in the recent European project, COMICS, and since then we have continued with attempts to make the assay both more reliable and more sensitive. The latter improvement involves the use of lesion-specific endonucleases that recognise oxidised, alkylated or otherwise altered bases. If adopted for testing novel chemicals for genotoxicity, this modified assay would diminish the frequency of 'false negative' results. The comet assay can be applied to the measurement of DNA repair, in two ways. In the 'challenge assay', cells are treated with a damaging agent, incubated, and samples analysed at intervals to follow the removal of the damage. In an alternative *in vitro* assay, a cell extract is incubated with a DNA substrate containing specific lesions and the initial event of repair, incision, is measured. The assays have been applied mainly to peripheral blood lymphocytes and cells in culture. We have recently modified the *in vitro* assay for use with extracts from animal and human solid tissues.

KEY WORDS: *chemical testing, genotoxicity, lesion-specific endonucleases, novel improvements, solid tissue extracts*

I-6

BISPHENOL A AND ITS ANALOGS - NEW ASPECTS OF THEIR BIOLOGICAL ACTIVITY

Marija SOLLNER DOLENC[1], Katra KOLŠEK[1], Janez MAVRI[2,3], Urban ŠVAJGER[4], and Matjaž JERAS[1]

University of Ljubljana[1], National Institute of Chemistry[2], EN-FIST Centre of Excellence[3], Blood Transfusion Centre of Slovenia[4], Ljubljana, Slovenia

Xenoestrogen bisphenol A (BPA) is a component of polycarbonate plastics and also found in various consumers product from reusable drink bottles to cell phones. It is an important component of epoxy resins lining the insides of food and drink cans. Chronic exposure to bisphenol A from food, drink and other sources is the reason that this chemical is detected at low levels in the urine of nearly everyone. A large number of studies on the toxicity and hormonal activity of BPA in laboratory animals have been published. Considerable discrepancies in outcome among these studies with respect to both the nature of the effects observed as well as the levels at which they occur have been reported. Bisphenol A is a known endocrine disruptor but it has other effects, including genotoxicity and immunotoxicity. To investigate the possible genotoxic mechanisms of bisphenol A we evaluated the chemical reactivity of the bisphenol A metabolite bisphenol A-3,4-quinone with deoxyguanosine by using density functional theory in conjunction with Langevin dipoles solvation model. The influence on adhesion of bisphenol A and its analogs was measured on HUVEC cell model. Some preliminary data indicated the interactions BPA and its analogs with dendritic cells and so confirmed their possible modulation of the immune system.

KEY WORDS: *chronic exposure, density functional theory, genotoxicity, Langevin dipoles salvation model, polycarbonate plastic, xenoestrogens*

I-7

MYCOTOXINS IN SOUTH AFRICAN FOODS: A CASE STUDY ON AFLATOXIN M_1 IN MILK

Michael F. DUTTON

University of Johannesburg, Johannesburg, South Africa

Mycotoxins are toxic products formed by filamentous fungi. They pose a problem in food if the food has been infected with a fungus which then produced its toxic product. As the infection can occur at many points in the food production chain, e.g., in the field or in storage, then it is very difficult to decide if a processed food product is contaminated and if it is, where the contamination arose from. In this case the contamination of milk with aflatoxin M_1 was considered. Here the mycotoxin is ingested by the milk producing animal as aflatoxin B_1, which is then metabolically converted to a hydroxy derivative in the animal, called aflatoxin M_1. Samples of feeds, forage, maize and milk were taken at the dairy farms supplying milk to the dairy whose milk was being evaluated. Samples of milk from various parts of the dairy were taken in the same time period as the supplying farm samples. The feeds are to be screened for fungi and mycotoxins and the milk samples for aflatoxin M_1. The milk from the farms supplying the dairy was positive for aflatoxin M_1 ranging from <1 ppb to 10 ppb by the Vicam milk assay and <1 ppb to 1.5 ppb by high-performance liquid chromatography (HPLC). Although this does not seem high, it is above regulated levels of several countries. Work is now continuing to discover the source of the parent aflatoxin, aflatoxin B_1.

KEY WORDS: *bio-tracing, dairy production, food chain, fungal metabolites, high performance liquid chromatography*

I-8

TOXICITY OF FUMONISIN FAMILY AND RELATED RISKS FOR CONSUMERS

Alberto RITIENI

Faculty of Pharmacy, University of Napoli Federico II, Napoli, Italy

Fumonisins are a well-known family of toxic and carcinogenic mycotoxins produced by *Fusarium verticillioides* that is a common fungal contaminant of many food based on corn. Contamination with fumonisin B1 (FB1) causes various animal diseases but the first barrier is the gastrointestinal tract that is exposed to the fumonisins. Consequently, the ingestion of fumonisin-contaminated food could expose the intestinal epithelial cells to a high concentration of toxin. FB1 is poorly absorbed and metabolized in the intestine, and it induces abdominal pain or diarrhoea and causes extra-intestinal organ pathologies such as pulmonary edema, leukoencephalomalacia, or neural tube defects. In literature, the main toxicological effect of FB1 reported is the accumulation of sphingoid bases associated with the depletion of complex sphingolipids. The interference on sphingolipid biosynthesis pathway is at the basis of observed toxicological effects such as an alteration in intestinal epithelial cell viability and proliferation, a modification of cytokine production, and a modulation of intestinal physical barrier function. One of the less investigated syndromes is the irritable bowel syndrome (IBS) widespread in adults and adolescents with considerable costs in economic and social terms. IBS is a multifactorial syndrome that triggers a series of morphological and metabolic changes of the dependent intestinal membrane by altering its operation and its structure. Junk food chain is a possible way to entrance for fumonisins that have toxic action in the enteric nervous system in analogy with the central nervous system.

KEY WORDS: *food toxicity, irritable bowel syndrome, mycotoxins, nervous system, toxic effects*

I-9

DRIVING UNDER THE INFLUENCE OF DRUGS, THE LESSONS OF THE *DRUID* PROJECT

Alain G. VERSTRAETE

Ghent University Hospital, Ghent University, Ghent, Belgium

The DRUID project, performed by 37 institutes from 17 EU Member States and Norway, studied driving under the influence of drugs, alcohol and medicines in Europe between 2006 and 2011. Experimental studies were carried out with administration of medicinal and illicit drugs. In the EU roadside survey 3.48 % (weighted EU average) of the drivers were positive for alcohol alone (>0.1 g L^{-1}), 1.90 % for illicit drugs, 1.36 % for medicinal drugs and 0.76 % for combinations. The percentage of drug and alcohol positive injured drivers varied from 28 % to 53 % and killed drivers from 30 % to 51%. The relative risk of serious injury was highest (20-200 times) for alcohol >1.2 g L^{-1} and combinations of alcohol and drugs and lowest (1-3 times) for cannabis and alcohol 0.1 g L^{-1} to 0.5 g L^{-1}. A categorisation system for medicines according to their influence on driving was made for more than 1500 drugs. Europe-wide standards and recommendations of good practice for DUI/DUID rehabilitation measures were developed for implementation, assessment or evaluation of rehabilitation programs. Recommendations on license withdrawal for the general driving population and specific problem groups such as DUI/DUID drivers, patients in substitution or other long-term treatment with psychoactive medicines were developed. The emphasis of risk communication towards young people should be given to drink driving prevention, targeting the age group 15-24 years. Preventive measures should be differentiated into general preventive approaches and special focused preventive measures for certain smaller subgroups. The results of the project are described in 50 deliverables that are available on *www.druid-project.eu*.

KEY WORDS: *alcohol, driving accidents, drugs of abuse, medicines, prevention*

I-10

PSYCHOACTIVE CONTROLLED SUBSTANCES - SITUATION IN SERBIA

Vesna MATOVIĆ

Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

There is widespread recognition that drugs of abuse jeopardize security and development of the world. Globally, some 210 million people use illicit drugs each year, and almost 200,000 of them die. Epidemiological studies carried out in Serbia during 2006 on 15,563 adults estimated that nearly 11 % of the population aged 20-59 years used marijuana and about 2 % ecstasy, while cocaine, inhalants, amphetamine and LSD were present in lower extent (1.3 %, 1.2 %, 0.9 % and 0.7 %, respectively). Among 6,553 secondary school first graders which participated in the survey conducted in 2008, 15.1 % used one of psychoactive controlled substances, at least once during lifetime with no significant differences in the use of illicit drugs between boys and girls, with prevalence of sedatives (7.6 %) and marihuana (6.7 %). About 3 % of study participants started to smoke marihuana before age of nine. In 2011 it was estimated that there are around 24,000 intravenous heroin addicts in Serbia (approximately one third in Belgrade) and several hundred thousand addicts to other drug types. Despite the efforts of Ministry of Internal Affairs of Serbia all drugs are currently available in Serbia though the price of cocaine still makes this addiction rather rare. We hope that National Drug Strategy adopted in February 2009, as well as new Law on Chemical Precursors and Law on Controlled Psychoactive Substance together with recently formed Commission for psychoactive controlled substances will contribute to the reduction of drugs of abuse. (Partly supported by Ministry of Science and Environmental Protection, Republic of Serbia, Grant No. III 460009)

KEY WORDS: *addiction, adolescents, drugs of abuse, legislation, prevention, statistical data*

I-11

HAZARD AND RISK ASSESSMENT IN CHEMICAL LABORATORY

Zdravko LOVRIĆ

Croatian Institute for Toxicology and Antidoping, Zagreb, Croatia

The importance of laboratory safety has been recognized for many years in industry. However, scientific and educational institutions have been slower to adopt such safety practices and programs. Chemical pollution caused by inappropriate conditions in laboratory and inappropriate handling with chemicals creates human illnesses preventing people from developing to their full potential. A healthy environment is crucial to achieve sustainable working conditions. Preventing pollution at the source, rather than cleaning it up at the end, is particularly good way to avoid toxic chemical problems. Unfortunately, chemical safety is very often neglected in chemical laboratory. Although there are small quantities of chemicals, the number of them is huge. This is the reason why incompatible chemicals very often come in contact, especially in storages and during collection of waste. It is important to include pollution prevention in mainstream capacity building services to laboratories. There are, also promising areas for collaborating among laboratories in creative arrangements such as sharing risk reduction information. The very important thing is to have appropriate tool to guides laboratory staff through the process of identifying hazards associated with a specific laboratory process or an entire laboratory and then helps to assign a numerical risk to the process or laboratory. The results of the risk assessment can then be used to identify specific hazards or controls which can be addressed to reduce risks.

KEY WORDS: *chemical safety, laboratory pollution prevention, sustainable working conditions*

I-12

EXPOSURE ASSESSMENT AND HUMAN HEALTH RISK ASSESSMENT IN THE PROCESS OF REGISTRATION OF PLANT PROTECTION PRODUCTS AND BIOCIDES

Rajka TURK

Poison Control Centre, Institute for Medical Research and Occupational Health, Zagreb, Croatia

Exposure of different population groups to agricultural pesticides and biocides is a major factor considered by the registration authorities in the European Union. Several calculation models are used to estimate the amount of exposure for various situations including occupational, but also non-dietary consumer and resident exposure during and after application. The predicted exposure is then compared to the acceptable exposure levels based on toxicity study data, to demonstrate the safe use of plant protection products (PPP) and biocidal products for both professional operators and consumers. The effect of pesticides during occupational exposure is mostly due to dermal exposure and methods of assessing dermal absorption used in risk assessments need to be improved. In this review, the preparation of new European guidance documents on PPP exposure assessment and dermal absorption is discussed and compared to biocidal products registration requirements regarding exposure evaluation. The approach taken by the Croatian regulatory authorities is also presented. The overall conclusion is that there is a need to develop a harmonised approach to exposure and risk evaluation of PPP and biocides to ensure the consistency of scientific and regulatory decisions.

KEY WORDS: *agricultural pesticide, biocidal product, dermal absorption, exposure, registration process*

I-13

CROATIAN TOXICOLOGY

Franjo PLAVŠIĆ and Alka WOLF ČOPORDA

Croatian Institute for Toxicology and Antidoping, Zagreb, Croatia

The pioneer days of Croatian toxicology began in the first half of the 20th century in the field of forensic toxicology and the study of chemical weapons. In the sixties, its development was accelerated and covered various fields. The first undergraduate course in toxicology was set up in 1967 and lingered on until today under different names. Furthermore, the sixties saw a rapid development of the toxicology of metals. The seventies, on the other hand, brought about the development of clinical toxicology (chronic and acute exposure), which required clinics and laboratories to be developed. This did not take place until the eighties when various laboratories, both experimental and clinical, were established. The turning point was 1991 when Toxicological Service was founded and most of toxicologists were united working for the country's defence in the Homeland War. Suddenly, the strength and diversity of the themes in toxicology were recognised. The Croatian Toxicological Society was founded and the first scientific postgraduate course of studies in toxicology was launched. After the first congress on the Toxicological Service was held, everything began to move quite intensely. A particular mention must be made of the foundation of undergraduate, postgraduate, and doctoral courses of studies in toxicology at different faculties and high schools, as well as the specialist postgraduate course of studies in analytical toxicology. The education of workers and engineers who work with chemicals was organised. The number of doctoral candidates increased, as did the number of scientific and professional papers of Croatian toxicologists. However, new problems arose from insufficient education on the essential concepts in toxicology. We will elaborate on the problem of misinterpreting laboratory findings, aggressive behaviour of ideological toxicologists who attempt to frighten the citizens and the problems encountered when applying European directives and regulations, which is again the problem of insufficient education at all levels.

KEY WORDS: *review, state of the art, toxicology in Croatia*

O-1

ECOTOXICOLOGICAL ANALYSIS OF RJEČINA RIVER AND BAKAR BAY SEDIMENT

Mirta SMODLAKA TANKOVIĆ[1], Sanja ŽIVKOVIĆ[2], Jasmina PILJAGIĆ[2], and Maja FAFANĐEL[1]

Center for Marine Research, Ruđer Bošković Institute, Rovinj[1], Public Health Institute of Primorje-Gorski Kotar County, Rijeka[2], Croatia

The ecotoxicological analysis is an important issue for the risk assessment of chemical contamination and its potential impact on water quality and biota of receiving marine ecosystems. Marine sediments are often a final sink for numerous anthropogenic contaminants and may impose serious effects on benthic organisms and ecosystem. This study tried to evaluate the environmental quality of the sediments at six sampling sites within the Rječina River and 10 sampling sites within Bakar Bay. Total PAHs content ranged from 28 μg kg^{-1} to 9000 μg kg^{-1} DW, metal pollution index for 10 metals ranged from 12 to 67, and PCB content was up to 48 μg kg^{-1} DW. Sediments from selected sites displayed different dynamics of chemical compounds and potential toxicity. Integrated analysis of investigated parameters showed a significant difference between non-urban, urban (traffic, wastewater outlets), and industrial zones (ex-coke plant, harbour). The sampling sites could be divided into three groups: i) sediments with high pollutant load and low potential toxicity, ii) sediments with the highest potential toxicity and intermediate concentration of pollutants, and iii) the rest of the sampling sites characterised by very low to intermediate pollutant load and site-specific pollutant content and potential toxicity. Sampling sites in groups i) and ii), three sites in Bakar Bay and one site in Rječina estuary, were considered for further pollutant characterisation, biological impact investigation, and corresponding remediation activities.

KEY WORDS: *ecotoxicology, marine sediments, polycyclic aromatic hydrocarbons, polychlorinated biphenyls*

O-2

ULTRASTRUCTURAL CHANGES IN FISH GILLS AS A SYMPTOM OF WATER POLLUTION BY LOW CONCENTRATIONS OF FUNGICIDES

Hanna LUTNICKA[1], Agnieszka LUDWIKOWSKA[1], Anna PECIO[2], and Olga WOŹNICKA[3]

Faculty of Animal Science, University of Agriculture in Krakow[1], Department of Comparative Anatomy[2], Department of Cell Biology and Imaging[3], Institute of Zoology, Jagiellonian University, Krakow, Poland

Fungicides are widely used in agriculture. Their residues can be detected in surface waters, which are the main source of fish culture. They can affect fish organs and their health. Ultrastructural changes in fish gills are very useful for the study of the influence of low concentrations of toxic substances. Carp *Cyprinus carpio* L. (mean weight ± standard deviation): (50±10) g was exposed for 14 days, in aquaria conditions, to three fungicides: mancozeb, prochloraz, and tebuconazole (one group of fish was exposed to one fungicide), at concentrations 0.1 mg L^{-1}, 0.1 mg L^{-1}, and 0.25 mg L^{-1}, respectively. After exposure, fish were transferred to clean water for 30 days for a possible recovery. Gills were sampled after 3 and 14 days of exposure and at the end of the recovery period. Ultrastructural changes were studied by scanning electron microscopy. The experiments showed that all fungicides caused ultrastructural changes in fish gills, especially in the middle part of secondary lamellae. Cells of affected part of lamellae larger in size compared to the controls and cells in unaffected areas. The surface of lamellae surface was flat, either without microridges or the microridges were small in number (one or two), but big in size. Cellular infiltrations were observed between secondary lamellae, especially on their surfaces. These infiltrations resulted from the fusion of the secondary lamellae. Mucous secretion was intense. These changes were more significant at the end of exposure and for prochloraz. The time of recovery was too short for a complete regeneration.

KEY WORDS: *carp, mancozeb, prochloraz, scanning electron microscopy, tebuconazole*

O-3

UPTAKE AND IMPACT OF ENGINEERED NANOPARTICLES ON EMBRYONAL DEVELOPMENT AND STRESS RESPONSE IN SELECTED MARINE ORGANISMS

Petra BURIĆ, Martin PFANNKUCHEN, Željko JAKŠIĆ, Filip STIPIĆ, and Daniel M. LYONS

Centre for Marine Research, Ruđer Bošković Institute, Rovinj, Croatia

Engineered nanoparticles are finding use in an ever increasing range of consumer and industrial applications ranging from cosmetics and clothing to catalysis and soil remediation. However, questions regarding their accumulation and impact on the environment are, somewhat belatedly, only now being posed. Indeed, data on the impact of such nanoparticles on living organisms in aquatic systems is relatively scarce and the details on the transfer of nanoparticles between trophic levels remain essentially unknown. The aim of this work was therefore to investigate the fate of selected engineered nanoparticles and probe their impact on mussels and sea urchins, both *in vivo* and *in vitro*. Here we present preliminary data on the uptake and impact of a range of silver and silica nanoparticles on mussel *Mytilus galloprovincialis*. Mussels displayed a nanoparticle dose-dependent stress response for nanoparticle concentrations in the 1 ppb to 100 ppb range on the level of the entire organism (stress-on-stress test; animal death endpoint), on lysosomal membrane integrity in haemocytes (neutral red leakage from 50 % of cells) and on acetylcholinesterase activity in gills. Nanoparticle uptake in gills has been found to be relatively rapid with accumulation of nanoparticles in cells and apparent contact with the nucleus. Further, data on the retardation of embryonic development of *Arbacia lixula* and *Paracentrotus lividus* (sea urchin embryo development test) by these nanoparticles at concentrations >50 ppb are also presented.

KEY WORDS: *mussels, sea urchin embryo development, silica and silver nanoparticles, uptake in gills*

O-4

CYTOTOXIC AND GENOTOXIC EFFECTS OF TITANIUM DIOXIDE NANOPARTICLES ON HUMAN NEURONAL CELLS

Gözde KILIÇ[1,2], Vanessa VALDIGLESIAS[1,3], Carla COSTA[4], Vyom SHARMA[5], Eduardo PÁSARO[1], Alok DHAWAN[5], Joao Paulo TEIXEIRA[4], and Blanca LAFFON[1]

Department of Psychobiology[1], Department of Cell and Molecular Biology[2], University of A Coruña, A Coruña, Spain, IRCCS San Raffaele Pisana, Roma, Italy[3], National Institute of Health, Porto, Portugal[4], Indian Institute of Toxicology Research, Lucknow, India[5]

Rapid development of nanotechnology is accompanied by promises of substantial benefits that will have significant economic and scientific impacts. But there are also increasing concerns on the potential adverse effects that the production and subsequent exposure to nanoparticles (NPs) might pose on human health. Titanium dioxide (TiO_2) NPs are among the most frequently used; they are present in a variety of consumer products, including food industry in which they are employed as an additive. The potential toxic effects of these NPs on mammalian cells have been extensively studied in the last years. However, studies regarding the potential neurotoxicity and specific effects on neuronal systems are very scarce, and no studies on human neuronal cells have been reported so far. Thus, the main objective of this work was to investigate the effects of two types of TiO_2 NPs on human SHSY5Y neuronal cells. After NP characterisation, a battery of assays was performed in order to evaluate the viability, cytotoxicity, genotoxicity, and oxidative damage in TiO_2 NP-exposed SHSY5Y cells. Results showed similar behaviour for both types of NPs; they did not reduce the viability of neuronal cells but were effectively internalised by the cells and were found to induce dose-dependent cell cycle alterations, apoptosis by intrinsic pathway, and genotoxicity not related with double strand break production. Furthermore, all these effects were not associated with oxidative damage production and, consequently, further investigations are required to elucidate the specific mechanisms underlying the genotoxic and cytotoxic effects observed.

KEY WORDS: *apoptosis, cell cycle alterations, exposure to nanoparticles, oxidative damage, SHSY5Y neuronal cells*

O-5

INFLUENCE OF PLATINUM AND ZINC ON ACTIVITY OF SUPEROXIDE DISMUTASE IN HUMAN ERYTHROCYTES *IN VITRO*

Blanka TARIBA, Tanja ŽIVKOVIĆ, and Alica PIZENT

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Platinum-containing complexes are widely used in chemotherapy of various malignancies, although some side effects and resistance could occur. It has been indicated that reactive oxygen species may be involved in these effects. Cu-, Zn-superoxide dismutase (SOD1) is an antioxidant enzyme that catalyses the dismutation of superoxide anion and protects cells from damage induced by free radicals. The aim of the study was to evaluate the *in vitro* effect of platinum and zinc exposure on the SOD1 activity at doses (final concentrations) that can be found in human body fluids. Aqueous solution of zinc (0.5 mg L^{-1}, 0.7 mg L^{-1} or 1.0 mg L^{-1}) was added to erythrocytes of a healthy, non-smoking man (age 24). After incubation for 60 min at 37 °C, aqueous solution of platinum (1.0 mg L^{-1}) was added to pre-treated samples, followed by incubation for another 30 min at 37 °C. Addition of zinc to erythrocytes slightly decreased SOD1 activity, while the addition of platinum to erythrocytes pretreated with zinc resulted in a further concentration-dependent activity decrease. Inhibition of SOD1 activity was the highest when the added concentrations of zinc and platinum were both 1.0 mg L^{-1}. The results indicate that the inhibitory action of platinum depends on the status of zinc in the erythrocytes. Reduction of SOD1 activity may induce a decline of the defence potency of cells against the toxicity of reactive oxygen species generated by *cis*-platinum used in cancer treatment, and accelerate the manifestation of the toxic effects of this drug.

KEY WORDS: *cancer treatment, inhibition of enzyme activity, reactive oxygen species, toxicity*

O-6

PROOXIDATIVE-ANTIOXIDANT BALANCE AS A NEW PARAMETER IN INVESTIGATION OF CADMIUM-INDUCED OXIDATIVE STRESS

Aleksandra BUHA[1], Zorica BULAT[1], Vitomir ĆUPIĆ[2], and Vesna MATOVIĆ[1]

Faculty of Pharmacy[1], Faculty of Veterinary Medicine[2], University of Belgrade, Belgrade, Serbia

Among the multiple mechanisms of cadmium (Cd) toxicity, one of the most studied is the indirect induction of oxidative stress which, as our recent studies have shown, may be limited by pretreatment with magnesium (Mg). The aim of this study was to determine the levels of prooxidative-antioxidant balance (PAB) in plasma of rats exposed to Cd/Cd+Mg single treatment. Furthermore, the correlation between commonly used parameters of oxidative stress and PAB was estimated. The experiment was performed on male albino Wister rats (n=40) randomly divided into: control group, Cd_{or} group (30 mg kg^{-1} b. w. of Cd by oral gavage), $Cd+Mg_{or}$ group (30 mg kg^{-1} b. w. of Cd + 50 mg kg^{-1} b. w. of Mg orally), Cd_{ip} group (1.5 mg kg^{-1} b. w. of Cd intraperitoneally) and $Cd+Mg_{ip}$ group (1.5 mg kg^{-1} b. w. of Cd + 3 mg kg^{-1} b. w. of Mg intraperitoneally). Rats were sacrificed after 24 h and PAB was measured in plasma by method with 3,3',5,5'-tetramethylbenzidine chromogen. Levels of PAB were significantly higher in both groups treated with Cd when compared to controls, with more pronounced negative effects after intraperitoneal treatment. This Cd-induced effect on PAB was reduced by simultaneous Mg administration. Prooxidative-antioxidant balance showed strong positive correlation with commonly used parameters of prooxidant status - total oxidative status and malondialdehyde (ρ=0.679, ρ=0.771; $p<0.001$) and strong negative correlation with commonly used parameter of antioxidant status - enzyme superoxide dismutase activity (ρ=-0.654; $p<0.001$). These findings show that PAB as a parameter that simultaneously determinates both prooxidant and antioxidant levels can be used as a biomarker of Cd-induced oxidative stress. (Supported by the Ministry of Science and Environmental Protection, Republic of Serbia, Grant No. III 460009)

KEY WORDS: *cadmium toxicity, magnesium, plasma, prooxidant and antioxidant levels, rats*

O-7

THE EFFECT OF ALUMINIUM AND SILICON ON THE PLANARIAN *POLYCELIS FELINA* (DALY.)

Nives RAJEVIĆ[1], David JELENKOVIĆ[1], Gordana GREGOROVIĆ[1], Goran KOVAČEVIĆ[1], Ivan NEMET[2], Sanda RONČEVIĆ[2], Lejla KNEZOVIĆ[3], Mirjana KALAFATIĆ[1], and Ivna KOCIJAN[4]

Division of Biology[1,4], Division of Chemistry[2], Faculty of Science, University of Zagreb, Zagreb, Croatia, Department of Biology, Faculty of Science and Education, University of Mostar, Mostar, Bosnia and Herzegovina[3]

The most frequent metalloid and metallic elements in the Earth's crust are silicon and aluminium. Silicon is an essential element in lower life forms, while its biological function in higher forms of life is not known. Acidification of water ecosystems and lower pH value increase the solubility of metal ions e.g. aluminium, which becomes toxic at higher concentrations. Aluminium is a known neurotoxin, which interferes with the synthesis of neurotransmitters in the central nervous system of mammals. We analysed the effect of aluminium (80 mg L^{-1}, 100 mg L^{-1}) in neutral and acidic media (pH 7.1 and 5.2) and sublethal doses of silicon (0.17 g L^{-1}, 0.20 g L^{-1}, 0.23 g L^{-1}, 0.25 g L^{-1}, 0.27 g L^{-1}, 0.30 g L^{-1}) on *Polycelis felina* (Daly.). We found a toxic effect of aluminium at applied concentrations. Aluminium in acid media induced higher mortality, stronger morphological and behavioural changes, and more extensive tissue damage. Depending on the dosage of the applied concentration, silicon also caused mortality, decephalisation, depigmentation, morphological and cytohistological changes. We found a significant amount of silicon in the body tissue using high-performance liquid chromatography (HPLC). Most intensive damages were established on the first and second day after the treatment in the experiment with aluminium in acidic media and on the second and third day after the treatment in the experiment with silicon. We found identical changes on the histological slides of planarians treated with either aluminium or silicon: damages to the epidermis, degradation of tissue, decomposition of mucous layer, and an increased number of rhabdites and neoblasts.

KEY WORDS: *behavioural changes, cytohistological changes, high-performance liquid chromatography, morphological changes, pH, regeneration, toxic effect*

O-8

ANDROGEN RECEPTOR $(CAG)_N$ REPEAT LENGTHS AND SPERM CHROMATIN INTEGRITY: POSSIBLE RISK FACTORS FOR MALE INFERTILITY?

Erdem COSKUN[1], Bensu KARAHALIL[1], Ayse Basak ENGIN[1], Cihan KABUKCU[2], Stefano BONASSI[3], Valentina DALL'ARMI[3], and Ali Esat KARAKAYA[1]

Faculty of Pharmacy, Gazi University[1], Gynaecology and Obstetrics Clinic[2], Ankara, Turkey, IRCCS San Raffaele Pisana, Rome, Italy[3]

Today, it is widely acknowledged that almost half of infertility cases can be attributed to the male factor. Regardless of the type of disorder, male-factor infertility is strongly related to genetics. Almost in all cases diagnosed as primary infertility, there is an underlying genetic reason, which points to the phenotype as a disorder. For the treatment, and ultimately for restoring fertility, it is crucial to clarify the genetic causes of male infertility. Although numerous gene polymorphisms are related to male infertility, many of them are not yet proven as clinically significant. The most important confounding factor for male infertility is thought to be the effect of environment, especially xenobiotics targeting directly sperm chromatin. Therefore, similar to other population studies, polymorphism studies of male infertility should also be carried out with the chromatin damage assessment of sperm cells as a biomarker of the effect of exposure. In our study, we have assessed (1) sperm parameters, (2) sperm chromatin integrity by comet assay and (3) CAG trinucleotide repeats in the exon 1 sequence of androgen receptor gene in 82 infertile patients and 63 healthy controls. These parameters were evaluated for any possible link with sperm counts, motilities, and morphologies, and examined for any correlation between the AR Exon-1 (CAG)n repeat polymorphism and sperm DNA integrity. The ultimate goal of this study is to contribute to finding novel biomarkers for individualised treatments of male infertility. (Financed by the Research Council of Gazi University, Project No: 02/2009-2)

KEY WORDS: *androgen receptor gene, comet assay, gene polymorphism, male-factor infertility, sperm parameters*

O-9

LIGHT AND TRANSMISSION ELECTRON MICROSCOPICAL OBSERVATIONS ON RAT SCIATIC NERVE INDUCED BY ELECTROCUTION

Salah H. AFIFI, Adel SHEHATA ISMAIL, Mahmud ABD EL-NASER, Omar H. BAYUOMI, and Doaa SAFWAT

Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

Electrocution induces several alterations of the heart, skin, blood vessels, muscles, and nerves. The main objective of the present study was to investigate possible alterations of the sciatic nerve of rats exposed to 220 V for 5 seconds by light and transmission electron microscope (TEM). Electric current was applied on the thigh region near the gastrocneamus muscles of rats. The sciatic nerve and muscles were taken immediately and fixed in 10 % neutral buffered formalin and 4 % cold glutaraldehyde. They were then processed for both light and TEM, stained by toulidine blue and lead acetate, respectively, and photographed by image software. Light microscope showed irregularity of the shape with elongation of the sciatic nerve compared to the control. Moreover, annulations of the myelin sheath were detected and mast cell infiltration was observed around the myelin sheath, which suggested a response of the nerve tissue to injury. TEM showed that the myelin sheath of non-exposed rats had no remarkable morphological changes: the thickness was in the range (1.41±0.7) μm. On the contrary, the exposed nerve showed a remarkable increase in thickness (1.69±0.8) μm. The exposed nerves were fragmented either in localised areas of the nerve where they appeared "bulby" or onion-like or in the surroundings of the entire nerve. No changes were observed in Schwann cells. Mast cells were detected around the affected nerves and showed empty vesicles, which suggested degranulation. These results can be a helpful tool in forensic toxicology.

KEY WORDS: *electric current, forensic toxicology, microscopy, morphological changes, myelin sheath*

O-10

DEREGULATION OF CALCIUM SIGNALLING IN FUMONISIN B_1 NEUROTOXICIY

Ana-Marija DOMIJAN[1], Stjepana KOVAC[2,3], and Andrey A. ABRAMOV[2]

Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia[1], Institute of Neurology, University College London, London, United Kingdom[2], Department of Neurology, University of Muenster, Muenster, Germany[3]

Fumonisin B_1 (FB_1) is a neurotoxic mycotoxin that contaminates maize and maize-based food all around the world. Molecular mechanism underlying FB_1 toxicity as well as neurotoxicity is still not known. We explored the effect of FB_1 on calcium signalling and mitochondrial membrane potential in the cells of neuronal origin. In our study, FB_1 (0.5 μmol L^{-1}, 5 μmol L^{-1}, and 50 μmol L^{-1}) in astrocytes and neuroblastoma cells was able to significantly increase calcium signal compared to control cells and produce mitochondrial membrane depolarisation in a concentration-dependent manner. These results indicate that FB_1 primarily targets mitochondria causing depolarisation of mitochondrial membrane that leads to calcium deregulation and presumably to cell death. To confirm this finding we checked the possible impact of FB_1 on physiological and pathological conditions in the brain that are characterised by calcium deregulation and mitochondrial membrane depolarisation. In all three tested models, the low glutamate model, the glutamate excitotoxicity model, and the low magnesium model of epilepsy, pre-treatment of hippocampal neurones with FB_1 (0.5 μmol L^{-1} and 10 μmol L^{-1}) increased or changed cytosolic calcium level simultaneously with mitochondrial membrane depolarisation. Since FB_1 alone induced mitochondrial depolarisation and limited mitochondrial ability to uptake calcium, the higher calcium signal in neurons after pre-treatment with FB_1 in tested models can be explained by lower calcium uptake in mitochondria. Taken together, our results indicate that FB_1, even at very low concentrations that humans can be exposed to, deregulates calcium signalling and can act as a trigger to cell death.

KEY WORDS: *calcium deregulation, hippocampal neurones,* in vitro, *mitochondrial membrane, neurotoxic mycotoxin*

O-11

A NEW HPLC/DAD/FLD/MS^N METHOD FOR QUANTIFYING LOVASTATIN AND CITRININ IN FOOD AND VARIOUS RED YEAST RICE PRODUCTS

Miranda SERTIĆ, Ana MORNAR, and Biljana NIGOVIĆ

Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

Cardiovascular diseases are the leading cause of death worldwide, with cholesterol as a primary risk factor. Statins, cholesterol lowering drugs, are among most often prescribed prescription drugs. People that can not use statin therapy because of their side effects or wish to try a non-medicament therapy, tend to use red rice products. The main active ingredient of these products is monacolin K, which is actually lovastatin, registered as a drug in 1987. However, marketed as dietary supplements, they do not comply with strict regulations as medicines do. Hence, there are significant differences among manufactures and even between lots of the same manufacture. Citrinin, a highly nephrotoxic compound, can also be present in red rice products. We developed and validated a new high-performance liquid chromatography (HPLC) method for simultaneously determining lovastatin, its acid form, and citrinin in red rice food and dietary supplements. HPLC separation technique was coupled to three detectors - diode array detector, fluorescence detector, and electrospray ionisation ion trap analyser - to ensure selectivity and sensitivity, and to enable identification of unknown ingredients. Method was validated in terms of accuracy (98 % to 104 %), precision (RSD<3.77 %), linearity (1 μg mL^{-1} to 500 μg mL^{-1} for lovastatin, 0.001 μg mL^{-1} to 10 μg mL^{-1} for citrinin), and LOQ = 0.001 μg mL^{-1}, 0.05 μg mL^{-1}, and 0.1 μg mL^{-1} for citrinin, lovastatin lactone, and hydroxy acid, respectively. The method was successfully applied to red rice food and various dietary supplements. Significant discrepancies were found between the labelled values and determined content (2 % to 157 %) of lovastatin. Citrinin was found in two products, below the EU permitted level (95 ppb and 98 ppb). Four additional monacolins were identified using ESI-MS/MS technique.

KEY WORDS: *development and validation of a method, dietary supplement, food, nefrotoxic mycotoxin, statin*

O-12

NEW SCARFOLDS OF OXIME-ASSISTED ACETYLCHOLINESTERASE REACTIVATORS FOR TREATMENT IN TABUN EXPOSURE

Zrinka KOVARIK[1], Jarosław KALISIAK[2], Nikolina MAČEK[1], Maja KATALINIĆ[1], Suzana BEREND[1], Zoran RADIĆ[3], Valery V. FOKIN[2], Barry K. SHARPLESS[2], and Palmer TAYLOR[3]

Institute for Medical Research and Occupational Health, Zagreb, Croatia[1], Skaggs Institute for Chemical Biology, The Scripps Research Institute[2], Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego[3], La Jolla, CA, USA

The copper-catalysed azide-alkyne cycloaddition reaction enables an efficient and reliable synthesis of libraries of new oximes that were screened for the reactivation activity of tabun-inhibited human acetylcholinesterase (AChE), its mutants, and butyrylcholinesterase (BChE). Fifty-three out of 100 oximes reactivated wild type AChE, but only 14 of them restored its full activity. It appears that an approximate distance equivalent to 8 methylenes between two quaternary nitrogens achieved an optimal level of AChE reactivation. The mutant, Y337A, at the choline binding site was reactivated by more than 80 % with only 13 oximes. The most efficient reactivators of Y337A appeared to be 2PAM analogs, with maximal reactivation rate constants k_{max} up to 10-times faster than those determined for the most efficient reactivator of AChE wild type. Although introducing an additional mutation into the Y337A choline binding site in double mutant Y337A/F338A reduced the enhancement observed in the Y337A mutant, the most efficient Y337A/F338A reactivators also contained the 8 methylene equivalence between two quaternary nitrogens as found for the wild type. Since all oximes were designed as reactivators of phosphorylated AChE, a limited reactivation capacity for BChE was expected. However, 37 oximes reactivated tabun-inhibited BChE more efficiently than the standard antidote 2PAM, and five reached maximal reactivation of 70 %. In addition, toxicity and antidotal studies with lead reactivators in mice showed significantly improved protective indexes compared to 2PAM. Therefore, our findings offer a platform for further development of more potent congenic antidotes in tabun exposure. (Supported by the CounterACT Program NIH, Grant Number U01 NS058046)

KEY WORDS: *antidotes, butyrylcholinesterase, CNS, mutants, nerve agents, organophosphorus compounds, reactivation*

O-13

ACCREDITATION OF MEDICAL, BIOMEDICAL, ANIMAL DIAGNOSTIC LABORATORIES, AND ANIMAL PRODUCTION UNITS

Zdenko FRANIĆ and Gina BRANICA

Institute for Medical Research and Occupational Health, Zagreb, Croatia

The use of accredited procedures and certified animals in toxicological and in biomedical research and practice has become standard procedure in the last few decades. Accreditation is a process in which credentials of qualification, competency, authority or credibility of the laboratory are presented by an impartial third party that has a relevant or *de facto* authority or assumed competence to do so. Accreditation of laboratories is a seal of approval, offering customers the chance to review the operation, streamline it, and improve not only quality but also the efficiency of testing; accreditation is therefore a marketing tool as well. Accreditation, although voluntary, is sometimes stipulated by national legislation. Accreditation of medical laboratories is performed according to HRN EN ISO 15198 standard - (Medical laboratories - Particular requirements for quality and competence) specifying the quality management system and technical requirements particular to medical laboratories. Animal diagnostic laboratories and production units are mostly accredited according to HRN EN ISO/IEC 17025 standard (General requirements for the competence of testing and calibration laboratories), as the main standard used by testing and calibration laboratories, and Federation of Laboratory Animal Science Associations recommendations (FELASA). In Republic of Croatia, are six medical/biomedical laboratories accredited according to ISO 15189 standard and none of the animal diagnostic units. This work presents a review of the quality of infrastructure in Croatia relevant to the accreditation of laboratories and certification of standardisation for laboratory animals. Special emphasis has been put on Quality Control and Quality Assurance of data, results, and procedures.

KEY WORDS: *accredited procedures, ISO standards, quality assurance, quality control, quality of infrastructure*

O-14

RADIATION PROTECTION IN A MIXED CONTAMINANT CONTEXT, RISK ASSESSMENT METHODOLOGIES

Ivica PRLIĆ

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Often little is known about the mid- and long-term health and ecological consequences of simultaneous exposure to a mixture of potential stressors. The consideration of chronic low-level mixed exposures presents considerable challenges for methodology and data interpretation. Determining mixture effects is complex as the contaminants may interact at different levels. Co-contaminants may affect the mobility, absorption, distribution, storage, biotransformation, elimination, and evolution of toxic effects of other contaminants. To fully understand the effects of multiple stressors on life-history responses such as growth, reproduction, and survival requires a multidisciplinary approach. Radionuclides never occur in isolation. Radiation exposure conditions entail a number of radionuclides which are treated in impact and risk assessments in an additive way although considering different weighing factors for different radiation types. However, radiological exposure situations are essentially mixed contaminant exposure situations with a mix of radionuclides, heavy metals, metalloids or organic pollutants. We experienced this fact while performing the radiological research at several existing industrially polluted sites. Objective was to research and understand the mechanisms by which mixtures of contaminants interact to induce adverse effects on biota and the environment to determine if radiation protection criteria are protective enough when considering a mixed contaminant context. Knowledge on the transfer of pollutants between different environmental compartments, and on the impact of cumulative stressors is to be gathered by developing and using improved assessment tools and novel models, to reduce uncertainty in current risk assessment, for example by improving the scientific basis for setting safety factors. This will facilitate human and ecosystem health monitoring by providing the link with information on the condition of air, water, soil, and the built environment.

KEY WORDS: *co-contaminants, mixed exposure, novel models, radiation protection criteria*

P-1

ENVIRONMENTAL CONTAMINANT BISPHENOL: A TOXICITY STUDY IN SWISS ALBINO MICE MODEL

Sameya ANJUM and Sheikh RAISUDDIN

Jamia Hamdard University, New Delhi, India

Bisphenol A (BPA) enters environment as an industrial or domestic waste or byproduct of industrial processes. BPA is a monomer of polycarbonate plastic used to manufacture plastic baby bottles and lining of food cans. It has endocrine-disrupting potential and exerts both toxic and estrogenic effects on mammalian cells. The aim of this study was to investigate BPA-induced oxidative stress and toxicity in the testicular mitochondria of adult male mice. Mice were exposed to standardised dose of BPA (5 mg kg^{-1}, 10 mg kg^{-1}, 100 mg kg^{-1} body weight), orally for 14 days. BPA caused lipid peroxidation (LPO) and a decrease in glutathione (GSH) content of testicular mitochondria. Significant differences $p<0.01$ were observed in the LPO and GSH parameters when compared with control values. BPA caused a significant decrease in the activities of marker mitochondrial enzymes such as succinate dehydrogenase, malate dehydrogenase, and isocitrate dehydrogenase as compared to the control group. Besides, it also affected the activities of antioxidant enzymes such as superoxide dismutase, glutathione reductase, and glutathione peroxidase. Significant differences $p<0.01$ were also observed in the SOD, GR, and GPx parameters when compared with control values. These effects increased as the dose of BPA increased. Ultra structural changes observed by transmission electron microscopy showed that BPA caused abnormalities like deformed acrosome and nucleus of spermatids and apoptotic cells were observed in the testes of treated animals. Hence we can conclude that BPA induced oxidative stress in testicular mitochondria of exposed group and the results were further confirmed by the observations of transmission electron microscopy.

KEY WORDS: *Bisphenol A, endocrine disrupting chemicals, lipid peroxidation, marker mitochondrial enzymes, oxidative stress*

P-2

IMPACT OF WATER POLLUTION ON CYTOSOLIC METALS AND BIOMARKER RESPONSES IN PIKE *ESOX LUCIUS* AND ROACH *RUTILUS RUTILUS*

Dušica IVANKOVIĆ[1], Domagoj ŠEGREGUR[1], Zrinka DRAGUN[1], Damir ĐERMIĆ[2], and Marijana ERK[1]

Division for Marine and Environmental Research[1], Division of Molecular Biology[2], Ruđer Bošković Institute, Zagreb, Croatia

Different chemicals from waste water are continuously discharged into the aquatic environment where they can lead to various adverse effects on aquatic organisms. We investigated the impact of inorganic contaminants on pike (*Esox lucius*) and roach (*Rutilus rutilus*) caught in the Drava River, upstream and downstream from a point source of water contamination. Potential biological impact of aquatic contaminants could be assessed using various biomarkers. Metallothioneins and cytosolic metals in liver were used as biomarkers of metal exposure. Serum concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were used as possible biomarkers of liver damage while condition index was used to assess the general condition of fish. In water samples, the total dissolved metals and physico-chemical parameters of water quality were determined. The concentrations of several dissolved elements in river water and the values of liver metallothioneins were higher at downstream location, indicating increased metal exposure. Elevated levels of Cd, Cs, V, Mn, Fe, Co, Zn, and As in hepatic cytosol of pike and of Se, Rb, Mo, Pb, Zn and Cu in hepatic cytosol of roach were found at downstream location. There were no differences in ALT and AST values between sampling locations for either species. The condition index of fish was slightly lower at downstream location indicating the possible harmful effects of pollution. Thus, our results indicate that the combination of selected indicators in pike and roach may be useful for assessing the biological impact of water pollution, even in weakly contaminated waters.

KEY WORDS: *alanine aminotransferase, aquatic pollution, aspartate aminotransferase, fish, inorganic contaminants, metallothioneins*

P-3

THE INFLUENCE OF IMIDACLOPRID AND ITS TRANSFORMATION PRODUCT 6-CHLORONICOTINIC ACID ON MXR ACTIVITY IN AMPHIPOD *GAMMARUS FOSSARUM* AND EARTHWORM *EISENIA FETIDA*

Olga MALEV[1], Roberta SAUERBORN KLOBUČAR[2], Anamaria ŠTAMBUK[3], Maja ŠRUT[3], Natalija TOPIĆ POPOVIĆ[3], Ivančica STRUNJAK PEROVIĆ[3], Polonca TREBŠE[1], Goran I. V. KLOBUČAR[2], and Rozelindra ČOŽ-RAKOVAC[3]

University of Nova Gorica, Nova Gorica, Slovenia[1], Ruđer Bošković Institute[2], Faculty of Science, University of Zagreb[3], Zagreb, Croatia

The multixenobiotic resistance (MXR) defence system is mediated by different efflux transporters and its activity has been confirmed in many organisms, both prokaryotes and eukaryotes. MXR transporters confer resistance by preventing xenobiotics from entering the cells and by their extrusion into the extracellular environment. Chemicals known as chemosensitizers (inhibitors, modulators) can inhibit MXR function and thus represent specific environmental contaminants. A wide range of pesticides have been shown to act as MXR inhibitors. Presence of chemosensitizers in the environment may increase the absorption of xenobiotics and consequently their cellular toxic influence in aquatic and soil organisms. In this study, MXR activity and its inhibition have been measured using a dye exclusion assay in amphipod *Gammarus fossarum* and earthworm *Eisenia fetida* in order to assess the chemosensitizing potential of imidacloprid (IMI) and its transformation product 6-chloronicotinic acid (6CNA). In amphipods, 6CNA was proved to be a potent inhibitor of MXR at low concentrations, while IMI presented no significant inhibition compared to control. Interestingly, co-exposure of amphipods to IMI or 6CNA and the common fungicide copper sulphate resulted in a greater additive inhibition effect of MXR activity. Earthworms exposed to tenfold lower concentration of IMI and 6CNA (because higher concentrations were lethal) did not exhibit inhibition of efflux pumps. These results highlight the need of further investigation of the effects of neonicotinoids as chemosensitizers, as they enhance the toxicity of other co-occurring contaminants and may thus have significant consequences for aquatic and soil organisms.

KEY WORDS: *chemosensitizer, invertebrates, multixenobiotic resistance (MXR) defence system, neonicotinoids, rhodamine B*

P-4

CYTOTOXICITY OF ENVIRONMENTAL ESTROGENS IN FISH CELL LINE - IMPACT OF SERUM

Kristina RADOŠEVIĆ, Sanja LAŠKARIN, Igor SLIVAC, and Višnja GAURINA SRČEK

Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

Fish cell lines are becoming increasingly important in the aquatic ecotoxicology. The discrepancy between whole fish (acute toxicity) and fish cell lines (cytotoxicity) is mostly attributed to the lower bioavailability of tested substances *in vitro*. During the *in vitro* testing, the cells are cultivated in the media with serum, which can modify the outcome of cytotoxicity studies. This is mainly due to the binding of the test substance to serum proteins, which affects the concentration-effect ratio. In this study, we tested the impact of serum on diethylstilbestrol (DES) and 17*α*-ethynylestradiol (EE2) cytotoxicity in CCO (Channel Catfish Ovary) cell line. The cells were treated with DES and EE2 (0.1 μg mL^{-1} to 10 μg mL^{-1}) in DMEM medium with 2.5 %, 5 % or 10 % (v/v) foetal bovine serum (FBS) or serum stripped with charcoal (Cs-FBS). After 72 hours of treatment, the viability of CCO cells was determined by WST-1 method. DES (0.1 μg mL^{-1}) induced stimulatory effect in the range of 15 % to 40 % compared to control, while higher concentrations (5 μg mL^{-1} and 10 μg mL^{-1}) had inhibitory effects by 15 % to 60 % and 40 % to 70 %, respectively. The same was observed for EE2, the stimulation of proliferation (15 % to 30 %) with 0.1 μg mL^{-1} and inhibition of 5 μg mL^{-1} and 10 μg mL^{-1} (20 % to 35 % and 30 % to 50 %). Calculated EC_{50} values have shown that the influence of DES and EE2 on CCO cells depends on the type and quantity of serum. Further research is necessary to elucidate which culture conditions better correlate with *in vivo* toxicity and thus improve the prediction of human acute toxicity, which is the ultimate goal of alternative *in vitro* testing.

KEY WORDS: *CCO cells, diethylstilbestrol, EC_{50}, 17α-ethynylestradiol, foetal bovine serum*

P-5

ASSESSMENT OF NITROFURANTOIN AS ECOTOXICANT

Roberta SAUERBORN KLOBUČAR[1], Anamaria BROZOVIĆ[2], Anamaria ŠTAMBUK[3], Maja ŠRUT[3], Natalija TOPIĆ POPOVIĆ[1], Ivančica STRUNJAK PEROVIĆ[1], Margita JADAN[1], Josip BARIŠIĆ[1], and Rozalindra ČOŽ RAKOVAC[1]

Division of Materials Chemistry[1], Division of Molecular Biology[2], Ruđer Bošković Institute, Faculty of Science, University of Zagreb[3], Zagreb, Croatia

Antibiotic nitrofurantoin (NF) and its derivatives are used in veterinary and human medicine. The impact of NF on the aquatic environment is largely unknown and its ecotoxicity is still under investigation due to conflicting opinions on its use. Increasing evidence suggests that NF can cause various adverse effects in living cells. In this investigation, we used three different fish cell lines (PAC2, PLHC-1, R1) to evaluate cytotoxicity of NF towards fish. PAC2 cells were shown to be the most sensitive to NF in an MTT colorimetric assay, exhibiting an EC_{50} of 15 μmol L^{-1} (3.57 mg L^{-1}) NF. Furthermore, we determined DNA damage and apoptosis in PAC2 cell line depending on the concentration and duration of treatment. We also determined growth inhibition of unicellular algae *Scenedesmus subspicatus* and mutagenic action towards Salmonella strains (TA98 and TA100) using classic Ames test. A significant decrease in the growth of unicellular algae after 24 h exposure to NF (1 μmol L^{-1}, 10 μmol L^{-1}, 30 μmol L^{-1}, 100 μmol L^{-1}, 200 μmol L^{-1}) was observed. Mutagenicity test responses revealed a large number of revertants following exposure to NF. These results indicate a substantial risk of exposure to NF for the health of aquatic ecosystems

KEY WORDS: *antibiotic, aquatic environment, cytotoxicity, genotoxicity, growth inhibition*

P-6

CADMIUM IN BROWN BEARS FROM CROATIA: AGE AND SEX DIFFERENCES

Maja VIHNANEK LAZARUS[1], Ankica SEKOVANIĆ[1], Jasna JURASOVIĆ[1], Đuro HUBER[2], and Slaven RELJIĆ[2]

Institute for Medical Research and Occupational Health[1], Veterinary Faculty, University of Zagreb[2], Zagreb, Croatia

Levels of cadmium (Cd) in the environment should be continuously monitored because of its ubiquity and ability to accumulate and exhibit toxic effects in the organism. Brown bears are suitable for Cd monitoring because of their feeding habits (omnivores) and relative longevity compared to other wild animals. There are no published data on metals in Dinara-Pindos bear population of which Croatian bears are a part. In this study, Cd was measured in the muscle, liver, and kidney cortex of 111 brown bears (*Ursus arctos*) hunted in Gorski kotar and Lika during 2009 and 2010 according to "The Brown Bear Management Plan for Croatia". Cadmium content was determined using inductively coupled plasma-mass spectrometry. Distribution of Cd across tissues was as follows (median; range): muscle [0.008 μg g^{-1}; (0.0007 to 1.55) μg g^{-1}] wet mass, liver [1.02 μg g^{-1}; (0.003 to 8.35) μg g^{-1}], kidney [16.5 μg g^{-1}; (0.774 to 139.5) μg g^{-1}]. Cadmium in all three tissues was 1.5 to 2.2 times higher in females than in males ($p<0.01$). Age group (young: ≤3 years; old >3 years) had significant influence on Cd in muscle ($p<0.05$), liver ($p<0.001$), and kidney ($p<0.001$). Old bears of both sexes contained more Cd in target organs of accumulation than the young ones. Factors causing sex-differences in Cd levels could be hormones, reproductive state, and size variations, all affecting food intake, the principal source of Cd for bears. Regarding maximum levels of Cd allowed in meat, liver and kidney for consumption, bear meat is considered suitable for human consumption but offal should be avoided.

KEY WORDS: *accumulation, monitoring, tissue, toxic metal,* Ursus arctos

P-7

FLUORIDE CONCENTRATION IN DRINKING WATER AND URINE SAMPLES OF SCHOOLCHILDREN IN RITOPEK, SERBIA

Evica ANTONIJEVIĆ[1], Nemanja MILIĆEVIĆ[1], Zoran MANDINIĆ[2], Marija STANKOVIĆ[3], Marijana ĆURČIĆ[1], and Biljana ANTONIJEVIĆ[1]

Faculty of Pharmacy[1], School of Dentistry[2], University of Belgrade, Institute of Public Health[3], Belgrade, Serbia

Although optimal levels of fluorides have a beneficial effect on dental health, chronic intake of fluoride above optimal levels during enamel formation may cause dental fluorosis. In this study, we determined the fluoride content in drinking water and urine samples from schoolchildren living in Ritopek, suburban settlement of Belgrade, Serbia. This study was approved by the Ethical Committee of Faculty of Dentistry, University of Belgrade. Forty-eight healthy schoolchildren of both genders participated in the study. Fluoride concentration in drinking water (n=27) and urine samples (n=48) was determined directly using composite fluoride ion-selective electrode. In addition, dental examination was performed. Mean fluoride concentration in drinking water samples was 0.59 ppm. In three water samples fluoride levels were 3.81 ppm, 3.75 ppm and 4.14 ppm, which was significantly higher than the levels recommended by WHO (0.7 ppm to 1.2 ppm). Mean urinary fluoride concentration was 0.44 ppm. Almost all values of urinary fluoride concentration were up to 1 ppm, whereas four values ranged from 1 ppm to 3 ppm. However, clinical examination revealed occurrence of dental fluorosis in most cases. It seems necessary to identify precisely the source of exposure and to establish the relationship between the concentrations of fluoride in urine and the occurrence of dental fluorosis. (Partly supported by the Ministry of Education and Science, Serbia, Grant III 46009).

KEY WORDS: *composite fluoride ion - selective electrode, dental examination, dental fluorosis exposure*

P-8

TISSUE-SPECIFIC RESPONSE OF ACID DNASE ACTIVITY IN MUSSEL *MYTILUS GALLOPROVINCIALIS* EXPOSED TO MIXED POLLUTANTS IN SEA-WATER ORGANIC EXTRACTS

Ines KOVAČIĆ[1,2], Lorena PERIĆ[2], and Maja FAFANĐEL[2]

Juraj Dobrila University of Pula, Pula[1], Centre for Marine Research, Ruđer Bošković Institute, Rovinj[2], Croatia

New biomarkers of high sensitivity and low cost are widely investigated for marine biomonitoring purposes. Our previous investigations showed that the exposure of mussels to individual model marine pollutants caused an increase in the acid DNase activity in haemocytes and hepatocytes indicating acid DNase activity as a promising biomarker. For further validation, mussels were exposed to composite organic extracts of seawater from the Adriatic (Vranjic, industrial area - high potential toxicity, Rijeka, harbour - moderate potential toxicity, Ploče, small rural - low potential toxicity) and acid DNase activity was measured in the gills and digestive glands. The patterns of influences in both tissues are site-specific and related to different potential toxicity of sea-water extracts. In referent mussels, the acid DNase activity was higher in the gills than in the digestive glands. The extract from the station with low potential toxicity (Ploče) did not induce statistically significant change in the acid DNase activity in either tissue. Enzyme activity changed in the gills of mussels exposed to the extract with moderate potential toxicity (Rijeka) and in the digestive glands of mussels exposed to the extracts with high potential toxicity (Vranjic). In both cases, enzyme activity increased already after 3 h and lingered for 48 h. Advantages/disadvantages of using acid DNase activity in the mussel gills and/or digestive glands as a biomarker of toxic pollutant presence are discussed.

KEY WORDS: *biomarker, digestive glands, enzyme activity, gills, marine pollutant*

P-9

DETERMINATION OF 24- AND 48-HOUR LC_{50} VALUES OF DIAZINON IN *GAMBUSIA AFFINIS* (BAIRD & GIRARD, 1853)

Utku GÜNER

Faculty of Sciences, Trakya University, Edirne, Turkey

Diazinon (Basudin 60 EM) is a non-systemic organophosphate insecticide formerly used to control cockroaches, silverfish, ants, and fleas in residential, non-food establishments. Although the aquatic environment is not the target of the use of such pesticides, the results of a number of monitoring studies have evidenced the presence of diazinon and its metabolite, diazoxon, in lakes and rivers. The purpose of this study was to determine value of lethal concentration 50 % (LC_{50}) for diazinon (IUPAC name: O,O-diethyl O-[4-methyl-6-(propan-2-yl)pyrimidin-2-yl] phosphorothioate) during 24- and 48-hour periods in the mosquito fish *Gambusia affinis* (Baird & Girard, 1853). The LC_{50} values were determined under static test conditions for 24- and 48-hour periods. Animals were exposed to 9 different sub-lethal (0.1 g L^{-1}, 1 g L^{-1}, 1.5 g L^{-1}, 2 g L^{-1}, 5 g L^{-1}, 7.5 g L^{-1}, 10 g L^{-1}, 15 g L^{-1}, 20 g L^{-1}) diazinon doses. Experiments were performed at 23.0 °C under normal light-dark cycle in 1 L volume containers filled with water that was filtered through an Ion Exchange Cartridge with a total hardness value of 50 mS. The results were evaluated by the statistical analysis programme AnalystSoft Inc., BioStat v2009, to determine the LC_{50} values. The LC_{50} value for *G. affinis* was found to be 10.73 g L^{-1} for the 24-hour period and 8.50 g L^{-1} for the 48-hour period.

KEY WORDS: *LC_{50}, LD_{50}, mosquito fish, organophosphate insecticide*

P-10

DOES THE MIXTURE OF CADMIUM AND DECABROMINATED DIPHENYL ETHER INFLUENCE LIPID PEROXIDATION IN RAT LIVER?

Marijana CURCIC[1], Maja DZOKOVIC[1], Marko ANTUNOVIC[1], Snezana DJORDJEVIC[2], Vesna KILIBARDA[2], Slavica VUCINIC[2], Ksenija DURGO[3], and Biljana ANTONIJEVIC[1]

Faculty of Pharmacy, University of Belgrade[1], Military Medical Academy[2], Belgrade, Serbia, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia[3]

Decabrominated diphenyl ether (BDE-209) and cadmium (Cd) are chemicals present in environment, food, and consequently in animal and human tissues, mostly as a result of anthropogenic activities. A toxicity mechanism of their mixture is still under examination so the aim of this study was to determine the effect of subacute exposure to the mixture of Cd and BDE-209 on lipid peroxidation in the liver. For this experiment we used male Wistar rats divided in 17 groups. Animals, weighing 200 g to 240 g (seven weeks old, eight animals in each group) were exposed to doses of BDE-209 (1000 mg kg^{-1} b. w. day^{-1}, 2000 mg kg^{-1} b. w. day^{-1} or 4000 mg kg^{-1} b. w. day^{-1}) and Cd (2.5 mg kg^{-1} b. w. day^{-1}, 7.5 mg kg^{-1} b. w. day^{-1} or 15 mg kg^{-1} b. w. day^{-1}) (as a mixture or individually plus control and solvent groups) by gavage during 28 days. We measured lipid peroxidation in the liver homogenates by measuring malondialdehyde (MDA) production (assayed by the reaction with thiobarbituric acid). Different doses of BDE-209 seem to reduce the effect of Cd in terms of destruction of the cell's lipid layer or sub cellular lipid structures, primarily membranes. However, in the groups treated by single poisons (BDE-209 or Cd), as well as with a medium dose of BDE-209 in combination with all doses of Cd, dose dependent increase of MDA contents was observed indicating that the mixture of Cd and BDE-209 may influence lipid peroxidation in rat liver. (Partly supported by the Ministry of Science and Education, Republic of Serbia, Grant III 460009)

KEY WORDS: *anthropogenic contaminants, malondialdehyde, subacute exposure, toxicity, Wistar rats*

P-11

LUMINESCENCE RESONANCE ENERGY TRANSFER AS A NEW DETECTION TECHNIQUE FOR MARINE BIOTOXINS

Filip STIPIĆ[1], Galja PLETIKAPIĆ[2], Vesna SVETLIČIĆ[2], Željko JAKŠIĆ[1], Leo FRKANEC[3], Petra BURIĆ[1], and Daniel M. LYONS[1]

Centre for Marine Research, Ruđer Bošković Institute, Rovinj[1], Department of Marine and Environmental Research[2], Department of Organic Chemistry and Biochemistry[3], Ruđer Bošković Institute, Zagreb, Croatia

Shellfish can pose a significant threat to human health due to their susceptibility to contamination by a range of algal toxins including okadaic acid, saxitoxin, and domoic acid. As the current reference toxin detection method (mouse bioassay) has a number of significant drawbacks, both the EU and WHO have encouraged the development of new and more sensitive techniques as a replacement. Thus, we report a first step in the development of a sensitive nanoparticle-based luminescence technique for the detection of the marine toxin okadaic acid. Luminescent composite LaF3:Ce,Tb nanoparticles were prepared by hydrothermal synthesis, to which okadaic acid was subsequently conjugated. These tailored toxin-nanoparticle composites were then captured by fluorophore-labelled anti-okadaic antibodies. Non-radiative resonant energy transfer between the nanoparticle donor and fluorophore acceptor was achieved after exciting the complex at a wavelength of 280 nm, through the overlap of nanoparticle emission and dye excitation bands at 490 nm, and measuring emission at 518 nm. The intensity of the emission was found to be proportional to the number of binding events between the okadaic acid-decorated nanoparticles and the labelled antibodies.

KEY WORDS: *algal toxins, luminescent composite nanoparticles, shellfish, toxin detection*

P-12

MERCURY IN EARTHWORMS *LUMBRICIDAE* IN THE VICINITY OF NATURAL GAS TREATMENT PLANT IN CROATIA

Andreja PREVENDAR CRNIĆ[1], Emil SREBOČAN[1], Jelena ŠURAN[1], Jasna JURASOVIĆ[2], and Zdravko ŠPIRIĆ[3]

Faculty of Veterinary Medicine, University of Zagreb[1], Institute for Medical Research and Occupational Health[2], OIKON Ltd. Institute for Applied Ecology[3], Zagreb, Croatia

During the last two decades (1990 to 2012), earthworms were collected from four locations in the vicinity of the natural gas production and treatment plant Molve, Croatia, as a part of a comprehensive ecosystem monitoring programme. Their tissues were analysed for total mercury concentration by cold vapour AAS. The range of median mercury concentration values (wet weight) in earthworms were (0.004 to 0.283) μg g^{-1}; (0.013 to 0.260) μg g^{-1}; (0.005 to 0.317) μg g^{-1}; and (0.009 to 0.191) μg g^{-1} for Molve 9, Molve 10, Molve 11, and Molve 12 location, respectively. Although the results of mercury measurements in earthworm tissues from different locations vary in a wide range of values, integrated results of all measurements during the last twenty-year period demonstrate a small but constant decline in concentration values, especially after 2004. Comparing our results with the results published in the available data from Europe and some areas of Croatia on mercury concentration in earthworms, it can be concluded that the area investigated in this research belongs to moderately mercury-contaminated region. Nevertheless, further eco-monitoring and mercury measurements in various biological samples are important for a more complete interpretation of results and these will be continued.

KEY WORDS: *cold vapour atomic absorption spectrometry, contamination with mercury, environmental monitoring, Molve, tissue*

P-13

HEAVY METAL CONTENT IN ROE DEER ANTLERS IN SELECTED REGIONS OF SLOVAKIA

Svätoslav HLUCHÝ[1], Norbert BOLCSÓ[1], Pavel FĽAK[2], Róbert TOMAN[1], and Jozef GOLIAN[1]

Slovak University of Agriculture[1], Research Institute for Livestock Production[2], Nitra, Slovak Republic

We investigated the content of heavy metals (Fe, Mn, Zn, Cu, Ni, Pb, Cd, and Hg) in deer antlers in two regions of Slovakia (Nitra and Gemer). We found that the amount of Fe (109.10±13.27) mg kg^{-1} was the highest and the amount of Hg (±0.0189 mg kg^{-1}) was the lowest in both regions. However, we found high variability and asymmetry relative to Fe, Mn, and Hg in particular, the last two being in a statistically highly significant excess. The only statistically significant difference between regions was found for Cu (7.62±0.39) mg kg^{-1} in Nitra and (6.15±0.22) mg kg^{-1} in Gemer). Statistically high and moderate correlations were found only between Pb and Cd, Zn and Hg. In both regions there was a similar correlation between Cu and Ni, Cd and Fe, Mn and for Pb, Zn and Hg.

KEY WORDS: *correlations between metals, deer, heavy metals, variability*

P-14

MERCURY IN HARE TISSUES *LEPUS EUROPAEUS* PALLAS IN POLLUTED AND UNPOLLUTED AREAS

Željko LINŠAK[1,2], Dijana TOMIĆ LINŠAK[2], Zdravko ŠPIRIĆ[1,3], Emil SREBOČAN[4], and Čedomila MILIN[5]

Department of Environmental Medicine[1], Department of Chemistry and Biochemistry[5], School of Medicine, University of Rijeka, Teaching Institute of Public Health Primorsko-Goranska County, Rijeka[2], OIKON Ltd. Institute for Applied Ecology[3], Faculty of Veterinary Medicine, University of Zagreb[4], Zagreb, Croatia

The aim of this study was to quantify the concentration of total mercury in tissues of hares *Lepus europaeas* Pallas, that are used as bioindicators of environmental pollution by heavy metals due to the complete integration of the biotope. The study conducted during 2010, included two study groups of hares, one from a polluted and the other from an unpolluted area. The polluted area was located near the largest natural gas production and treatment plant Molve, in Croatia. Their organs (brain, muscle, liver and kidney) were analysed for total mercury concentration using advanced mercury analyser AMA 254 (Leco, USA). The range of the median mercury concentration values in hare organs from the polluted area were (0.022 to 0.102) μg g^{-1} d. w., (0.013 to 0.046) μg g^{-1} d. w., (0.058 to 0.189) μg g^{-1} d. w., and (0.138 to 0.406) μg g^{-1} d. w. for the brain, muscle, liver, and kidney, respectively. The range of the median mercury concentration values in hare organs from the unpolluted area were [(0.017 to 0.040), (0.006 to 0.022), (0.011 to 0.031), and (0.019 to 0.223)] μg g^{-1} d. w. for the brain, muscle, liver, and kidney, respectively. The concentration of total mercury was significantly higher in the liver ($p=0.012$) and kidney ($p=0.022$) of hares from contaminated areas than in the liver and kidney of hares from the unpolluted area. When comparing the results obtained in this study with the results published in the available literature on mercury concentration in hare's tissue, it can be concluded that the polluted area investigated in this research belongs to low mercury-contaminated area.

KEY WORDS: *bioindicator, brain, kidney, liver, muscle, mercury concentration*

P-15

INFLUENCE OF THE CYANOBACTERIAL TOXIN CYLINDROSPERMOPSIN ON APOPTOSIS AND PROLIFERATION IN HepG2 CELLS

Alja ŠTRASER, Metka FILIPIČ, and Bojana ŽEGURA

National Institute of Biology, Ljubljana, Slovenija

The cyanobacterial toxin cylindrospermopsin (CYN) is increasingly being found in fresh water bodies worldwide. It is a potent protein synthesis inhibitor and has been reportedly implicated in human intoxications and animal mortality. The toxin induces genotoxic effects in metabolically competent test systems *in vitro* and *in vivo*, while its carcinogenic activity remains to be elucidated. Our previous studies have shown that CYN (0.1 μg mL^{-1} to 0.5 μg mL^{-1}) induces DNA damage (DNA strand brakes, and micronuclei, nuclear bud, and nucleoplasmic bridge formation) and modulates mRNA expression of DNA damage-responsive genes in HepG2 cells. Further experiments, presented here, showed that it reduced cell viability after prolonged exposure (48 h to 96 h) and propidium iodide staining and flow-cytometry analysis demonstrated that the toxin affected the cell cycle. The results of several approaches measuring cellular processes associated with apoptosis (anexin V staining and flow-cytometry analysis, activity of caspase 3/7, changes in mitochondrial membrane potential MMP) showed that CYN induced one of the initiating reversible steps (changes in MMP). Reduction in caspase 3/7 activity however indicated that apoptosis could even be inhibited at later stages, which would enhance the hazard of CYN. The results indicate complex effects of CYN on cellular processes that are involved in cancer initiation and progression.

KEY WORDS: *cell-cycle, cell death, cell proliferation, cyanotoxin, human hepatocellular liver carcinoma cell line*

P-16

POTENTIAL OF METALS TO ACT AS ENDOCRINE-DISRUPTING CHEMICALS IN HUMAN REPRODUCTION

Martina PIASEK

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Synthetic endocrine disrupting (ED) chemicals can mimic, enhance, or inhibit the action of endogenous hormones that are responsible for maintaining homeostasis and controlling normal development. They have been a focus of major concern since the early 1990s in mammals including humans. Reproductive and developmental toxicology studies have yielded increasing evidence that metals and metalloids (cadmium, lead, mercury, arsenic, uranium, and other) have the insidious ED potential and may act as metalloestrogens in reproductive tissues and during foetal development (Henson MC, Piasek M et al., In: Endocrine Toxicology 3rd Ed., 2010. p. 256-79). As for cadmium, within the last two decades human and animal studies have demonstrated its potential to affect gonadotropins, steroidogenesis, and placental leptin that may adversely impact male and female reproductive functions. Our own research work in experimental animals under *in vivo* and *in vitro* exposure conditions and in cadmium-exposed tobacco smoking postpartum women has added considerably to the evidence identifying cadmium as an endocrine disrupting chemical in the female reproductive system (Piasek M et al., IAOEH 2002;75:S36-44). This is scientifically still a rather controversial issue. A better understanding of ED effects of metals and metalloids bears great clinical relevance as they constitute an important part of our ecosystem and lifestyle and their production and use is unlikely to be discontinued in the foreseeable future. Complementary results attained from different experimental paradigms should all be carefully evaluated. Future research should assess critical exposure levels, reliable endpoints, and underlying mechanisms of ED action in humans.

KEY WORDS: *cadmium exposure, metals and metalloids, reproductive and developmental toxicity*

P-17

EXPOSURE TO CADMIUM AND EFFECTS ON PLACENTAL STEROIDOGENESIS IN RATS: COMPARING ORAL *VERSUS* PARENTERAL ROUTE OF EXPOSURE

Anja MIKOLIĆ[1] , Saša KRALIK OGUIĆ[2], Vesna KUŠEC[2], and Martina PIASEK[1]

Institute for Medical Research and Occupational Health[1], University Hospital Centre Zagreb[2], Zagreb, Croatia

Cadmium is a toxic environmental pollutant with potential to act as an endocrine-disrupting chemical in reproductive organs. Main sources of cadmium exposure in general population are contaminated food, water, and tobacco smoke. Up to 50 % of the inhaled cadmium and an average of 5 % (<1 % to >20 %) of cadmium oral intake is absorbed, which depends on age, nutrition, and physiological state. In exposed mammals, including humans, cadmium accumulated in placental tissue can disrupt its vital function of hormones' synthesis during gestation. We compared effects of oral *vs.* parenteral cadmium route of exposure on placental steroidogenesis in laboratory rats. Previous investigation showed cadmium effects on placental progesterone production in rats (Sprague Dawley) after 19-day exposure during pregnancy at total doses of 3 or 5 mg Cd kg^{-1} b. w. subcutaneously by osmotic pumps (Piasek et al., In: TEMA 10, 2000. p. 809-12). In our new investigation, rats (Wistar) with regular four-day oestrous cycle were exposed orally to 50 ppm Cd (as chloride; 7.26±0.86 mg Cd kg^{-1} b. w.) from gestation day 1 to 20. Mother rats were then euthanized and placentas dissected, weighed, and prepared for cadmium analysis (by atomic absorption spectrometry) and steroid hormone assay (by enzyme-immunometric method). Cadmium increased in placentas of exposed rats by both routes of exposure. While parenteral cadmium exposure decreased placental progesterone, oral exposure increased progesterone concentrations in rat placentas. In conclusion, cadmium disrupts placental progesterone synthesis, which may compromise pregnancy outcome and foetal viability. This effect depends on the route of exposure during pregnancy.

KEY WORDS: *cadmium exposure, placental progesterone, pregnancy, steroid disruption*

P-18

ACTIVITY OF COPPER-ZINC SUPEROXIDE DISMUTASE IN HUMAN ERYTHROCYTES EXPOSED TO CADMIUM AND ZINC: *IN VITRO* STUDY

Tanja ŽIVKOVIĆ, Blanka TARIBA, and Alica PIZENT

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Antioxidant enzyme copper-zinc superoxide dismutase 1 (SOD1) is a scavenger of superoxide radicals. Since zinc acts as a co-factor responsible for its structural stability, the interaction of cadmium with zinc can affect the enzyme activity due to binding competition to the same sites. There is no data related to the influence of simultaneous exposure to cadmium and zinc on SOD1 activity. The aim of this study was to investigate such effects in human erythrocytes *in vitro* at doses that can be found in human body fluids. Blood erythrocytes obtained from healthy male non-smoking donor (age 24) were incubated with aqueous solution of cadmium (2 μg L^{-1}) and zinc (0.5 mg L^{-1}, 0.7 mg L^{-1} or 1 mg L^{-1}) at 37 °C for 60 minutes. Instead of metal solutions, phosphate buffer (0.1 mol L^{-1}, pH 7.0) was used in control samples (SOD1 activity was 100 %). Treatment of erythrocytes with zinc alone resulted in slight decrease, whereas treatment with cadmium alone resulted in slight increase of SOD1 activity. When erythrocytes were incubated with both cadmium and zinc, SOD1 activity initially slightly increased, but addition of 1.0 mg L^{-1} of zinc resulted in a sharp drop of SOD1 activity (remaining activity was (79±10) %. Our results have shown that simultaneous exposure to cadmium and zinc under *in vitro* conditions may differently affect SOD1 activity compared to each metal alone.

KEY WORDS: *antioxidant enzyme, enzyme activity, simultaneous exposure, superoxide radicals, metals*

P-19

ANALYSIS OF COBALT AND CHROMIUM IN BLOOD FRACTIONS OF PATIENTS WITH HIP IMPLANTS

Ankica SEKOVANIĆ[1], Tatjana ORCT[1], Domagoj DELIMAR[2], and Jasna JURASOVIĆ[1]

Institute for Medical Research and Occupational Health[1], University Hospital Centre Zagreb[2], Zagreb, Croatia

In some patients with metal-on-metal hip implant systems, particularly when cast molded Cr-Co alloys were used, the joints wear increase debris formation to a significant level. Increased circulating metal ions could reach high levels in blood and serum and therefore adversely affect patient's health. We assessed Co and Cr status in 20 patients after hip arthroplasty (aged 63±11.4 years; >1 year after operation). To estimate the most appropriate biomarker of Co and Cr status, we analyzed levels of Co and Cr in different blood fractions (whole blood, serum and erythrocytes) using ICP-MS. Median and range blood, serum and erythrocyte Co levels were 1.38 μg L^{-1} [(0.21 to 134) μg L^{-1}], 1.53 μg L^{-1} [(0.21 to 113) μg L^{-1}] and 0.46 μg L^{-1} [(0.09 to 50.9) μg L^{-1}] of blood, while Cr levels were 2.05 μg L^{-1} [(0.74 to 42.1) μg L^{-1}], 2.64 μg L^{-1} [(0.76 to 68.6) μg L^{-1}] and 0.55 μg L^{-1} [(0.33 to 1.34) μg L^{-1}] of blood. Concentrations of the metal between blood fractions were highly correlated (R=0.83 to R=0.99; $p<0.001$). The results have shown that Co is distributed equally between plasma and red blood cells, while Cr ions are in very low concentrations in red blood cells. As only highly toxic Cr(VI) form crosses cell membranes easily, our results suggest rather Cr(III) than Cr(VI) exposure in patients with Cr-alloy hip implants. Both serum and whole blood could be used for assessment of Co and Cr status and metal wear debris rate formation in patients with metal-on-metal hip implants. However, because of dominant Cr distribution in serum and easier measurements using simpler matrix, serum might serve as a better indicator of the true metal status and implant failure.

KEY WORDS: *erythrocytes, ICP-MS, levels of Co and Cr, metal-on-metal hip implants, serum, whole blood*

P-20

ASSESSMENT OF DIETARY METHYLMERCURY INTAKE AND BLOOD MERCURY VALUES IN WOMEN FROM CONTINENTAL *VERSUS* COASTAL CROATIA

Antonija SULIMANEC[1], Ankica SEKOVANIĆ[1], Tatjana ORCT[1], Sandra STASENKO[2], Judita GRZUNOV[3], Marijana MATEK SARIĆ[4], Jasna JURASOVIĆ[1], and Martina PIASEK[1]

Institute for Medical Research and Occupational Health[1], University Hospital Merkur[2], Zagreb, Institute of Public Health Zadar[3], University of Zadar[4], Zadar, Croatia

Fish is nutritionally highly valuable source of omega-3 fatty acids, proteins, vitamins, and selenium, which are essential for optimal cardiovascular and brain function. It is beneficial to maternal and foetal health during pregnancy. Fish and seafood are also main sources of exposure to methylmercury, a highly toxic mercury form, which bears potential to affect early brain development with immediate neurodevelopmental or delayed neurobehavioral effects. Cultural tradition, cost, and fish availability determine food patterns in different country areas. Our investigation aimed at assessing dietary intake of methylmercury in 290 healthy postpartum women (29±4.7 years) from the continental (Zagreb area, n=194) and coastal part (Zadar County, n=96). The participants filled out a short questionnaire by self-estimating monthly consumption of fish, shellfish, and canned fish. Estimated portion sizes were: fresh fish ca. 175 g; shellfish 50 g; canned fish 80 g. Personal and lifestyle data were also recorded. Total mercury (T-Hg) in maternal venous blood, determined by AAS and ICP-MS methods, was used as a biomarker of mercury exposure. Even though patterns of fish consumption did not vary, maternal T-Hg was significantly higher in women from coastal than those in continental Croatia; consumption: 23 *vs.* 17 g fresh fish day^{-1}; T-Hg: 2.75±3.40 μg L^{-1} *vs.* 0.9 3±0.79 μg L^{-1}. Multiple regression results showed that fish consumption was dominant source of maternal mercury exposure. In conclusion, to provide reliable data about mercury exposure through fish consumption, the questionnaire should be improved to include detailed instructions on how to assess the exact portion size and consumed seafood species.

KEY WORDS: *fish consumption, food questionnaire, mercury exposure, pregnancy*

P-21

THE EFFECT OF CADMIUM AND COPPER ON LARVAL DEVELOPMENT OF IDE *LEUCISCUS IDUS* L.

Malgorzata WITESKA and Ewelina KOWAL

University of Natural Sciences and Humanities, Siedlce, Poland

Ide (*Leuciscus idus* L.) is a cyprinid fish of relatively high environmental requirements. However, sensitivity of this species to toxic agents is almost unknown. The aim of present study was to evaluate toxicity of cadmium and copper to the ide larvae. The fish were exposed to cadmium or copper (0.1 mg L^{-1}) during embryonic (CdK and CuK) or larval development alone (KCd and KCu) or during the entire early development (embryonic and larval: CdCd and CuCu). The control group was constantly kept in metal-free water (K). Fish survival, growth (as body length and body perimeter area) and development (yolk sac and swim bladder perimeter areas) were measured during 30 days of larval development. Ten fish from each group were photographed daily and all measurements were done in scaled photographs. The fish were weighed at the end of the experiment. Both metals caused a significant decrease in larval survival, even if the ide were subjected to intoxication only during the embryonic period. Growth and development retardation was also observed in the intoxicated groups: yolk utilisation and swim bladder inflation slowed down and the final body mass lowered in all metal-exposed groups compared to the control. The obtained results showed that cadmium was more toxic to the ide larvae than copper. Lower larval mortality and higher growth and development indices in CuCu group compared to KCu indicate that exposure to this metal during embryonic development induced acclimation (probably by early activation of detoxication mechanisms such as metallothionein synthesis), which was not observed in the case of cadmium.

KEY WORDS: *ide larve, heavy metal toxicity, teratogenesis*

P-22

INFLUENCE OF SELENIUM PRETREATMENT ON ESSENTIAL ELEMENT CONTENT AND LIPID PEROXIDATION IN SUCKLING RATS EXPOSED TO INORGANIC MERCURY

Tatjana ORCT, Maja BLANUŠA, Maja VIHNANEK LAZARUS, Ankica SEKOVANIĆ, Jasna JURASOVIĆ, and Martina PIASEK

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Concentrations of copper, zinc, iron, and manganese in body tissues reflect the nutritional status of laboratory animals. The changes in these essential metals may reflect nutritional deficit or interaction with toxic metals and metalloids. Lipid peroxidation can serve as a biomarker of cellular oxidative stress and has been observed after increased mercury Hg^{2+} or selenium Se^{4+} exposure. We investigated the effect of selenium oral supplementation together with oral mercury exposure on the level of essential metals and lipid peroxidation in tissues of neonatal rats. Wistar suckling rats, seven days old, were pretreated with selenium (Na_2SeO_3, 0.51 mg Se kg^{-1} b. w. day^{-1}) during three days. Following four days, equimolar amounts of mercury ($HgCl_2$, 1.29 mg Hg kg^{-1} b. w. day^{-1}) were given together with selenium. Pups were euthanized 24 h after the last dose. Their liver, kidneys and brain were dissected and tissue was prepared for the analyses. Copper and zinc were measured by atomic absorption spectrometry after digestion with nitric acid. Amount of lipid peroxides (determined by malondialdehyde, MDA) in tissue homogenates was quantified using the thiobarbituric acid reactive substances (TBARS) assay. The $HgCl_2$ exposure alone increased concentrations of Cu and Zn in kidneys while oral supplementation with Na_2SeO_3 completely prevented these increases. Exposure to mercuric chloride significantly increased TBARS production in hepatic and brain tissue preparations; this effect was only partially prevented by oral selenium supplementation. To conclude, our results show that cautious oral selenium supplementation could prevent the toxic effects of inorganic mercury in mammals at a very early age.

KEY WORDS: *essential micronutrients, mercury exposure, oral selenium supplementation, TBARS assay*

P-23

EFFECT OF MAGNESIUM PRETREATMENT ON WHOLE CADMIUM POOL IN ORGANS OF MICE EXPOSED TO ACUTE CADMIUM INTOXICATION

Danijela ĐUKIĆ ĆOSIĆ, Zorica BULAT, Aleksandra BUHA, and Vesna MATOVIĆ

Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

It is well known that toxic metal cadmium (Cd) is involved in all of the major diseases of our time, including cancer, diabetes, arthritic syndromes, heart and kidney disease, etc. However, experimental studies indicate that bioelements may antagonise some of Cd toxic effect. The aim of this study was to investigate the influence of increased oral magnesium (Mg) pretreatment on the whole Cd pool in the organs of mice exposed to acute Cd intoxication. The experiment was performed on Swiss albino mice divided into two groups: a Cd group of animals (controls) was given a single oral dose of 20 mg kg^{-1} b. w. of Cd and a Mg + Cd group of mice pretreated with 40 mg kg^{-1} b. w. of Mg 1 hour before Cd intoxication. Cadmium concentration in mineralised organs was determined by AAS 4, 6, 12, and 24 hours after Cd intoxication. Using the concentrations of Cd assessed in the soft tissues, we calculated the whole Cd pool (kidneys + liver + heart + lung) for each investigated interval. The obtained results show that the whole pool of Cd in organs of Mg + Cd group was lower after 4 h, 6 h, and 24 hours by 3 %, 10 %, and 15 %, respectively if compared with the group exposed to Cd only. Contrary to this, after 12 hours, the total Cd content in the studied organs of co-exposed mice was higher if compared with the Cd group. These results suggest that supplementation with Mg can reduce Cd absorption and prevent deposition of this toxic metal. (Partly supported by the Ministry of Education and Science, Grant III46009)

KEY WORDS: *acute intoxication, atomic absorption spectrometry, supplementation with magnesium, toxic metal*

P-24

THE EFFECT OF CADMIUM AND COPPER ON HEMATOPOIETIC ACTIVITY IN COMMON CARP *CYPRINUS CARPIO* L.

Elżbieta KONDERA

University of Natural Sciences and Humanities, Siedlce, Poland

Groups of common carp (21.6±8.3) g were exposed for four weeks to waterborne cadmium (0.65 mg L^{-1}) or copper (0.075 mg L^{-1}). The concentrations of both metals were 10 % of 96 h LC_{50} previously calculated from the results of the survival test. Five fish from each group were killed weekly and main hematopoietic organs - head kidneys were sampled. Proliferating and apoptotic hematopoietic precursor cells were detected using immunocytochemical methods with monoclonal anti-PCNA or anti-caspase 3 antibodies, respectively. The cells undergoing proliferation or apoptosis were counted, and their frequency was calculated for 500 hematopoietic cells in each preparation. Additionally, the frequency of early blast cells was calculated in head kidney preparations stained with May-Grünwald and Giemsa solutions. Peripheral blood was also collected and subjected to standard hematological analysis. Both metals, copper and cadmium, caused a similar and significant increase in the rate of precursor cell proliferation and apoptosis, which was accompanied by an increase in the frequency of early blast cells. However, increase in cell death rate was stronger than induction of cell division, which resulted in a significant reduction of hematopoietic activity. On the other hand, precursor cell turnover rate (proliferation/apoptosis) was in all cases higher than 1 and no visible structural damage was observed. No significant reduction in basic peripheral blood parameters occurred. The obtained results indicate that fish hematopoietic system is sensitive to sublethal intoxication with heavy metals but also shows a high homeostatic potential.

KEY WORDS: *apoptosis, fish, head kidney, sublethal intoxication with heavy metals*

P-25

OCCUPATIONAL EXPOSURE TO TOXIC METALS IN THE GALVANISATION PROCESS

Vesna LAZAREVIĆ[1], Ivan KRSTIĆ[2], and Snežana IVEZIĆ ĐORĐEVIĆ[3]

Institute for Preventive and Medical Protection[1], Faculty of Occupational Safety, University of Niš[2], Niš, Poison Control Centre, Military Medical Academy, Belgrade[3], Serbia

Occupational poisoning with toxic metals in the galvanisation process is almost exclusively of chronic character, which has brought about a need to study their harmful effects. In the process of galvanisation, toxic metals occur in the form of toxic dust and vapours, both in the elementary and oxide forms. In order to analyse the harmful effects of toxic metals, an investigation of human material has been performed. The data have been collected from the annual reports of the department of social medicine and statistics, the data from patients′ medical records in primary health care and employee health care, as well as expert findings of the Public Health Institute in Nis. The concentrations of toxic metals in the human material have been determined by atomic absorption spectrophotometry. Statistical analysis of the results has been performed by the software packages Excel, MATLAB, and SPSS19.0. The research has shown that the increased concentration of toxic metals in human material causes negative health effects to the exposed groups, which is an important indicator of toxicological risks.

KEY WORDS: *harmful effects of toxic metals, occupational poisoning, toxicological risks*

P-26

ANALYSIS OF SYNTHETIC DYES IN SELECTED CONFECTIONERY

Jozef GOLIAN[1], Róbert TOMAN[2], and Svätoslav HLUCHÝ[2]

Faculty of Biotechnology and Food Sciences[1], Faculty of Agrobiology and Food Resources[2], Slovak University of Agriculture, Nitra, Slovak Republic

In this study, samples of confectionery containing synthetic dyes were analysed. Analyses were carried out in 2009 to 2011. Samples were analysed by qualitative and quantitative methods using high-performance liquid chromatography (HPLC). In 2009, 101 samples were analysed for the content of synthetic dyes: seven samples contained an amount of synthetic dyes under the limit of quantification (LOQ) and no sample contained synthetic dyes above the value of 100 mg kg^{-1}. In 2010, 82 samples were analysed: in five cases the content of synthetic colours in confectionery was below LOQ and four samples had a value of 100 mg kg^{-1}. The most frequent dye was E 124, which was found in four cases, and three samples were found to contain dyes E 104 and E 110. In 2011, 109 samples were analysed: the dye content in 10 of them was determined to be below 100 mg kg^{-1} and no sample contained synthetic dyes above the value of 200 mg kg^{-1}. The dye E 133 was detected in seven samples and the dye E 129 in two samples. Our results show that none of the synthetic dyes exceeded the maximum permissible limits in analysed confectionery. These results indicate the decreasing trends in synthetic dye occurrence in confectionery and therefore an improvement in toxicological safety for consumers.

KEY WORDS: *food additives, food safety, high performance liquid chromatography, synthetic colour*

P-27

RESVERATROL REDUCES OXIDATIVE DAMAGE IN THE RAT LIVER CAUSED BY OCHRATOXIN A AND CITRININ

Dubravka RASIC and Maja PERAICA

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Humans and animals are continuously exposed to mycotoxins ochratoxin A (OTA) and citrinin (CTN) which are often found together because they are produced by the same strains of moulds. One of the possible mechanisms of their toxicity is the increase of reactive oxygen species and induction of oxidative stress. It is already known that one of the best known antioxidants is resveratrol (RSV) a polyphenol found in grapes and red wine. The aim of our study was to check whether RSV treatment could decrease the oxidative stress in OTA and CTN treated animals. In this study, adult male Wistar rats were given orally daily for 21 days RSV only, and combination of RSV, OTA and CTN. The results were compared with those obtained in animals given the combination of OTA and CTN. All animals were sacrificed 24 hours after the last treatment and liver tissue was collected and frozen until the analysis of malondialdehyde (MDA). The concentration of MDA in the liver of rats treated with RSV was lower than in controls given 51 mmol L^{-1} $NaHCO_3$ (0.482±0.063) µmol g^{-1} of tissue and (0.666±0.042) µmol g^{-1} of tissue, respectively. MDA concentration in the liver of animals treated with mycotoxins and RSV was higher than in rats treated only with RSV (0.538±0.066) µmol g^{-1} of tissue in treated animals, but lower than in control rats treated with 51 mmol L^{-1} $NaHCO_3$ (0.666±0.042) µmol g^{-1} of tissue. These results indicate that RSV may significantly reduce oxidative lipid damage caused by OTA and CTN.

KEY WORDS: *antioxidants, high performance liquid chromatography, mycotoxins, Wistar rats*

P-28

SAMPLING AND HOMOGENISATION AS THE MOST IMPORTANT STEPS IN ANALYSING MYCOTOXINS IN FOOD

Jasna BOŠNIR, Maja KOVAČ, Dinko PUNTARIĆ, Dario LASIĆ, Slavko ANTOLIĆ, and Sanja OŽIĆ

Institute of Public Health "Dr. Andrija Štampar", Zagreb, Croatia

Mycotoxins are one of the most important parameters on food safety. The analytical procedures for determining the presence of mycotoxins in foods are not simple. This is why today great attention is paid to proper sampling, sample homogenisation, and division of bulk sample in laboratory samples. This is especially evident when it comes to huge shipments (tens of tons), and the shell fruit. Because of significant damage, respecting all points of the analytical procedure is of great importance. This article described the procedure for sampling peanut shell samples of the series weighing 50 tons and the procedures that had to be implemented for the purpose of proper homogenisation of the bulk sample and the distribution of laboratory specimens. The procedures were performed in accordance with the existing legislation, the EU recommendations, and scientific literature. The process of homogenisation of the sample used involved the so-called "slurry mixing" method. The described sampling and homogenisation processes proved to be both demanding and reliable methods for obtaining safe results. Also, division of the bulk sample in laboratory samples, whose number depends on the mass of the bulk sample and the results obtained on the presence or absence of mycotoxins in them, unambiguously point to the correctness or incorrectness of the sampled series.

KEY WORDS: *analytical procedure, food safety, presence of mycotoxins in food, sampling procedure*

P-29

ISOLATION METHODS FOR MYCOTOXINS - THE GOOD, THE BAD AND THE UGLY

Bojan ŠARKANJ

Faculty of Food Technology, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

In most cases, isolation methods for mycotoxins are composed of several processes: sample preparation, mycotoxin extraction, purification, and concentration. Due to low concentration of mycotoxins in food, analysis is impossible without sample pre-treatment, excluding enzyme linked immunosorbent assay (ELISA) and highly sensitive LC-MS/MS devices, following a simple dilute-and-shoot method. Sample preparation is one of the most important steps, which must meet all analytical criteria and legislation requirements. There are different strategies used to extract mycotoxins dependent on the sample matrix and targeted mycotoxin physicochemical properties. Since raw extracts can contain a lot of impurities from sample matrix, they can be removed using various approaches. The most common are: solid phase extraction (SPE) in the form of immunoaffinity columns (IAC), ion exchange columns (IEC) filled with adsorbents or normal phase (NP) reverse phase (RP) filled solid phase columns. Newer SPE techniques comprise the usage of aptamers (short DNA or RNA strains which are able to detect target molecules) and molecular imprinted polymers (MIP). There is also a possibility of liquid/liquid extraction (LLE), which is used mainly for complex matrices due to some difficulties such as the use of a high amount of organic and chlorinated solvents, formation of emulsions, and co-extraction of impurities. There is no simple answer as to which isolation method is the best for your mycxotoxin(s) of interest - it depends on its physicochemical properties and used detection method, but generally they can be classified as "the good, the bad, and the ugly".

KEY WORDS: *detection of mycotoxins in food, extraction methods, sample preparation*

P-30

URINE OCHRATOXIN A AND OCHRATOXIN ALPHA IN PREGNANT WOMEN FROM CROATIA

Tomislav KLAPEC[1], Bojan ŠARKANJ, Ines BANJARI[2], Ivica STRELEC[1], and Novela MARINOVIĆ VITEZ

Faculty of Food Technology, Josip Juraj Strossmayer University of Osijek, Osijek[1], Belje Inc., Darda[2], Croatia

This study determined exposure of pregnant women to ochratoxin A (OTA). Forty samples of first-void urine samples from Croatian women in the third trimester of pregnancy were analysed for OTA and its major metabolite ochratoxin alpha (OTα). The subjects filled a short food frequency questionnaire (FFQ). Analysis was performed by HPLC-FLD following liquid-liquid extraction. All samples were subjected in parallel to enzymatic treatment (β-glucuronidase / aryl sulfatase) to release OTA and OTα from the conjugates. The median urinary levels of OTA and OTα before treatment were 0.02 ng mL^{-1} [range: (nd to 1.07) ng mL^{-1}] and 0.16 ng mL^{-1} [(nd to 1.86) ng mL^{-1}]. The concentrations after enzyme hydrolysis were 0.02 ng mL^{-1} [(nd to 1.11) ng mL^{-1}] and 1.18 ng mL^{-1} [(0.11 to 7.57) ng mL^{-1}]. While OTα levels increased significantly following enzymatic treatment, evidence for OTA conjugation was weak. The ratio of urinary OTα medians after and before hydrolysis was 1.5 times higher than previously reported for nonpregnant female subjects, possibly indicating upregulated metabolism and/or elimination of mycotoxins and metabolites in pregnancy. The mean daily dietary OTA intake calculated from FFQs [(1.08±0.57) ng kg^{-1} b. w.] was well below the provisional tolerable daily intake and the greatest contributors to intake were cereal products, fruit juices, chocolate, and coffee.

KEY WORDS: *detection of ochratoxins, HPLC-FLD method, mean daily dietary intake, pregnancy*

P-31

ORGANOCHLORINE PESTICIDES (OCPs) AND POLYCHLORINATED BIPHENYLS (PCBs) IN *MURAENA HELENA* FROM THE ADRIATIC SEA NEAR DUBROVNIK, CROATIA

Domagoj ĐIKIĆ[1], Ana MOJSOVIĆ ĆUIĆ[2], Gordana JURAK[3], Dario LASIĆ[3], Daria SKARAMUCA[1], Sanja MATIĆ SKOKO[4], Pero TUTMAN[4], Jasna BOŠNIR[3], and Boško SKARAMUCA[5]

Faculty of Science, University of Zagreb[1], University of Applied Health Studies[2], Institute of Public Health "Dr Andrija Štampar"[3], Zagreb, Institute of Oceanography and Fisheries, Split[4], University of Dubrovnik[5], Dubrovnik, Croatia

The work demonstrates why Moray eels are good bioindicator organisms for the local studies of bioaccumulation or biomagnifications. Morays were caught in the East Adriatic Sea near Dubrovnik in what is believed to be an unpolluted area. Not a single animal was recorded to be free of OCPs or PCBs. Regardless of the season, minimally 5 to 11 pollutants were detected in each fish in the summer and 5 to 9 in the winter period. Out of these, at least 1 to 5 were PCB congeners in the summer and 3 to 4 congeners in the winter caught fish. Levels of bioaccumulated p-DDT were similar to those in other biota caught from the Italian side of central and southern Adriatic or same as average values for the Mediterranean biota. Other OCPs had lower (but detectable) levels than in the rest of the Adriatic or Mediterranean (endrine > ppDDE > heptachloreepoxide >heptachlore > aldrine > lindane > βHCH > δHCH > endrine aldehyde). During winter season, 20 % higher concentrations of endrine were present in fish. Aldrine was detected only in the summer caught fish while δHCH and endrine aldehyde were detected only in the winter caught fish. Other OCPs were present in both seasons in different proportions. We detected PCB congeners 77, 105, 114, 118, 125, 156, 169 which is again fewer than the usual average in the fish of the Adriatic and Mediterranean. PCB 105 and 169 were the only two congeners found in the same proportion in caught fish regardless of the season. A congener 169, which is considered to be of a particular hazardous potential, was detected in all analysed fish regardless of the season.

KEY WORDS: *bioindicator organism, Moray eel, PCB congeners, pollutants, summer, winter*

P-32

TOXICITY ASSOCIATED WITH THE RECOVERY OF BIOGENIC AMINES AND ENDOTOXIN FROM MARINE SOURCES

Ljerka PRESTER[1], Dubravka KIPČIĆ[2], Jelena VUKUŠIĆ[2], Tatjana ORCT[1], and Jelena MACAN[1]

Institute for Medical Research and Occupational Health[1], Croatian National Institute of Public Health[2], Zagreb, Croatia

Decomposition of fish and shellfish is associated with the recovery of bioactive compounds such as biogenic amines and endotoxins. It is associated with adverse reactions in humans due to ingestion, inhalation or skin contact with contaminated fish or fish products. Long-term persistence of histamine (>3 years) in stored fish-meal was recorded, confirming it as long-term health hazard for animals and workers manipulating it. Biogenic amines (histamine, putrescine, tyramine, cadaverine) are indicators of spoilage of seafood. Endotoxin is a lipopolysaccharide (LPS) component of the wall membrane of Gram-negative bacteria, and can be used for the estimation of bacterial contamination in food. In our studies, the level of selected biogenic amines and endotoxin were measured in finfish (sardine, mackerel, hake), cephalopods (squid, musky octopus), crustaceans (Norway lobster) and bivalves (mussels) caught off the Adriatic coast. All fresh fish and shellfish samples had low levels of all biogenic amines and no elevated endotoxin load (<1 EU mg^{-1}). After 12 h at room temperature, histamine was highest in dark-muscle fish (mackerel and sardine, range 40 mg kg^{-1} to 67 mg kg^{-1}) and exceeded the permissible level of 50 mg kg^{-1} established by Food and Drug Administration. At the same time, the histamine levels were significantly lower in whitefish (hake) and shellfish, with putrescine and cadaverine as the most objective indicators of quality for these organisms. After 12 h, endotoxin level increased in all fish and shellfish species indicating spoiled condition. These results pointed out the importance of proper seafood storage (<8 ºC) and potential of endotoxin as an indicator of freshness.

KEY WORDS: *biogenic amines level in seafood, bivalves, cephalopods, crustaceans, endotoxin load, finfish*

P-33

VIRGIN OLIVE OILS - HEALTHY OR HARMFUL EFFECT IN LIGHT OF EVERYDAY LIFE

Zlatka KNEZOVIĆ, Jadranka MARUŠIĆ, Angela STIPIŠIĆ, Sanja LUETIĆ, and Zrinka MAJIĆ

Public Health Institute, Split, Croatia

Virgin olive oils are the oils obtained from olives only by mechanical procedures (without using enzymes or organic solvents for extraction). Their quality is prescribed by the Ordinance on Olive and Olive Pomace Oils (Official Gazette 7/2009). Nutritionists and cardiologists often recommend them as the main source of fat for a balanced ratio of unsaturated fatty acids, and as a significant source of protective substances (polyphenols, tocopherols, carotenoids). Numerous papers have been published on the positive effect of virgin olive oils, especially in the prevention of cardiovascular diseases. Different studies on experimental animals confirmed the absorption of fatty acid oxidation products and secondary products of oxidation in the liver and consequent hypertrophy and dysfunction of the liver; the inhibitory effect of hydroperoxides on some enzymatic systems, etc. Even if there is no absorption, the intake of oxidised food is a risk for intestinal mucosa damage. What is the real state of play of virgin olive oil market in our country? This paper presents the results of quality parameters determination in the samples of virgin olive oil on the Croatian market - with an emphasis on the Split-Dalmatia County. Analysed oils were either part of the official controls, or were those which producers/consumers brought on their own for the analysis. The parameters taken as the indicators of quality were free fatty acids (oleic), peroxide value, absorbency in the UV light, and sensory analysis. All methods were standardised.

KEY WORDS: *health impact, oxidation, quality of virgin olive oil*

P-34

TESTING HAIR FOR DRUGS OF ABUSE - FOURTEEN YEARS OF OUR EXPERIENCE

Irena BRČIĆ KARAČONJI and Nataša BRAJENOVIĆ

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Hair analysis for drugs of abuse has become very popular because it provides information on long-term exposure to illegal drugs over a period of few weeks to months, depending on the length of hair collected. Since 1999, we have analysed at the Institute over 1000 hair samples for different drugs of abuse. Gas chromatography/mass spectrometry (GC/MS) methods have been developed for determining opiates (heroin, 6-acetylmorphine (6-MAM), codeine, morphine), methadone, cocaine, and amphetamines [amphetamine, methamphetamine, 3,4-methylene-dioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA-Ecstasy), 3,4-methylenedioxyethylamphetamine (MDEA)] in hair. Hair samples were taken in the Unit (39 %), delivered by mail (20 %) or a third person brought them to the Unit (41 %). Samples were identified only by code in 69 % of cases because subjects, who belong mainly to the younger population, wanted to remain anonymous. In 29 % of hair samples, one or more illegal drugs were detected. Considering single drug uses, the highest percentage of positive hair samples was found for MDMA (Ecstasy) (18 % of all positive samples) and cocaine (18 %), followed by heroin (confirmed by positive 6-MAM) (11 %) and amphetamine (9 %). Two or more substances from different groups were detected in 17 % of positive hair samples. The most frequent combinations were amphetamines with cocaine (6 % of all positive samples) and opiates with cocaine (3 %). Our experience has shown that interest in hair testing for drugs of abuse in Croatia continues to increase.

KEY WORDS: *amphetamines, cocaine, gas chromatography/mass spectrometry, hair analysis, opiates*

P-35

MASS SPECTROMETRIC METHOD FOR DETERMINING 11-NOR-Δ^9-TETRAHYDROCANNABINOL-9-CARBOXYLIC ACID IN URINE

Snezana DJORDJEVIC[1], Krsta SULIC[2], Marijana CURCIC[3], Vesna KILIBARDA[1], Dragana RANCIC[1], Gordana BRAJKOVIC[1], Nadezda KRSTIC[1], Natasa PERKOVIC VUKCEVIC[1], and Jasmina JOVIC STOSIC[1]

Poisoning Control Centre, Military Medical Academy, Belgrade[1], Public Health Institute, Pozarevac[2], Faculty of Pharmacy, University of Belgrade[3], Belgrade, Serbia

Psychoactive substance abuse has increased in recent years. The detection of five most commonly abused drugs (opiates, cannabinoids, metamphetamines, cocaine, and benzodiazepines) is performed on urine samples, because of their fast metabolism and low concentration in blood. The first step in the analysis of these compounds is immunochromatographic test. Each positive result has to be confirmed by analysing samples using some of chromatographic techniques. We have developed a liquid chromatography with mass spectrometry (LC-MS) method for determining 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) as a major THC metabolite in urine. Hydrolysis of glucuronide from urine samples was performed with KOH at 60 °C. After adjusting pH at 3 and adding 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid-D3 as an internal standard, THC-carboxylic acid was extracted with a mixture of n-hexan-ethylacetate (7:1). THC-COOH was determined by LC-MS method in the single-ion monitoring mode (SIM) at m/z 345, 327, and 299 for THC-COOH, and 348, 330, and 302 for IS. Chromatographic separation was performed on XTerra®RP18 column, using a gradient of acetonitrile/acetic acid 1 % and acetate buffer pH 3.5 as the mobile phase. Linearity was achieved in the range 10 ng mL^{-1} to 100 ng mL^{-1}. Retention times of THC-COOH and IS were 21.54 minutes and 21.52 minutes, respectively. Limit of detection and limit of quantitation for THC-COOH were 2.65 ng mL^{-1} and 8.82 ng mL^{-1}, respectively. Coefficient of variation was 7.62 %. The described LC-MS method for the analysis of THC-COOH in urine is a precise, accurate, reproducible, and reliable method for confirming cannabis abuse.

KEY WORDS: *cannabis abuse, development of LC-MS method, limit of detection, limit of quantification*

P-36

THE EFFECT OF METHAMPHETAMINE ON ATAXIA AND BRAIN MALONDIALDEHYDE LEVELS IN THE RAT MODEL OF DOPAMINERGIC NEUROTOXICITY

Jelena ŠURAN

Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

A single, neurotoxic methamphetamine dose administered to rats represents a valuable rodent model of dopaminergic neurodegeneration whose pathogenesis is associated with lipid peroxidation in the brain, especially in nigrostriatum, and is characterised by ataxia and impaired motorics. The effect of three neurotoxic doses of methamphetamine [(10, 20, and 40) mg kg^{-1} b. w., *s.c.*] on development of ataxia and malondialdehyde (MDA) levels in striatum, frontal cortex, cortex, hippocampus, diencephalon, and cerebellum of thirty Wistar rats was evaluated. Atactic phenotype was scored 24 hours after methamphetamine administration. Total MDA in brain tissue supernatants was measured using high performance liquid chromatography with UV detection. Atactic phenotype was dose dependent, being more pronounced in animals administered 40 mg kg^{-1} (9±2) mg kg^{-1}, mean score ± standard deviation) than in animals administered 20 mg kg^{-1} (8.25±0.9) mg kg^{-1} and 10 mg kg^{-1} (7±1.4) mg kg^{-1} of methamphetamine. Methamphetamine decreased MDA levels in most brain regions; the lowest level was measured 2 hours after methamphetamine dose of 40 mg kg^{-1} in striatum [(12.8±2.4) nmol g^{-1} of tissue] in comparison to saline-injected control [(27.2±2.6) nmol g^{-1} of tissue], but it increased 24 hours later [(24.9±4.4) nmol g^{-1} tissue]. Similar trend was present after methamphetamine doses of 20 mg kg^{-1} and 10 mg kg^{-1}, however mainly without exceeding control levels significantly. These results question the role of MDA in the mechanisms of methamphetamine-induced neurotoxicity, but nevertheless confirm the effectiveness of these mechanisms in rendering an animal model of monoamine disruption with a pivotal role in the development of new therapeutic approaches to Parkinson disease.

KEY WORDS: *ataxia, central nervous system stimulant drug, lipid peroxidation, rodent model of dopaminergic neurodegeneration*

P-37

BENCHMARK DOSE DERIVATION FOR THE EFFECTS OF BDE-209 ON THYROID HORMONE LEVELS IN RAT SERUM

Vesna MILOVANOVIC[1], Marijana CURCIC[2], Aleksandra BUHA[2], Vesna JACEVIC[3], Slavica VUCINIC[3], and Biljana ANTONIJEVIC[2]

Serbian Chemicals Agency[1], Faculty of Pharmacy, University of Belgrade[2], Poison Control Centre, Military Medical Academy[3], Belgrade, Serbia

Decabrominated diphenyl ether (BDE-209), a widely used flame retardant, is considered to be an endocrine disrupting chemical. The aim of the study was to evaluate a dose-response relationship for the effects of BDE-209 on thyroid hormone levels using Benchmark dose (BMD) approach, and to derive the BMD05 for the effects. In the study, eight groups (n=7 per group) of the adult male Wistar rats were treated orally with BDE-209 in the range of 31.25 mg kg^{-1} b. w. day^{-1} to 4000 mg kg^{-1} b. w. day^{-1} for 28 days. Thyroxine (T4), free thyroxine (fT4), triiodothyronine (T3), and free triiodothyronine (fT3) levels were determined in rat serum using commercial tests on Roche Elecsys 2010. Using PROAST software, a dose-response relationship was confirmed for the influence of BDE-209 on T4, T3, and fT3 homeostasis, but not on fT4, and associated BMD05 and their lower confidence limits (BMDLs) derived from the fitted models were: 859 mg kg^{-1} b. w. day^{-1} and 449.8 mg kg^{-1} b. w. day^{-1} for T4, 40 mg kg^{-1} b. w. day^{-1}, and 14.84 mg kg^{-1} b. w. day^{-1} for T3, and 451 mg kg^{-1} b. w. day^{-1} and 149.8 mg kg^{-1} b. w. day^{-1} for fT3, respectively. BMD05/BMDL ratio for the effects on T4, T3, and fT3 levels was under 10, which was in agreement with very recent EFSA scientific opinion. Results obtained in this study indicate that the critical effect of BDE-209 on thyroid hormone homeostasis is the one exerted on T3 levels, leading to its significant decrease towards control value.

KEY WORDS: *decabrominated diphenyl ether, dose-response relationship, thyroid hormone homeostasis, Wistar rats*

P-38

ANALYSIS OF REGISTERED ACUTE POISONINGS IN THE REPUBLIC OF CROATIA FROM 2007 TO 2010

Daniela ČEPELAK DODIG, Irena Zorica JEŽIĆ VIDOVIĆ, Franjo PLAVŠIĆ, Alka WOLF ČOPORDA, Davor GRETIĆ, and Saša ĐURAŠEVIĆ

Croatian Institute for Toxicology and Antidoping, Zagreb, Croatia

Longitudinal reports on poisoning types and dynamics serve to monitor the preventive measures against exposure to toxic substances. To analyse the data of the Croatian National Institute of Public Health (CNIPB) about the number of hospitalised persons due to acute poisoning during a four-year period, 2007 to 2010. CNIPB data about the number of poisoned hospitalised persons during the period 2007 to 2010 were used. The methods of descriptive epidemiology were implemented. The total number of hospitalised poisoned persons decreased gradually. In 2010 there were by 29 % less hospitalised poisoned persons than in 2007. Annual incidence (cases per 100,000 inhabitants) of poisoning in 2007 was 46.8, in 2008 it was 44.7, followed by a significant decrease in 2009 to 35.0. At the end of the period, in 2010, the incidence decreased to 33.3. Among the total number of persons admitted to hospitals, there were more children than adults. Most poisoning cases were caused by alcohol. In adults, the second most frequent cause were toxic animals, and in children, chemicals used in the home. The highest incidence was recorded in the following counties: Osječko-baranjska, Sisačko-moslavačka, and Požeško-slavonska, and the lowest in Dubrovačko-neretvanska. Longitudinal monitoring programmes for poisoning allow for the preventive measures to be implemented for population protection purposes.

KEY WORDS: *annual incidence of poisoning, descriptive epidemiology, poisoning types and dynamics, the counties of Croatia*

P-39

COMPARISON OF HEADSPACE SOLID PHASE MICROEXTRACTION AND LIQUID-LIQUID EXTRACTION FOR DETERMINING NICOTINE AND COTININE IN CHILDREN'S URINE BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Irena BRČIĆ KARAČONJI[1], Nataša BRAJENOVIĆ[1], and Lukrica ZIMIĆ[2]

Institute for Medical Research and Occupational Health[1], Children's Hospital Srebrnjak[2], Zagreb, Croatia

Environmental tobacco smoke (ETS) is a widespread pollutant with adverse health effects on nonsmokers. Nicotine and its main metabolite cotinine are commonly used as biomarkers of ETS exposure. Cotinine is mostly determined in urine, and appears to be the biomarker of choice for ETS exposure. Although exposure to ETS often involves determining nicotine and cotinine in urine, there are no data on the comparison of liquid-liquid extraction (LLE) and headspace solid phase microextraction (HS-SPME) as the extraction techniques for assessing the exposure to ETS. The aim of this study was to compare these two procedures used to extract nicotine and cotinine from nonsmokers' urine and use selected procedure, together with gas chromatography/mass spectrometry (GC/MS) to quantify urinary nicotine and cotinine. Both methods showed linearity in the range 1 μg L^{-1} to 200 μg L^{-1}, good accuracy (>90 %) and precision (RSD≤8.5 %), and low detection limits (0.15 μg L^{-1} to 0.25 μg L^{-1}). In comparison with LLE-GC/MS, HS-SPME-GC/MS was simpler and faster and thus applied for the quantitative analysis of nicotine and cotinine in urine samples collected from 157 children. Children exposed to environmental tobacco smoke (n=81) had significantly higher levels of nicotine ($p<10^{-10}$) and cotinine ($p<10^{-16}$) in urine than nonexposed children (n=76). No need for solvent use and sample preparation, high sensitivity and rapidity make the HS-SPME-GC/MS method more convenient for determining nicotine and cotinine in nonsmokers' urine.

KEY WORDS: *biomarkers of exposure, comparison of the extraction procedures, environmental tobacco smoke, levels of nicotine and cotinine*

P-40

ANALYTICAL CONFIRMATION OF LEVAMISOLE IN A COCAINE OVERDOSE FATALITY: CASE REPORT

Gordana BRAJKOVIĆ, Dragana RANČIĆ, Gordana BABIĆ, Vesna KILIBARDA, Milica ZLATKOVIĆ, and Nadežda KRSTIĆ

Poisoning Control Centre, Military Medical Academy, Belgrade, Serbia

Levamisole is an imidazothiazole derivative used as a veterinary anthelminthic. It has been encountered as an adulterant in street-grade cocaine. We present a case of death caused by cocaine "body-packing". The Drug Enforcement Administration (DEA) reports that levamisole is recognised as an adulterant in cocaine and is known to cause severe adverse reactions. We developed a liquid chromatography-mass spectrometry (LC-MS) method for detecting levamisole in biological fluids. This method was used to obtain results presented in the present report. A 26-year-old man was taken to the Emergency Department at the Military Medical Academy, from the airport, with no vital signs. CPR was initiated but with no response. Toxicological-chemical analysis demonstrated high concentrations of cocaine, its metabolite benzoylecgonine, and levamisole in the blood sample. The objective was to identify and quantify levamisole, cocaine, and benzoylecgonine. Their concentrations were determined using liquid chromatographic method with mass spectrometry (LC-MS). Drugs were isolated using a liquid-liquid extraction with chloroform/izopropanol (9:1). Separation was achieved on the XTerra® RP18 column with a mobile phase of 5 mmol L^{-1} ammonium formate (pH 3.5), acetonitril with 0.1 % formic acid. The extraction efficiencies of levamisole from plasma ranged from 75 % to 81 %, while the limit of detection was set at 10 ng mL^{-1}. Toxicological-chemical analysis using liquid chromatography LC-MS method identified cocaine, its metabolite benzoylecgonine, and levamisole at toxic concentrations of 5.19 mg L^{-1}, 3.11 mg L^{-1}, and 1.57 mg L^{-1}, respectively.

KEY WORDS: *cocaine "body-packing", detection of levamisole in biological fluids, liquid chromatography-mass spectrometry method*

P-41

MICROBIAL ETHANOL PRODUCTION IN *POST-MORTEM* URINE SAMPLE

Marina NESTIĆ[1], Zdravko KOVAČIĆ[1], Stjepan GUSIĆ[1], Tajana MLINAREK[1], Sanda SARDELIĆ[2], Ilza SALAMUNIĆ[3], and Davorka SUTLOVIĆ[4]

School of Medicine, University of Zagreb[1], Zagreb, Department of Clinical Microbiology[2], Department of Clinical Diagnostic Laboratory[3] Department of Forensic Medicine[4], University of Split, Split, Croatia

We present a case in which *post-mortem* blood ethanol concentration was 0.02 g kg^{-1} and acetone concentration was 0.51 g kg^{-1} while urine ethanol concentration was 6.0 g kg^{-1} and acetone concentration was 0.63 g kg^{-1}. The presence of acetone in blood and urine indicated the presence of ketoacidosis, which can lead to ketotic diabetic coma. The concentrations acetone may reach in diabetics are almost independent from the *post-mortem* interval. Sodium fluoride was not added to the urine sample in which we continued to observe a remarkable increase in isopropyl alcohol concentration. The result was not logical and suggested that the alcohol concentration in urine was not due to consumption of alcoholic beverages. External contamination was excluded. Glucose levels were determined a few days after blood and urine samples were collected. Species of bacteria and yeasts, including *Candida albicans*, were isolated from both samples. A few days after the autopsy, we received the information that the patient suffered from *Diabetes Mellitus* and did not receive insulin therapy regularly. To prevent *post-mortem* ethanol production and thus making wrong assumptions of the cause of death, it is necessary to add sodium fluoride in blood and urine samples from patients with diabetes.

KEY WORDS: *ethanol and acetone concentrations in blood and urine, ketoacidosis, prevention of post-mortem ethanol production, sodium fluoride*

P-42

TRENDS IN METHADONE-RELATED DEATHS IN SPLIT-DALMATIA COUNTY

Davorka SUTLOVIĆ[1], Ivo VISKOVIĆ[2], Helena ŠUŠNJAR[2], Maja VERŠIĆ[2], Marija DEFINIS GOJANOVIĆ[1], Antonio ALUJEVIĆ[1], and Šimun ANĐELINOVIĆ[1,3]

Department of Forensic Medicine[1], Department of Forensic Sciences[3], University of Split[2], Split, Croatia

Methadone has a long and successful history in the treatment of opioid addiction. In Split-Dalmatia County, 33 methadone related deaths were identified in the period from 2001 to 2011. Data on fatal intoxications among drug addicts were submitted for medico-legal autopsy and toxicological analysis. Results of chemical-toxicological testing were based on: blood, bile and urine samples, kidney and liver tissues, nose swab and hair samples. After solid phase extraction, samples were tested by gas chromatography - mass spectrometry (GC-MS). Alcohol analyses had been carried out with GC-FID (headspace). A total of 166 cases (5.3 % of all performed autopsies) showed positive results for drugs of abuse in the *post-mortem* toxicological analysis. Combined drug intoxication was identified in 32 of 45 methadone positive individuals. Benzodiazepines were found in 20 cases, among them the most frequent were diazepam, nordazepam, and oxazepam. In 13 cases, only methadone was found. Alcohol was present in 31 of 45 cases (69 %). Most methadone related death cases were identified in the last two years, 6 in 2010 and 10 in 2011. The individuals were predominantly male (93 %) and ranged from 19 to 40 years (87 %). Methadone is becoming a major cause of death among drug users. According to data from the Public Health Institute in Split-Dalmatia County, 45 % were enrolled in the methadone treatment programme. This could indicate that despite strict control, methadone is still used as an illegal drug.

KEY WORDS: *fatal intoxications among drug addicts, forensic medicine, forensic toxicology*

P-43

SPE-GC-MS DETERMINATION OF THC, THC-OH AND THC-COOH IN URINE SAMPLES

Marina NESTIĆ[1], Sandra BABIĆ[2], Dragana MUTAVDŽIĆ PAVLOVIĆ[2], and Davorka SUTLOVIĆ[3]

School of Medicine[1], Faculty of Chemical Engineering and Technology[2], University of Zagreb, Zagreb, School of Medicine, University of Split, Split[3], Croatia

Cannabis is the most commonly used illicit drug in the world. Therefore, there is a great demand for reliable and sensitive methods for determining their presence in biological samples. Its use is detected by determining the presence of the main active compound Δ9-tetrahydrocannabinol (THC) and its metabolites 11-hydroxy-Δ9-tetrahydrocannabinol (THC-OH) and 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH). An analytical method based on solid-phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS) has been developed and validated for confirming THC, THC-OH and THC-COOH in urine samples. Analytes were extracted from urine using molecularly imprinted polymer (MIP) with methacrylic acid (MAA) as functional monomer (130 mg) and THC-OH as target molecule. Before extraction, urine samples were base-hydrolysed, acidified to pH 3, and diluted to total volume of 6 mL. Elution was performed with chloroform and ethyl acetate (60:40 v/v). Dry extracts were silylated with BSTFA+1 % TMCS at room temperature for 30 minutes. One μL of silylated extract was injected to the GC-MS instrument. Detection and quantification were made in the single-ion recording mode. The developed method was linear over the range from LOQ to 150 ng mL^{-1} for all three analytes. LOD for THC, THC-OH, and THC-COOH was 2.5 ng mL^{-1}, 1.0 ng mL^{-1}, and 1.0 ng mL^{-1}, and LOQ was 3 ng mL^{-1}, 2 ng mL^{-1} and 2 ng mL^{-1}, respectively. The precision, accuracy, recovery, and matrix effect were investigated at 5 ng mL^{-1}, 25 ng mL^{-1}, and 50 ng mL^{-1}. In the investigated concentration range, recoveries were 76.1 % to 79.4 % for THC, 77.7 % to 81.9 % for THC-OH and 68.1 % to 72.8 % for THC-COOH. Optimised and validated method was successfully applied to 15 *post-mortem* urine samples positive to cannabinoides.

KEY WORDS: *development and validation of analytical method, extraction, metabolites, molecularly imprinted polymer, post-mortem urine samples, tetrahydrocannabinol*

P-44

POISONING BY METHANOL - ACCIDENT OR SUICIDE ATTEMPT: CASE REPORT

Maja VUJOVIĆ[1,2], Biljana MILOSAVLJEVIĆ[2], and Miroslav MILIĆ[2]

School of Medicine, University of Niš[1], Institute of Forensic Medicine[2], Niš, Serbia

Methanol poisoning accidents most frequently occur by drinking it, mistakenly believing it to be ethanol. Methanol poisoning is not due to the effects of methanol itself, but due to toxicity of its metabolites. A 47-year old female was found collapsed in the afternoon at her house by her husband. A bottle with unknown liquid was found near her body. On arrival at hospital she was comatose. She was intubated and subjected to oxygen inhalation, and her stomach was lavaged. The gastric lavage solution did not give any characteristic smell of alcohol. One hour after admission, the arterial blood showed pH at 6. Fluids and electrolytes were infused to treat metabolic acidosis and ethanol was administered as an antidote. Despite five days of aggressive resuscitative efforts, she expired after she had lapsed into cardiopulmonary arrest. All samples were sent for the toxicology analysis. For the quality analysis of methanol and ethanol, HS/GC/MS methods were employed. A quantitative method is presented for GC/FID analysis in all samples using headspace extraction. The body did not reveal any signs of violent death. Unknown liquid was a liquid for washing car windows without certification. Toxicological parameters were as follows: blood: 5.96 ‰ methanol; urine: 2.74 ‰ methanol, stomach content: 5.09 ‰ methanol. Unknown liquid: 24.81 % methanol. *Post-mortem*: blood: 0.0 ‰ methanol, 0.21 ‰ ethanol; urine: 0.0 ‰ methanol, 0.13 ‰ ethanol, *vitreous humour:* 0.0 ‰ methanol, 0.24 ‰ ethanol. The examination of brain preparations determined the existence of malignant brain oedema, fresh haemorrhages around blood vessels within the brain tissue and the soft meninges. The lungs were also found to be swollen. The signs of expressed liver parenchymatous degeneration were found, and acute renal tubular necrosis was also discovered. Fresh mucous haemorrhages were found in stomach preparation. The signs of blood stagnation were evident in all organs. According to the police investigation, toxicology findings, and histopathology results the cause of death was methanol poisoning and the manner of death was accidental.

KEY WORDS: *GC/FID analysis, histopathology, liquid for washing car windows, toxicological parameters in body fluids*

P-45

POISONING IN CHILDREN - 30-YEAR TRACK RECORD IN CHILDREN'S HOSPITAL ZAGREB

Arnes REŠIĆ[1], Nenad JAKUŠIĆ[2], and Nevenka HUZJAK[3]

Department of Clinical Toxicology and Pharmacology[1], Centre for Paediatric and Adolescent Mental Health Care[2], Children's Hospital Zagreb, Zagreb, Croatia, General Hospital Brežice[3], Brežice, Slovenia

The frequency and causes of children's acute poisoning in Croatia are not sufficiently known. Assessments are made based on the hospital track records on morbidity and range between 2 % to 5 % in some institutions, and up to 9 % of all hospitalised patients are children. We are presenting the results of a monitoring programme for children hospitalised in the Department of Clinical Toxicology, Children's Hospital Zagreb, in the period 1982 to 2011 (30 years). We monitored all children hospitalised due to acute poisoning, a proportion of intentional poisoning, breakdown by age and gender, and type of means that led to poisoning. The total number of children hospitalised due to acute poisoning in the reported period was 6,415. In 91 % of cases, poisoning was accidental, the average age of patients was 5 years, and 55 % of poisoned children were boys. Intentional poisoning accounted for 9 % of all intoxications, average age of patients was 16 years, and 82 % of cases involved girls. According to the type of intoxication, most frequent was poisoning with drugs (91 %), followed by alcohol intoxications (27 %), chemicals (13 %), pesticides (5 %), inhalation agents - gas (4 %), herbs (1 %) and other (1 %). Poisoning in children's age was usually accidental and happened at home in more than 90 % of all cases. Monitoring the frequency and causes related to poisoning enables an objective assessment of children's poisoning and planning of preventive measures. For this reason, it is necessary to establish a national register of poisoning in children.

KEY WORDS: *acute poisoning, monitoring programme, Republic of Croatia*

P-46

ERYTHROCYTE GLUTATHIONE IN SUBJECTS HIGHLY EXPOSED TO ARSENIC VIA DRINKING WATER

Suzana ĆAVAR[1], Tomislav KLAPEC[2], Ivana ČEPELAK[3], and Marija MILIĆ[4]

Institute of Public Health for Osijek-Baranya County[1], Josip Juraj Strossmayer University of Osijek[2], Clinical Hospital Osijek[4], Osijek, University of Zagreb, Zagreb[3], Croatia

Glutathione is an important thiol-containing intracellular antioxidant with the fundamental role in redox homeostasis and detoxification processes. Exposure of inhabitants to arsenic-contaminated drinking water has been a major concern at several localities in eastern Croatia. We investigated whether there was a relationship between glutathione in erythrocytes and arsenic exposure. Erythrocyte total glutathione concentration (GSH), was determined in 37 subjects exposed to high levels of arsenic from drinking water [mean arsenic concentration (611.9±10.1) μg L^{-1}]. Twenty-five control subjects were exposed to low arsenic concentration in drinking water (37.9 ± 4.8) μg L^{-1}. Urinary arsenic concentration (814.40 μg L^{-1} *vs.* 27.64 μg L^{-1}) and nails (10.68 μg L^{-1} *vs.* 0.31 μg L^{-1}) were used as biomarkers of exposure. Decreased GSH levels [(2.94±1.15) μmol g^{-1} Hb] compared to control group [(5.14±1.06) μmol g^{-1} Hb] support the hypothesis of increased oxidative stress resulting from exposure to arsenic. A subsample of 20 exposed subjects took part in a 60-day supplementation trial with vitamin C (500 mg day^{-1}) and E (200 mg day^{-1}). GSH levels [(2.77±1.18) μmol g^{-1} Hb *vs.* (2.97±1.24) μmol g^{-1} Hb)] were not significantly affected by antioxidant supplementation (p=0.56; only 6 subjects had higher GSH levels after trial). The results of this study indicated that high arsenic exposure significantly lowered erythrocyte GSH levels and an ameliorating effect of antioxidant vitamins was not detected although further studies with larger sample size might be needed to confirm this.

KEY WORDS: *arsenic in water, glutathione level, vitamin supplementation*

P-47

DETERMINATION OF FLUORIDE IN FINGERNAIL CLIPPINGS OF CHILDREN USING HEADSPACE GAS CHROMATOGRAPHY

Majda ZOREC KARLOVŠEK[1], Antonela LAH[1], Špela BURNIK[1], Rok KOSEM[1], and Marija SOLLNER DOLENC[2]

Medical Faculty[1], Faculty of Pharmacy[2], University of Ljubljana, Ljubljana, Slovenia

A fast headspace gas-chromatography method was developed for monitoring fluoride exposure in fingernail clippings in children. Fluoride was extracted from fingernail clippings by the hexadimethyldisiloxane (HMDS) - facilitated diffusion in a headspace vial followed by gas chromatography/flame ionisation detection (GC/FID) of volatile trimethylfluorosilane (TMFS) and methylethylketone as an internal standard. An Agilent 7694 headspace sampler and Hewlett Packard 5890 Series II chromatograph equipped with a 6-ft x 2-mm i.d. glass column (with a temperature programme) containing 0.2 % Carbowax 1500 on Carbopack C, 60-80 mesh, allowed a good separation of TMFS (2.075 min), and internal standard (4.617 min) from other interferences (7.604 min and 11.23 min). Linear regression of peak-area ratios of TMFS to methylethylketone, versus fluoride concentration, yielded an average intraday correlation coefficient of R^2>0.998, LOD=4.7 ng mL^{-1}, LOQ=9.5 ng mL^{-1}. Within run, accuracy and precision determined at 19 ng mL^{-1}, 146 ng mL^{-1}, and 285 ng mL^{-1} did not exceed 12.5 % deviation from target and the RSD did not exceed 7.7 %. Inter day precision did not exceed 10.9 %. Fingernail clippings were obtained from a group of 6-to-8-month-old children. They were collected on two occasions: before (first sampling, n_1=205) and 4 to 6 months after the regular use of fluoridated toothpastes (second sampling, n_2=205). The fluoride concentrations in fingernail clippings for the n_1 group were in the range 0.03 ng mg^{-1} to 3.13 ng mg^{-1}, mean 0.60 ng mg^{-1} and median 0.44 ng mg^{-1}. The corresponding data for the n_2 group were in the range 0.04 ng mg^{-1} to 5.14 ng mg^{-1}; mean 0.99 ng mg^{-1} median 0.79 ng mg^{-1}. With the use of Wilcoxon paired-sample test we confirmed a statistically significant (p<0.01) increase in fluoride concentration in fingernails after a regular use of fluoride-rich toothpaste.

KEY WORDS: *accuracy, development of analytical method, fluoride exposure, increase in fluoride concentration, inter day precision, regular use of fluoridated toothpaste*

P-48

NEW DRUGS IN THE REPUBLIC OF CROATIA

Borka DROPULIĆ[1] and Davorka SUTLOVIĆ[2]

University Centre for Forensic Sciences[1], Department of Forensic Medicine[2], University of Split, Split, Croatia

The last two years have seen a record number of new substances identified for the first time in Europe. Also, in recent times in Croatia, the use of psychoactive substances has increased. In the last two years, at least 35 new substances appeared, most of which belong to three major groups. A group of synthetic cannabinoids is the largest group of new substances. A common characteristic of these substances is the functional similarity with the Δ9-tetrahydrocannabinol. The following is a group of synthetic cathinones, substances which have the structure of cathinone as a common characteristic and act as stimulants. This group also includes compounds which contain pyrrolidine ring other than cathinone structure, which makes pyrovalerone structure their common characteristic. The last significant group is a group of compounds with the basic structure of phenylethylamine. Substances in this group are divided into two subgroups, hallucinogenic and stimulant substituted phenylethylamines. Of other compounds, there are one aliphatic amine and substances whose structure is based on benzodiazepines, aminoindanes or aminobenzoates.

KEY WORDS: *compounds with the basic structure of phenylethylamine, forensic toxicology, psychoactive substances, synthetic cannabinoids, synthetic cathinones*

P-49

IMPACT OF AGE, GENDER, SMOKING, BPb, ALAD, EP, B12, AND FOLATE CONCENTRATION ON STABILE DNA DAMAGE IN THE GENERAL POPULATION IN CROATIA

Vilena KAŠUBA, Ružica ROZGAJ, and Mirta MILIĆ

Institute for Medical Research and Occupational Health, Zagreb, Croatia

It is well documented that, in addition to exposure to different genotoxic agents, DNA damage can occur "spontaneously" in healthy, nonexposed subjects. To investigate the possible influence of some common factors such as smoking, exposure to low concentrations of lead in environment, as well as age and gender, CBMN assay was used. Concentrations of folate and vitamin B12 in serum were also included due to their protective role in DNA damage. The study was conducted on a group of volunteers, 66 men and 14 women, aged 19 to 65 years (average 40 years). The frequency of DNA damage and NDI was assayed in lymphocytes obtained from peripheral blood. Poisson regression was used to analyse the association between the frequency of MN, nuclear buds, and nucleoplasmic bridges as dependent variables and age, gender, smoking, B12 and folate concentration in serum, and BPb, ALAD and EP as covariates. Both uni- and multivariate regression analyses indicated the age as a significant factor for MN and nucleoplasmic bridges. Folate and B12 showed significant influence on MN frequency in univariate analysis. Smoking (multivariate) and age (univariate) significantly lowered NDI. The exposure to lead at allowed concentrations did not show the influence on any of the examined types of damage.

KEY WORDS: *blood, cytochalasin B-blocked micronucleus assay, healthy subjects, lead in environment, lifestyle factors, vitamin status*

P-50

EVALUATION OF c-Myc AND *TP 53* GENE INTEGRITY USING FISH-COMET IN EXTENDED-TERM LYMPHOCYTE CULTURE TREATED WITH GLYPHOSATE AND ITS FORMULATION

Marin MLADINIC, Nevenka KOPJAR, and Davor ZELJEZIC

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Increased genome damage has been linked with prolonged pesticide exposure, which has shown an elevated risk of cancer development. We tried to evaluate low concentrations of glyphosate and its formulation which are considered to be safe (ADI) or may occur in occupational exposure (OEL) in extended-term cultures of human lymphocytes. Peripheral blood lymphocytes from three healthy young volunteers were exposed for 14 days to 0.50 μg mL^{-1} ADI and 3.50 μg mL^{-1} OEL of glyphosate and its formulation with and without the presence of metabolic activation (S9). DNA damage was monitored using comet assay while distribution of c-Myc and *TP 53* gene signals in comets was detected using fluorescence in situ hybridisation (FISH-comet). At both tested concentrations, the observed percentage of DNA in tail was significantly elevated with and without metabolic activation compared to control (range 4.5 % to 9.5 % *vs.* 1.0 % to 1.5 %). We detected lower structural integrity of *c-Myc* and *TP 53* genes as enhanced migration of signals into the comet tail. Dose-dependent increase in appearance of signals in the tail of the comets was observed for glyphosate and its formulation, with and without S9. These results suggest that prolonged exposure to low concentrations of glyphosate or its formulation may cause direct damage to this particular proto-oncogene and tumour suppressor gene.

KEY WORDS: *comet assay, distribution of gene signals, fluorescence* in situ *hybridisation, human lymphocyte,* in vitro*, prolonged pesticide exposure*

P-51

EFFECTS OF LONG-TERM EXPOSURE TO ANAESTHETICS ON THE HUMAN GENOME

Ružica ROZGAJ, Vilena KAŠUBA, and Mirta MILIĆ

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Genotoxicity of anaesthetic gases in occupationally exposed populations was investigated using micronucleus and bleomycin test. The study included 50 operating theatre medical workers (anaesthesiologists, technicians, and nurses) and 50 control subjects corresponding in sex, age, and smoking habit. The results were analysed using the Poisson regression. The exposed group revealed an increase in genome damage in both tests. The expected number of micronuclei in all observed professions was significantly higher than in controls. In addition to exposure to anaesthetics, univariate analysis identified duration of exposure and age as significant predictors of MN frequency. Vitamin B12 and folate status, as expected, were in negative correlation with MN frequency. Negative correlation was obtained between smoking and MN frequency both by univariate and multivariate regression analyses. Significant correlation between all followed professions and nuclear buds was obtained by univariate regression. Age proved to be a significant predictor of the incidence of nuclear buds in univariate analysis, while years of exposure and additional exposure to radiation showed significant influence on the incidence of micronuclei both in univariate and multivariate regression analyses. Multivariate analysis revealed the level of folates and vitamin B12 as a significant predictor for an increase in nuclear buds. Nucleoplasmic bridges positively correlated with sex. Apoptosis significantly increased in anaesthesiologists and nurses. Apoptotic activity was significantly increased in smokers. Age, years of exposure and vitamin status positively influenced the sensitivity to bleomycin. The obtained results call for further targeted investigation of exposure risk.

KEY WORDS: *bleomycin test, folate, genotoxicity, micronucleus test, occupational exposure, vitamin B12*

P-52

INFLUENCE OF hOGG1 AND XRCC1 POLYMORPHISM ON MDA CONCENTRATIONS IN OCCUPATIONALLY EXPOSED RADIATION WORKERS AFTER IRRADIATION

Mirta MILIĆ[1], Dubravka RAŠIĆ[2], Sabrina ANGELINI[3], and Maja PERAICA[2]

Mutagenesis Unit[1], Unit of Toxicology[1], Institute for Medical Research and Occupational Health, Zagreb, Croatia, University of Bologna, Bologna, Italy[3]

Individual differences in DNA damage response and in lipid peroxidation level after exposure to ionising radiation (IR) are also due to single nucleotide polymorphism (SNP) in genes involved in DNA repair pathways. In order to estimate the influence of SNP on malondyaldehyde (MDA), as a product of lipid peroxidation, 58 individuals professionally exposed to low doses of IR were genotyped for hOGG1 (8-oxoguanine DNA glycosylase,Ser326Cys) and XRCC1 (X-ray repair cross-complementing protein-group 1, Arg194Trp). Blood samples were irradiated with 2 Gy to 4 Gy (Co^{60}). One or two polymorphic alleles were combined in one group (POL). T-test showed no significance between groups before and after radiation in MDA (Mean ± S.E.) : [(7.30±0.39), (7.36±0.46), (7.02±0.38) μmol L^{-1}]. After comparison before, and after 2 Gy and 4 Gy, results were: XRCC1 POL: [(7.02±0.47), (7.20±0.52), (7.04±0.47) μmol L^{-1}]; XRCC1 HO: [(7.74±0.70), (7.61±0.87), (6.99±0.65) μmol L^{-1}]; hOGG1 POL: [(7.80±0.53), (7.10±0.70), (6.30±0.56) μmol L^{-1}]; hOGG1 HO: [(6.90±0.57), (7.58±0.63), (7.61±0.50) μmol L^{-1}]. We found significant correlation among XRCC1 POL before and after both doses, while for XRCC1 HO there was correlation between the results before and after 2 Gy, and between 2 Gy and 4 Gy. HOGG1 POL correlated before and after 4 Gy; and between 2 Gy and 4 Gy radiation. hOGG1 HO correlated in all three groups. After t-test and Mann Whitney U-test comparison, there was a difference between the groups but it was not statistically significant. ANOVA showed an almost significant difference for hOGG1 groups (p=0.069), but not for XRCC1. Our conclusion is that the group was simply too small to estimate the real risk of exposure and conclude whether MDA is or is not a good biomarker for estimating occupational exposure to IR.

KEY WORDS: *hOGG1 gene, ionising radiation exposure, malondialdehyde, single nucleotide polymorphism, XRCC1 gene*

P-53

ASSESSMENT OF ANEUGENIC POTENTIAL OF DIAZINON IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES BY USING FLUORESCENCE *IN SITU* HYBRIDIZATION

Fulya Dilek GÖKALP MURANLI

Faculty of Science, Trakya University, Edirne, Turkiye

In the present study, clastogenic and aneugenic effects of commercial formulation of an organophosphorous pesticide diazinon (Basudin 60EM) were investigated using cytokinesis-block micronucleus (CBMN) and fluorescence *in situ* hybridisation (FISH) method in cultured human peripheral blood lymphocytes. In order to discriminate MN produced by agents causing chromosome breakage (clastogens) from those arising following treatment with agents causing spindle malfunctioning (aneugen), FISH with centromere-specific α-satellite DNA probe was used. Cells were treated with 5 μg mL^{-1} and 10 μg mL^{-1} of diazinon for 48 hours. Vinblastine sulphate (0.1 μg mL^{-1}) was used as positive control. The slides that were prepared using CBMN assay were used in FISH assay. FISH was performed using an α-satellite probe for all human centromeres (PanCentromeric primeFISH® – DIAGEN). After slides were pretreated with RNase, HCL, and pepsin, denaturation of slide and probe was done by baking on a 70 °C hot block. They were hybridised for one night at 37 °C and counterstained with DAPI. The slides were observed with fluorescence microscope using 360 nm, 460 nm, and 510 nm excitation filters. The classification of MN was restricted to cells whose nuclei showed bright fluorescent spots. The presence of centromere-positive MN (C+MN) in treated cultures was compared with controls with a one-tailed Fisher's exact test. The percentage of C+MN values were (69.3±0.6) %, (82.0±2.8) %, (68.7±1.6) %, (81.0±5.5) % for negative control, positive control, 5 μg mL^{-1}, and 10 μg mL^{-1} diazinon, respectively. As a result of the study, diazinon has aneugenic effect at 10 μg mL^{-1} in cultured human peripheral blood lymphocytes.

KEY WORDS: *aneugen, centromere-specific-α-satellite DNA probe, cytokinesis-block micronucleus assay, organophosphorous pesticide*

P-54

GRILLED CHICKEN MEAT EXTRACT AND HETEROCYCLIC AROMATIC AMINES: MUTAGENIC POTENTIAL IN *S. TYPHIMURIUM* AND MODULATION OF GENE EXPRESSION IN HepG2 CELLS

Marko PEZDIRC[1], Bojana ŽEGURA[1], Ana KOPČAVAR[1], Tomaž POLAK[2], and Metka FILIPIČ[1]

National Institute of Biology[1], Biotechnical Faculty, University of Ljubljana[2], Ljubljana, Slovenia

Heterocyclic aromatic amines (HAAs) are carcinogens formed in meats during cooking at high temperatures. By applying AMES test we determined the mutagenic potential of four most abundant HAAs in well-done poultry: 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and 2-Amino-3,4,8-trimethyl-3H-imidazo[4,5-f]quinoxaline (4,8-DiMeIQx). 2-Amino-3-methyl-3H-imidazo[4,5-f]quinoline (IQ) is often below the analytical quantification limit but it is believed that it is the most potent of all. We also determined mutagenicity of HAAs extracted from the crust of grilled chicken breast (grilled meat extract - GME). The concentrations of HAAs in chicken GME were defined using LC-MS/MS. The highest mutagenic potential was exerted by 4,8-DiMeIQx followed by IQ, MeIQx and PhIP. At equimolar concentrations, chicken GME exerted higher mutagenic potential than individual HAA. HAAs exert their mutagenic and carcinogenic activity when they are metabolically activated. Bioactivation and detoxification of HAAs include phase I and II metabolic enzymes that include cytochromes P450 (CYP1A1 and CYP1A2), sulfotranferases (SULTs), N-acetyltransferases (NAT2), and UDP-glucuronosyltransferases (UGT1A1). In metabolically active human hepatoma HepG2 cells treated with pure HAAs, their combinations, and chicken GME we measured mRNA expression of selected phase I and phase II metabolic enzymes. In addition, we examined modulation of mRNA expression of genes regulated by *TP53* gene and involved in response to DNA damage (*CDKN1A, MDM2)* and apoptosis (*BAX* and *BCL2*). We noticed upregulated mRNA expression of *CYP1A1, CYP1A2,* and *UGT1A1*. The results of the expression of *CDKN1A*, *BAX,* and *BCL2* indicate the activation of cell-cycle check points and suppression of apoptosis.

KEY WORDS: *carcinogens in food, grilled meat, mRNA, mutagenicity, phase I and phase II metabolic enzymes*

P-55

ERADICATION OF TOXIC MATERIAL BY USING GREEN CHEMISTRY IN THE DEVELOPMENT OF ANTICANCER DRUGS

Mohd RASHID, Asif HUSAIN, Ravinesh MISHRA, Nadeem A. SIDDIQUE, Sameya ANJUM, and Shama PARVEEN

Faculty of Pharmacy, Jamia Hamdard University, New Delhi, India

Cancer, a disease of the cell cycle, being one of major health problems has received enormous biomedical attention over the past two decades. The effectiveness of many existing anticancer drugs is limited by their toxicity to normal rapidly growing cells. Organic synthesis by non-conventional/modern methods is rapidly gaining importance in view of the fact that the use of many toxic and volatile organic solvents contributes to pollution. Consequently, it is highly desirable to develop environmentally benign processes that will minimise global warming and improve health by being: conducted in aqueous media, solvent-free, and solid-supported. In view of these points, it was thought worthwhile to study new benzimidazoles clubbed with fused heterocyclic ring systems, triazolo-thiadiazoles and triazolo-thiadiazines moieties. These were rationally designed as bendamustine, and were synthesised in a microwave under solvent-free conditions. *In vitro* anticancer screening was conducted at the Development Therapeutic Program (DTP), National Cancer Institute (NCI), Chemotherapeutic Research Division, USA, against full NCI 60 cell line panel. Compound 5h (NCS: 760452) exhibited a remarkable activity with mean GI_{50}=1.04 μmol L^{-1}, TGI>100 and LC_{50}>100 compared to the standard drug (bendamustine, NSC: 138783, mean GI_{50}=60 μmol L^{-1}, TGI>100 and LC_{50}>100). It may possibly be used as lead compound for developing new anticancer agents.

KEY WORDS: *anticancer potential, bendamustine, benzimidazole*

P-56

CYTO/GENOTOXIC AND PROTECTIVE EFFECTS OF QUERCETIN ON HUMAN LYMPHOCYTES AND LARYNGEAL CARCINOMA CELL LINE

Ksenija DURGO[1], Maja IVELIĆ[1], Katarina KAPURALIN[2], Gordana RUSAK[3], and Nevenka KOPJAR[4]

Faculty of Food Technology and Biotechnology[1], School of Medicine[2], Faculty of Science[3], University of Zagreb, Institute for Medical Research and Occupational Health[4], Zagreb, Croatia

Due to the specific structure of quercetin and its hydrophobic character, this compound can act as prooxidant and antioxidant in dependence of concentration and cellular conditions. In this work we determined its ability to cause cyto/genotoxic, prooxidative, and antioxidative effects on human lymphocytes and laryngeal carcinoma cells (HEp2), depending on the time of exposure. Tested concentrations were 1 μmol L^{-1} to 100 μmol L^{-1}, while the incubation lasted for 2 h, 4 h, and 18 hours. Cytotoxicity was studied by neutral red and an acridine orange/ethidium bromide *in situ* fluorescent assay. The level of primary genome damage was studied using the alkaline comet assay. HEp2 cells were more susceptible to the toxic effect of quercetin; it caused cell death in time- and dose-dependent manner. Concentrations higher than 3 μmol L^{-1} decreased cell survival by 20 %, and EC_{50} value was 13 μmol L^{-1} after prolonged exposure. Apoptosis dominated over necrosis. In lymphocytes, quercetin (1 μmol L^{-1} to 100 μmol L^{-1}) predominantly induced apoptosis, while the incidence of necrosis increased after treatment with concentrations above 100 μmol L^{-1}. Alkaline comet assay showed higher genome sensitivity of cancer cells in comparison to lymphocytes. Quercetin (3 μmol L^{-1} to 20 μmol L^{-1}) prevented oxidative stress in lymphocytes. Prooxidative effects of quercetin on lymphocytes were observed after exposure to 20 μmol L^{-1} for 4 h and 18 h, while in HEp2 cells this effect was observed at a concentration of 1 μmol L^{-1}. Antioxidative effect of quercetin (1 μmol L^{-1} to 3 μmol L^{-1}) was observed during 2 hours of incubation. Future studies should verify whether other cancer cell types similarly respond to treatment with quercetin and clarify which mechanisms are involved in its cytoprotective action on normal cells.

KEY WORDS: *apoptosis, bioactive compound, DNA damage,* in vitro*, necrosis, oxidative stress*

P-57

CHOLINESTERASE ACTIVITY AND OXIDATIVE STRESS IN RATS AFTER PARAOXON POISONING AND OXIME THERAPY

Suzana BEREND and Ana LUCIĆ VRDOLJAK

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Among a large number of different types of pesticides available, organophosphates (OP) are the most frequent causes of professional, suicidal or accidental intoxications. It is generally known that OPs act as irreversible inhibitors of cholinesterases (ChE/AChE), yet there are still some open questions about their non-specific effects. Another issue concerning OP poisoning is the inability of conventional therapies to provide adequate protection. The present study was undertaken to examine the impact of possible oxidative stress on OP toxicity and the efficacy of the applied therapy. Rats were injected subcutaneously with sublethal dose of paraoxon, and treated intraperitoneally 1 min later with a combination of oxime K048 (25 % of its LD_{50}) and atropine (10 mg kg^{-1}). Plasma and brain samples were analysed for ChE/AChE activity and concentration of thiobarbituric reactive substances (TBARS) at four different time points (0.5 h, 1 h, 6 h and 24 h) following the treatment. It is important to stress that cholinesterase activity in both plasma and brain did not return to normal even after 24 h following the exposure to paraoxon. A combination of K048 and atropine afforded high potency in restoring the activity of ChE in plasma (50 % to 60 % up to 6 h) while an increase in AChE activity in the brain did not surpass 20 %. Although applied therapy efficiently counteracted paraoxon poisoning, determined TBARS concentrations suggested that these acute treatments resulted with free radical-mediated lipid peroxidation.

KEY WORDS: *acetylcholinesterase, brain, lipid peroxidation, organophosphates, oxime K048, plasma*

P-58

DISTRIBUTION OF ORGANOCHLORINE COMPOUNDS IN MUSSELS COLLECTED AT BREEDING FARMS ALONG THE CROATIAN ADRIATIC COAST

Snježana HERCEG ROMANIĆ[1], Darija KLINČIĆ[1], and Ivana UJEVIĆ[2]

Institute for Medical Research and Occupational Health, Zagreb[1], Institute of Oceanography and Fisheries, Split[2], Croatia

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) belong to the group of Persistent Organic Pollutants. Mussels are good geographical bioindicators of pollution due to their ability to filter seawater and accumulate contaminants. The aim of this study was to investigate the distribution of 17 PCB congeners and 7 OCPs in the edible tissue of the aquacultured *Mytilus galloprovincialis* and natural population *Venus verrucosa* collected along the Croatian Adriatic. Mussels were collected in January and July 2010 and prepared as lyophilisate. Lyophilisate was cold extracted with *n*-hexane and cleaned up with sulphuric acid. The analysis was done on two high resolution gas chromatographs with electron capture detectors (HRGC/ECD) on two capillary columns. Seventeen PCB congeners were analysed: PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180, PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167, PCB-170, PCB-189, PCB-60, and PCB-74 (numbered according to IUPAC), as well as the following OCPs: hexachlorobenzene (HCB), α-HCH, β-HCH, γ-HCH (α-, β-, γ-hexachlorocyclohexanes), 1,1-dichloro-2,2-di(4-chlorophenyl)ethylene (DDE), 1,1-dichloro-2,2-di(4-chlorophenyl)ethane (DDD) and 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT). All analysed OCPs found in all samples, were in the range between 0.08 ng g^{-1} d. w. for α-HCH and 8.76 ng g^{-1} d. w. for DDT. The concentration range of six indicator PCBs was between 0.09 ng g^{-1} for PCB-101 and 37.68 ng g^{-1} d. w. for PCB-138. The remaining 11 PCBs ranged between below determination limit (PCB-157 was not found at 2 sampling sites in January 2010) and 19.03 ng g^{-1} d. w. for PCB-123.

KEY WORDS: *Adriatic sea, biomonitoring,* Mytilus galloprovincialis*, organochlorine pesticides, persistent organic pollutants, polychlorinated biphenyls,* Venus verrucosa

P-59

EFFECT OF FUNGICIDES ON THE GILL STRUCTURE IN *CYPRINUS CARPIO*: A MICROSCOPIC STUDY

Agnieszka LUDWIKOWSKA[1], Hanna LUTNICKA[1], Anna PECIO[2], and Olga WOŹNICKA[2]

Faculty of Animal Science, University of Agriculture in Krakow[1], Institute of Zoology, Jagiellonian University[2], Krakow, Poland

The fish gill is a very important and multifunctional organ involving gaseous exchange, ionic (Na^+, Cl^-, Ca^{2+}) transport, acid-base balance and nitrogenous waste excretion. Its external location and intimate contact with water means that they are susceptible to damage by any irritant toxic substance dissolved in water. Histopathological examinations of the gills are very useful for evaluating the toxic effect of water pollutants. The experiment was conducted in aquaria conditions. Individuals of *Cyprinus carpio* (mean weight ± standard deviation), (50±10) g were exposed to fungicides for 14 days: mancozeb (group 1), prochloraz (group 2), and tebuconazole (group 3) at concentrations of 1 mg L^{-1}, 1 mg L^{-1} and 2.5 mg L^{-1}, respectively. After exposure, individuals were moved to clean water for 30 days for a possible recovery. The gills were sampled after 3 and 14 days of exposure and after recovery period. The histopathological changes in the structure of the gills were studied using light and scanning electron microscopies. In light microscopy, in all exposure groups, lifting of the lamellar epithelium was observed, as well as hyperplasia, hypertrophy, and fusion of secondary lamellae. Scanning electron microscopy supports the findings of light microscopy. In addition, increased process of mucous secretion was observed. A significant loss of microridges of the pavement cells was found in many areas. Prochloraz caused the most significant changes. The recovery period in the present study was too short for a complete regeneration of the organ.

KEY WORDS: *carp, mancozeb, prochloraz, scanning electron microscope, tebuconazole*

P-60

GENDER RELATED DIFFERENCES IN MOUSE BLOOD CELL RESPONSES TO GENOTOXIC PROPERTIES OF SUBCHRONIC *IN VIVO* EXPOSURE TO CARBENDAZIM

Ana MOJSOVIĆ ĆUIĆ[1], Vesna BENKOVIĆ[2], Irena LANDEKA[3], Ivan ANĐIĆ[2], and Domagoj ĐIKIĆ[2]

University of Applied Health Studies[1], Faculty of Science[2], Faculty of Food Technology and Biotechnology[3], University of Zagreb, Zagreb, Croatia

The pesticide and preservative carbendazim is a chemical widely used in experiments as a known aneugen. It remains unclear whether it has clastogenic properties. In this study it was demonstrated that the subchronic repeated exposure (28 days) of mice to 20 mg kg^{-1} carbendazim (1/2 of NOEL dose), caused increased DNA breakage in leukocytes of peripheral blood, proving that the subchronic repeated exposure may cause clastogenic activity in sensible cells such as leukocytes. A decrease in RBC count, whose antioxidant defence mechanisms increased, was also noted. However, these effects were more pronounced only in males and not females, indicating that the males were more sensitive to the described clastogenic properties and hematotoxicity. On the contrary, the micronucleus assay showed that in terms of aneugenic properties both genders responded similarly. Parallel to this, there was an increase in granulocyte number (neutrophils, eosinophils, and basophils) in both sexes, indicating possible immunotoxic (allergenic) effects of prolonged carbendazim exposure.

KEY WORDS: *aneugen, immunotoxic effects, leukocytes, micronucleus assay, red blood cells, Swiss mice*

P-61

SENSITIVITY OF TISSUE ACETYLCHOLINESTERASE OF COMMERCIALLY IMPORTANT BIVALVE SPECIES WARTY VENUS *VENUS VERRUCOSA* (LINNAEUS, 1758) AND NOAH'S ARK SHELL *ARCA NOAE* (LINNAEUS, 1758) TO ORGANOPHOSPHOROUS PESTICIDES

Lorena PERIĆ[1], Vedrana NERLOVIĆ[1], and Luka RIBARIĆ[2]

Centre for Marine Research, Ruđer Bošković Institute, Rovinj[1], Juraj Dobrila University of Pula, Pula[2], Croatia

Marine environment is subjected to the input of a wide range of chemical compounds. Among them, organophosphorous pesticides (OPs) that are commonly applied in agriculture could represent a considerable threat to cultivated and wildlife populations of marine non-target organisms in the near-shore coastal areas. These compounds selectively inhibit acetylcholinesterase (AChE), a serine hydrolase essential for the transmission of nerve signals. Kinetic properties of AChE and its sensitivity to OPs have been investigated in the gills and adductor tissues of two commercially important bivalve species: warty venus (*Venus verrucosa*) and Noah's ark (*Arca noae*) that are widely distributed and harvested for human consumption along the eastern Adriatic coast. Specific inhibitors eserine and BW284C51 significantly affected AChE activity in the gills and adductor of both species, revealing the similarity to vertebrate enzyme. The highest ratio V_{max} k_m^{-1} indicating the enzyme catalytic efficiency was found for *V. verrucosa* adductor and *A. noae* gills (0.024 mL min^{-1} mg prot^{-1}). The lowest AChE activity was detected in the gills of *V. verrucosa* (≤2 nmol min^{-1} mg prot^{-1}). *A. noae* adductor had the highest AChE activity (≥10 nmol min^{-1} mg prot^{-1}). The exposure of both bivalve species to OPs resulted in a dose-dependent inhibition of AChE. The potential for use of *V. verrucosa* and *A. noae* as indicators of exposure to neurotoxic compounds in marine environment, in particular within the areas not inhabited by other common bioindicator species such as mussels, is discussed.

KEY WORDS: *AChE activity, adductor, bioindicator species, dose-dependent inhibition, gills*

P-62

AMITRAZ INTOXICATION IN CROATIAN WARMBLOOD HORSE: CASE REPORT

Jelena SELANEC[1], Nika BRKLJAČA BOTTEGARO[2], Darko GRDEN[1], Emil SREBOČAN[3], and Vladimir MRLJAK[1]

Internal Medicine Clinic[1], Surgery, Orthopedics and Ophthalmology Clinic[2], Department of Pharmacology and Toxicology[3], Veterinary Faculty, University of Zagreb, Zagreb, Croatia

Amitraz (formamidine) is a veterinary and agricultural pharmaceutical drug that is used worldwide as an acaricide and insecticide in many species but is not approved for horses. In mammals, amitraz has a highly complex pharmacological activity that can be related to the stimulation of alfa-2 adrenergic receptors. A three-year-old male Croatian warmblood horse was admitted to the clinic with a history of acute abdominal pain, depression, and mild ataxia in the last 24 hours. He was sprayed with an amitraz product (Taktic®, 12.5 %, Intervet) two days prior to referral. Physical examination revealed depression, moderate dehydration, poor gut motility, normal peritoneal fluid and ingesta filled cecum, and firm pelvic flexure. A complete haematology and serum biochemistry blood profile showed no specific changes. The horse was thoroughly washed with chlorhexidine soap and treated symptomatically with IV polyionic crystalloid fluids, metamizol, flunixine meglumine, and multiple doses of mineral oil and water per os. The following day, the horse showed improved gut motility and softer pelvic flexure on rectal palpation. On day three, gut motility returned to normal, rectal palpation of pelvic flexure showed no abnormality, and there were no signs of ataxia or depression. The use of alfa-2 adrenergic antagonists and yohimbin in amitraz intoxication treatment is described in the literature, but in this case the patient recovered fully within 3 days of only medical therapy for colonic impaction after the initial wash.

KEY WORDS: *acute abdominal pain, depression, insecticide spray for horses, mild ataxia, recovery*

P-63

CASES OF INTOXICATION OF ANIMALS BY PESTICIDES IN BELGRADE, SERBIA FROM 2010 TO 2011

Dragana RANČIĆ[1], Gordana BRAJKOVIĆ[1], Snežana ĐORĐEVIĆ[1], Nadežda KRSTIĆ[1], Vesna KILIBARDA[1], and Biljana ANTONIJEVIĆ[2]

Military Medical Academy[1], Institute of Toxicology Academic Danilo Soldatović[2], Belgrade, Serbia

Data material for this retrospective study was taken from all pesticide analysis requests received between January 2010 and December 2011 by the Department of Toxicological Chemistry, Poison Control Centre, Belgrade, Serbia. Pesticide analyses were performed using tissue samples (brain, kidney, liver) or liquid samples (gastric contents) of different species (dogs, cats, horses, pigs, wild animals, fish, and honey-bees). The proof of presence or absence of a suspected pesticide was performed by liquid-liquid or solid-phase extraction, followed by separation and characterisation by chromatographic methods. These included high performance liquid chromatography using a photodiode array detector (HPLC/PDA). Confirmatory technique was gas or liquid chromatography with mass-spectrometry (GC-MS, LC-MS). In 40 samples analysed, we detected the following pesticides: dinitro-o-cresol (DNOC), carbofuran, warfarin, methomil, and pirimiphos-methyl. In one request for the analysis of a sample of honey-bees, cypermetrin was detected. Our study has shown that with 35 % of cases, dinitro-o-cresol intoxications are the most frequent examples of pesticide poisoning in animals. DNOC is one of the most common causes of fatal poisoning diagnosed in dogs. The most frequent carbamate was carbofuran with 20 % of positive pesticide analyses. The presence of anticoagulant rodenticides was confirmed in 15 % of cases, organophosphates in 10 %. Out of the total number of registered cases of suspected poisonings, 5 % were requests from local police departments, 10 % from the local Court of Justice and the highest number of 85 % referred to personal requests. As a consequence of our retrospective two-year study, it can be concluded that DNOC and carbofuran are among the most prominent agents causing intoxication of animals.

KEY WORDS: *carbofuran, dinitro-o-cresol, HPLC/PDA, pesticides in tissues and liquid samples, retrospective study*

P-64

MICELLE-ASSISTED KINETICS OF CHEMICAL WARFARE SIMULANT DECOMPOSITION USING TERTIARY OXIMES

Bhanushree GUPTA and Kallol K. GHOSH

School of Studies in Chemistry, Pt. Ravishankar Shukla University, Raipur, India

Nucleophilic cleavage of chemical warfare simulants, namely phosphate and carboxylate esters is significant for many biological detoxification reactions. Tertiary oximes are α-nucleophiles of special interest due to their good nucleophilic potency and reactivation efficiency for organophosphate-inhibited acetylcholinesterase (AChE). They are highly lipid soluble, hence are able to reactivate AChE within the central nervous system. In the present investigation, the kinetic studies on the nucleophilic substitution reactions of some stimulants using two tertiary oximes viz. 2,3-butanedione monoxime (BDMO) and monoisonitroso acetone (MINA) in the presence of novel cationic surfactants have been studied. The physicochemical properties like acid dissociation constant (pk_a) and lipophilicity (log *P*) of these α-nucleophiles have also been studied spectrophotometrically and by using software ACD Labs. All reactions were monitored spectrophotometrically and followed pseudo-first order kinetics. The outcomes of such investigations can be used to predict the effect of organised assemblies on nucleophilicity of oximes and other α-nucleophiles.

KEY WORDS: *AChE reactivation, acid dissociation constant, 2,3-butanedione monoxime, cationic surfactants, kinetic studies, lipophilicity, monoisonitroso acetone, nucleophilicity*

P-65

COMPUTER-ASSISTED SPERM MOTILITY ANALYSIS AND SPERM MORPHOLOGY CHANGES AFTER A PERORAL ADMINISTRATION OF DIAZINON AND CADMIUM IN RATS

Róbert TOMAN[1], Maria ADAMKOVICOVA[2], Svätoslav HLUCHÝ[1], Michal CABAJ[1], Jozef GOLIAN[2], Peter MASSANYI[2], Norbert LUKAC[2], and Monika MARTINIAKOVA[3]

Faculty of Agrobiology and Food Resources[1], Faculty of Biotechnology and Food Sciences[2], Slovak University of Agriculture, Faculty of Natural Science, Constantine The Philosopher University[3], Nitra, Slovak Republic

Sperm motility parameters and spermatozoa abnormalities were estimated in rats after a peroral intake of 30 mg L^{-1} of $CdCl_2$ (Cd-group), 40 mg L^{-1} of diazinon (DZN-group), and 30 mg L^{-1} of $CdCl_2$ + 40 mg L^{-1} of DZN (Cd/DZN-group) in drinking water for 91 days. Group C was the unexposed control. The males were one month old at the beginning of the experiment. Motility (%), progressive motility (%), distance average path (DAP; μm), curvilinear distance (DCL; μm), distance straight line (DSL; μm), velocity average path (VAP; μm s^{-1}), curvilinear velocity (VCL; μm s^{-1}), velocity straight line (VSL; μm s^{-1}), straightness (STR; %), linearity (LIN; %), wobble (WOB; %), amplitude of lateral head displacement (ALH; μm), and beat cross frequency (BCF; Hz), as well as abnormalities (%) in spermatozoa head and flagellum were evaluated using computer-assisted sperm analysis (CASA). All motility parameters in Cd-group significantly decreased. In DZN-group, significant ($p<0.05$) increases in VAP, VCL, and ALH ($p<0.001$) but a decrease in BCF ($p<0.01$) were detected. Most of the parameters in Cd/DZN-group significantly increased ($p<0.001$) except for a decrease ($p<0.001$) in STR, LIN, WOB, and BCF. The spermatozoa head abnormalities increased in Cd ($p<0.01$) and DZN ($p<0.05$) groups. The flagellum defects increased ($p<0.001$) in all experimental groups. In conclusion, cadmium negatively affects the spermatozoa motility, but DZN alone and a mixture of Cd and DZN increase important motility parameters. Sperm abnormalities have increased under all experimental conditions. Results indicate that cadmium, insecticide diazinon, and their mixture affect rat sperm motility parameters differently. (Supported by KEGA grant, No. 025UKF-4/2012)

KEY WORDS: *heavy metal, insecticide, mixture, peroral intake, sperm abnormalities*

P-66

MORTALITY OF THE EUROPEAN LEAFROLLER PARASITOID *BRACON (BRACON) VARIEGATOR* SPINOLA, 1808 (*HYMENOPTERA, BRACONIDAE*) EXPOSED TO FIVE DIFFERENT INSECTICIDES

Mitat AYDOĞDU

Faculty of Sciences, Trakya University, Edirne, Turkey

Bracon (Bracon) variegator Spinola, 1808 (the European leaf roller) is a specific gregarious ectoparasitoid of *Archips rosana* (Linnaeus, 1758), which are serious pests in Turkey causing various levels of economic losses in different crop plants. A laboratory bioassay was used to evaluate the effects of five insecticides on the mortality of *Bracon (Bracon) variegator*. Adult parasitoids were caged in Petri dishes that had been sprayed at the application rate of insecticides. Experiments were performed in five replicates and observed deaths were recorded periodically over a 24-hour period. Evaluations of the effectiveness of insecticides were done according to IOBC (International Organization for Biological Control) class values. Alpha-cyphermethrin caused a 100 % mortality rate at the end of the first 16-hour period, whereas lambda-cyhalothrin and delthamethrin caused the same mortality by the end of 24 hours. Meanwhile, insecticide treatments with diazinon and dichlorvos were assessed as harmful (>99 % mortality) after four hours of their application. The ranking according to their effects on mortality was: dichlorvos > diazinon> alpha-cypermethrin > delthamethrin > lambda-cyhalothrin. These results indicated that the parasitoid bioassay test contributed to the development of biological control methods against leaf roller pests.

KEY WORDS: *alpha-cypermethrin, deltamethrin, diazinon, diclorovos, efficacy, laboratory bioassay, lambda-cyalothrin, pest control*

P-67

POISONING INCIDENTS REPORTED TO THE POISON CONTROL CENTRE (PCC) IN ZAGREB FROM 2006 TO 2011

Željka BABIĆ and Rajka TURK

Poison Control Centre, Institute for Medical Research and Occupational Health, Zagreb, Croatia

Poisoning incidents, reported to the PCC in Zagreb from 2006 to 2011, were analysed to assess the current pattern of poisoning in Croatia. A total of 8,849 cases were reported, mainly by health professionals (91 %). The average age of patients was 16 years, and the most prevalent age groups were infants and preschool children (47 %). Men were more frequently involved than women (54 % *vs.* 46 %), except in adolescents (32 % *vs.* 68 %). The most prevalent causative agents were drugs (41 %) and household chemicals (31 %). Psychoactive drug overdose comprised 45 % of all drug poisonings. From the total number of cases with known clinical symptoms, 47 % were asymptomatic, 44 % had mild symptoms, and only 9 % had severe symptoms i.e. serious disturbances of the central nervous system, corrosive injuries, or severe respiratory symptoms. Fourteen fatal outcomes were recorded, the majority being suicidal ingestions of psychoactive drugs in adults. Severe clinical presentation in most cases was caused by drugs (49 %), household chemicals (13 %) and pesticides (10 %). The ratio between suicidal and accidental poisonings was greater than 1:3, except in the adolescent group with nearly 3 times more suicidal than accidental poisonings. Paracetamol, organophosphates, and ethylene glycol exposure occurred in 223, 52, and 27 cases, respectively, indicating that specific antidotes i.e. N-acetylcysteine, fomepizole, and oximes must be readily available. However, antidote supply is a long-term problem in Croatia which needs to be resolved to achieve a higher standard in medical management of poisoning.

KEY WORDS: *antidotes, Croatia, ethylene glycol, paracetamol, organophosphates*

P-68

MARINE RADIOECOLOGY AND RADIOTOXICOLOGY

Gina BRANICA and Zdenko FRANIĆ

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Radioecology, a multidisciplinary scientific discipline acting as a tool for assessing radioactive contamination and evaluating doses received by population, has for a long time been focused on environmental transfers through the environment to address human radioprotection and radiotoxicology. Marine radioecology, as a part of radioecology, began to develop after intensive atmospheric nuclear weapons testing in the 1950s and 1960s. For dose assessment, it is essential to know the effective dose as a specific parameter for risk assessment in radiotoxicology. However, due to different concentration factors (i.e. ratios between activity concentration in water and in organisms for respective radionuclides) respective marine organisms have different radio-sensitivities. Unfortunately, data on concentration factors and radio-sensitivity are not readily available due to limited field research data. Consequently, this limits the knowledge on dose assessment. In this work, we presented the overview of the ongoing radioecological studies in the Adriatic area carried out by the Radiation Protection Unit. In order to move from anthropocentric initial scope of radioecology to a more eco-centric view capable of assessing ecological risk mediated by ionising radiation, we also presented data for selected marine organisms that are regarded as good bioindicators of anthropogenic radionuclides, i.e., mussels (*Mytilus galloprovincialis*), octopuses, (*Ozaena moschata*), and pelagic fish, pilchards (*Sardina pilchardus*). In 2011, activity concentrations for ^{137}Cs, as the main fission product still present in the environment, ranged from 0.05 Bq kg^{-1} in pilchards, 0.07 Bq kg^{-1} in octopuses, and 0.4 Bq kg^{-1} in mussels, while the maximum activity concentration in the Adriatic Sea water was recorded to be 4 Bq m^{-3} in Split area. We generally argue that better knowledge of the environment components, from individual organisms up to populations of species and ecosystems, together with their interaction with the abiotic compartments is needed to fully understand marine radioecology issues, including radiotoxicology.

KEY WORDS: *Adriatic sea, bioindicator organism, ^{137}Cs, radiation protection*

P-69

A SIMPLE AND FAST CAPILLARY ELECTROPHORETIC METHOD FOR DETERMINING ACTIVE PHARMACEUTIAL INGREDIENT LOVASTATIN

Miranda SERTIĆ, Biljana NIGOVIĆ, and Ana MORNAR

Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

Red rice is well-known to contain statin-like compounds called monacolins which act as inhibitors of enzyme hydroxymethyl glutaryl Coenzime A reductase, the rate-controlling enzyme in the biosynthesis of cholesterol. In fact, monacolin K is structurally identical to lovastatin, registered as a cholesterol-lowering drug. Cosmetic products are not allowed to have active pharmaceutical ingredients or any substance with therapeutic action, in which case they should be registered as special purpose cosmetics. Therefore, quality control of such products is necessary. A simple and fast capillary electrophoretic (CE) method was developed to determine lovastatin in lactone and hydroxy acid form in red rice cosmetic products. The first step in the CE method development was to select buffer pH, which influenced the extent of ionization and the mobility of analytes, as well as the control of electroosmotic flow. Optimised separation conditions were obtained by a background electrolyte containing 20 mmol L^{-1} phosphate buffer pH 7.0 and 27.5 mmol L^{-1} sodium dodecyl sulphate (SDS). The addition of SDS micelles in the background electrolyte solutions played a key role in the separation of negatively charged species and neutral lactone form. Separation was performed at 26 kV and 25 °C. Detection was performed using diode array detector set to 237 nm. Method was validated according to ICH guidelines. The principal advantages of the proposed CE method over the well-established HPLC procedures include improvements in low sample volume required, low consumption of solvents and reagents, environmental friendliness, cost efficiency, simplicity, high resolution and short analysis time.

KEY WORDS: *cosmetic products, development of method, monacolins, quality control, red rice, validation*

P-70

NEW HSS-GC-FID METHOD FOR DETERMINING VOLATILE COMPOUNDS IN CHILDREN'S COUGH SYRUP

Ana MORNAR, Biljana LEPAN, Miranda SERTIĆ, and Biljana NIGOVIĆ

Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

Cough syrup can contain pharmaceuticals or different plant extracts with proven antitussic or expectorant properties. Generally used extraction solvents are different concentrations of ethanol. Methanol is the most common impurity in ethanol and is very hard to eliminate because their boiling points are similar. Ethanol and methanol are central nervous system depressants and have significant psychoactive and toxic effects. They can lead to impaired sensory and motor functions, blindness, unconsciousness, hypoxia, metabolic acidosis, and death. Therefore, content of these volatile compounds must be carefully determined, especially in products intended for children. The aim of our work was to develop a new, fast method for determining volatile compounds in cough syrup. Since cough syrup has a complex matrix and in order to avoid sample pre-treatment, we employed headspace sampler (HSS). In order to achieve complete extraction, temperature (50 °C to 100 °C) and equilibration time (5 min to 40 min) of the HSS were optimised. GC analysis was performed on Agilent 6850 equipped with flame-ionisation detector. We used column HP-1 (30 m x 0.32 mm, 0.25 μm) and nitrogen at the flow rate of 1 mL min^{-1}. The temperature programme 50 °C rising to 100 °C (10 °C min^{-1}) was applied. Newly developed HSS-GC-FID method was validated according to ICH guidelines and was proven to be accurate (99 % to 101 %) and precise (RSD<4.66 %). The developed method was applied to analyse children's cough syrup. Content of ethanol was found to be 0.53 % which is in accordance with the labelled value (0.5 %). Methanol was also found, but below methanol limit for extracts and tinctures permitted by the European Pharmacopoeia (0.05 %).

KEY WORDS: *development of analytical method, ethanol, impurity, methanol, validation*

P-71

ANALYSIS OF EVENTS IN THE TRAGIC CASE OF DEATH OF TWO EMPLOYEES OF PORTUN FERRUM *D.O.O.*

Davor GRETIĆ, Franjo PLAVŠIĆ, Zdravko LOVRIĆ, Alka WOLF ČOPORDA, Saša ĐURAŠEVIĆ, Daniela ČEPELAK DODIG, and Irena Zorica JEŽIĆ VIDOVIĆ

Croatian Institute for Toxicology and Antidoping, Zagreb, Croatia

The series of events that led up to these tragic consequences are unexplainable, but it is clear that two employees entered their wardrobe located in the basement of the building. They went to change before going home after their shift ended. Soon they lost consciousness and died. A third employee tried to help, but quickly felt faint and experienced symptoms of respiratory tract irritation. He left the area to save his life. At the scene, under the management of the fire and police departments, employees of Zagreb's gas company detected very low concentrations of oxygen at the basement entrance, while concentrations of carbon monoxide and methane were within normal limits. A call was made to the preparedness service of the Croatian Institute of Toxicology and Antidoping around 5 pm. Toxicologists arrived at the scene around 6 pm, and took responsibility for further actions. Unfortunately, as systems for such unexpected events were not in place, and the organisation of the intervention in early phases was poor, air sampling with the intent of measuring concentrations of carbon monoxide and detecting a possible third gas was done hours after the incident. Due to the compulsory ventilation of the basement area prior to the sampling, the analysis itself actually had no purpose. Solving this case, given the above mentioned reasons, will probably never be possible. Nevertheless, it is necessary to learn lessons from cases like these and amend errors and oversights in organisation systems, as to keep similar accidents from happening in the future.

KEY WORDS: *accident, fatal intoxication, reporting system*

P-72

CLENBUTEROL AND SALBUTAMOL BLOOD CONCENTRATIONS IN TREATED ANIMALS

Jelka PLEADIN[1], Ksenija DURGO[2], Ana VULIĆ[1], Nina PERŠI[1], Svjetlana TERZIĆ[3], and Željko MIHALJEVIĆ[4]

Laboratory for Analytical Chemistry[1], Faculty of Food, Technology and Biotechnology, University of Zagreb [2] Laboratory for Analyses of Veterinary Medicinal Products[3], Laboratory for Ruminant Pathology and Transmissive Spongiform Encephalopathy[4], Croatian Veterinary Institute, Zagreb, Croatia

The β-adrenergic agonist clenbuterol and salbutamol are pharmacologically active substances with anabolic effects, which bind to the specific β-adrenergic receptors found on cell membranes of the animal target tissues. The use of these substances as growth promoters for fattening purposes has been banned in the European Union. The aim of the present study was to determine clenbuterol and salbutamol blood concentrations, separately in the plasma and serum of animals after their exposure. The experimental group of guinea pigs (n=20) were treated with two dosages (0.25 mg kg^{-1} and 2.5 mg kg^{-1}) of clenbuterol (n=10) and salbutamol (n=10) for 7 days, respectively, whereas the control group of animals (n=10) were left completely untreated. Validation of applied ELISA method showed acceptable recovery (R>70 %) and repeatability (CV<10 %) for all serum and plasma samples to which clenbuterol and salbutamol were added. This has proven the method to be applicable for quantitative determination of both analytes and matrices, preferably in plasma. Study results pointed to significantly higher (about 14-fold) clenbuterol concentrations (1287±293) μg L^{-1} in comparison to salbutamol (95±20) μg L^{-1} in plasma, and generally higher concentrations of both analytes in plasma than in serum. Application of higher exposure dose resulted in about four-fold and three-fold higher clenbuterol and salbutamol concentrations, respectively, and pointed to a different rate of release of these two β-agonists.

KEY WORDS: *β-adrenergic agonist, clenbuterol, ELISA, guinea pigs, plasma, serum, salbutamol*

P-73

ANALYSIS OF THE AJKAI TIMFÖLDGYÁR ALUMINA PLANT ACCIDENT

Saša ĐURAŠEVIĆ, Franjo PLAVŠIĆ, Zdravko LOVRIĆ, Alka WOLF ČOPORDA, Davor GRETIĆ, Daniela ČEPELAK DODIG, and Irena Zorica JEŽIĆ VIDOVIĆ

Croatian Institute for Toxicology and Antidoping, Zagreb, Croatia

Taking into consideration the spatial extent, duration and severity of consequences, the dam break of Basin X of the alumina plant near Ajka on 7th October 2010 and the flood of enormous quantities of red mud is assumed to be the greatest environmental crisis ever in Hungary and the whole region. The spilt slurry initially reached several settlements and contaminated the valleys of the Torna creek and the Marcal river, almost reaching the river Rábu. Along the Torna and parts of Marcal practically all aquatic life was destroyed. The disaster left ten people dead and over 150 injured. About 1000 acres of land was polluted. The amount of emitted pollutants is estimated at about one million cubic meters. After the slurry began spreading, the biggest concern became the contamination of the Danube; the second longest river in Europe flows through eight countries, including Croatia. Under the management of the National Protection and Rescue Directorate, data collection from the Croatian flow of the Danube began. The Croatian Institute for Toxicology and Antidoping soon came up with a hypothesis: the immense flow of the Danube will completely neutralise the slurry, lowering the concentration of pollutants to about 0.05 % of their initial concentration. Later analysis resulted in only one exception - the concentrations of aluminium rose to about 10 times the average maximum values before the accident. Concentrations of other toxic metals such as arsenic, chromium, and mercury did not fluctuate significantly in the observed period and were much lower than allowed limits.

KEY WORDS: *aluminium, Danube, red mud, toxic metals, slurry*

P-74

STUDY OF PHYSICOCHEMICAL PROPERTIES OF SOME OXIME-BASED FUNCTIONALISED SURFACTANTS

Namrata SINGH and Kallol K. GHOSH

Pt. Ravishankar Shukla University, Raipur, India

The use of organised molecular systems for acyl transfer reactions is of fundamental significance in chemical and biological fields. Decomposition of ecotoxic substrates of organophosphate origin is a challenging area of research which underlines the synthesis and development of nucleophilic-based functional detergents. In the present investigation, some novel classes of oxime-based functionalised surfactants of the type 3-hydroxyimminomethyl-1-alkylpyridinium bromide (alkyl: $C_{10}H_{21}$, $C_{12}H_{25}$, $C_{14}H_{29}$) were synthesised and their physicochemical properties were investigated. Acid dissociation constant (pk_a) of these surfactants was studied spectrophotometrically and confirmed through kinetic investigations. Micellar and surface properties of these surfactants were studied by conductometric and surface tensiometric measurements. pk_a and CMC of functionalised surfactants decreased in the order $C_{10}H_{21} < C_{12}H_{25} < C_{14}H_{29}$. Based on this order, their structure and reactivity was discussed. Such investigations may help in designing and developing supernucleophilic functional detergents for their widespread applications in detoxification of hazardous chemicals.

KEY WORDS: *detoxification, functional detergents, novel classes of surfactants, organophosphates*

P-75

GLYCOLS IN TOOTHPASTE ON CROATIAN MARKET

Ivona VIDIĆ ŠTRAC[1], Irena ŽUNTAR[2], Nino DIMITROV[3], and Maja PUKLEK[2]

Consumer Products Unit[1], Food Contact Materials Unit[3], Croatian National Institute of Public Health, Faculty of Pharmacy and Biochemistry[2], University of Zagreb, Zagreb, Croatia

Diethylene glycol (DEG) (CAS 111-46-6) is a highly toxic organic solvent that may cause nausea, dizziness, kidney failure, breathing problems, convulsions, and even coma when ingested. The potential health risks from chronic exposure to DEG are a particular concern in specific vulnerable populations such as children and consumers with kidney or liver diseases. DEG is forbidden as a cosmetic ingredient but can be present as an impurity in glycerol and polyethylene glycols, common ingredients in oral care products. Maximum permitted concentration as impurity is 0.1 % (m/m). The objective of this study was to determine the presence of diethylene glycol in toothpaste on the Croatian market using a gas chromatography-mass spectrometry method. Linearity of the response of mass detector was tested by analysing standard solutions with increasing concentrations of monoethylene glycol (MEG) and DEG. Regression analysis of chromatographic data indicates that linearity of response in the detector was within the examined concentration area 0.5 mg L^{-1} to 50 mg L^{-1}, with correlation coefficients >0.998. Limit of detection (LOD) for DEG was <6 mg kg^{-1}, and for MEG <4 mg kg^{-1}. Testing was conducted on 30 samples of toothpaste produced and marketed in Croatia and EU. Obtained results were significantly below the permitted threshold of 0.1 % thus assuring consumer safety.

KEY WORDS: *diethylene glycol, gas chromatography – mass spectrometry, limit of detection, maximum permitted concentration, monoethylene glycol, oral care product*

AUTHOR INDEX / AUTORSKO KAZALO; VOLUMEN 63(SUPPL 2), 2012.

Under the Auspices of:

Croatian Institute for Toxicology and Antidoping

Institute for Medical Research and Occupational Health

Sponsored by:
Ministry of Science, Education and Sports of Republic of Croatia

General sponsor:
Instrumentalia Adria

Sponsors:
Lipomed GmbH
Medic
AlphaChrom d.o.o. (Agilent Technologies)
Biovit
LGC Standards GmbH
Merck Millipore
Shimadzu
Alltech Biotehnologija
Perceptive Instruments Ltd
Konceptmedia
Vita Lab Nova

instrumentalia

PRODAJNI PROGRAM

HRVATSKA
INSTRUMENTALIA Adria d.o.o.
T (01) 662 3883
F (01) 662 3884
E sales@instrumentalia.hr

SLOVENIJA
INSTRUMENTALIA, d.o.o.
T (01) 524 0196
F (01) 524 0198
E sales@instrumentalia.si

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Liquid Chromatography, SFC-Supercritical Fluid Chromatography, Mass Spectrometry Lab Informatics, SPE, HPLC Columns and Accessories, Analytical Standard Reagents
www.waters.com/waters/home.htm

TA

Thermal Analysis (DSC, TGA, DMA, DVS) Rheology, Microcalorimetry, Flash Diffusivity, Dilatometry, Thermal Conductivity, Simultaneous Thermal Analyzers, Rotational High Temperature Viscometer
www.tainstruments.com

Horizon technology

Automated Extraction System for trace organic contaminants in aqueous samples, SPE discs, Concentrator of Organic Extracts, Automated Extraction System for SPE cartridges
www.horizontechinc.com

PEAK SCIENTIFIC

Gas generators for GC and LC-MS
www.peakscientific.com

TOMTEC

Robotic Workstations, Sample Prep Automation (SPE, LLE, PPT), Liquid Handling Automation, Tissue Homogenizers, Cell Harvesters
www.tomtec.com

EUROPA

Europa Bioproducts-reagents for life science research (ProZyme Inc, Bio Inc, Immunotech Ltd, Moss Inc.)
http://www.europa-bioproducts.com/

Labhut .com

Syringe Filters, Dissolution Accessories
www.smi-labhut.com/

APPLIED RESEARCH EUROPE GmbH

Chromatography Accessories & Supplies
www.appliedresearch.de/home.html

RIGGTEK
Dissolution Test Systeme

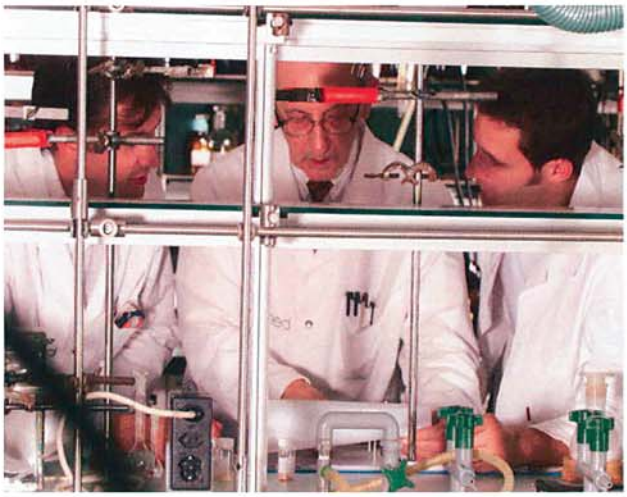
Dissolution Test Systems
http://www.riggtek.de/

Lipomed AG, Arlesheim Switzerland

lipomed

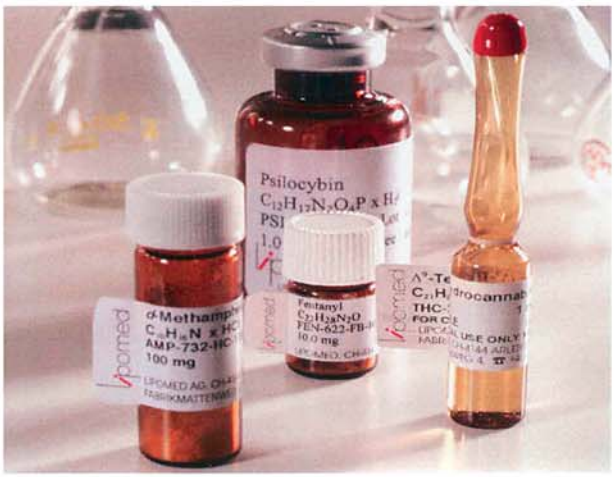
SERVICES TO HEALTH

Diagnostic Reference Standards



Lipomed AG is an ISO 9001:2008 certified private health care company that produces promotes and distributes Pharmaceuticals and Diagnostic Reference Standards.

To learn more about Lipomed AG and our products please sign up at:
www.lipomed.com



The new Diagnostic Reference Standards are available as calibrated solutions ● and as calibrated 1 mg powders ✹ and as bulk powders ■ in 10, 20, 50 & 100 mg and some bulk products also in 1 gram standard pack sizes.

Lipomed AG Fabrikmattenweg 4
CH-4144 Arlesheim
Switzerland
Tel: +41 61 702 02 00
Fax: +41 61 702 02 20
E-Mail: lipomed@lipomed.com

lipomed
SERVICES TO HEALTH

Distributed by:

MEDIC d.o.o.
za unutrašnju, vanjsku trgovinu i zastupanje

Exclusive distributor of Lipomed reference standards in Croatia

ALPHA CHROM

Agilent Technologies
Authorized Distributor

AlphaChrom d.o.o
Buzinski prilaz 10, 10000 Zagreb
Tel: 01 550 2222, Fax: 01 550 2299
www.alphachrom.hr

BIOVIT d.o.o.

Poduzeće za uvoz lijekova
i medicinske opreme

SIGMA-ALDRICH™

- Osnovne laboratorijske kemikalije, pribor i oprema

ALDRICH™ Chemistry

- Kemikalije za sintezu i specifične kemikalije

Fluka™ Analytical

- Analitički reagensi i laboratorijske kemikalije

SIGMA™ Life Science

- Biokemijski spojevi i istraživački reagensi

SUPELCO™ Analytical

- Kromatografija i priprema uzoraka

Grant SCIENTIFIC

- Laboratorijska oprema i instrumenti

MACS Miltenyi Biotec

- Separacija, uzgoj i analiza stanica

Biovit d.o.o. - Poduzeće za uvoz lijekova i medicinske opreme
tel. 042.260.001, fax. 042.260.021, info@biovit.hr, http://www.biovit.hr

Raising standards
√ Quality
√ Compliance
√ Commitment
√ Competence
√ Choice
providing solutions

LoGiCal®

produced by LGC

A new generation of reference materials produced by LGC

Quality

Certified reference materials produced under ISO Guide 34 and 17025 accredited systems to satisfy your accreditation and certification requirements.

Commitment

In-house forensic, clinical and analytical experts in combination with in-country representatives to support your use of these reference materials.

Compliance

Fully satisfy national and international labelling and other requirements for transportation and health and safety.

Competence

Expertise based on over 30 years of reference material production and over 70 years of forensic and clinical experience to support your current requirements and anticipate your future needs.

Choice

A broad and continually developing product range to ensure you can obtain the reference materials for your intended use.

LoGiCal Standards: the most up-to-date range of quality assured drug reference materials.

www.logical-standards.com **askus@logical-standards.com**

www.lgcstandards.com

LGC Standards

Excellence through measurement

LGC Quality - ISO Guide 34 • GMP/GLP • ISO 9001 • ISO/IEC 17025

3189/SS/0312

Top Klasa

Da bi osigurale maksimalnu zaštitu svojih potrošača, industrije hrane i pića jednako kao i prehrambene, kemijske te proizvodne industrije u svojim proizvodnim lancima zahtijevaju pouzdanu kontrolu kvalitete. Što je bolje njihovo poznavanje spojeva i sastava, to se nalaze na sigurnijem tlu u okolini visoko-zahtjevnih standarda, odredaba i smjernica.

Kao jedan od pokretača analitičke instrumentacije, Shimadzu proizvodi vodeće analitičke sustave. Temelji njihovog razvoja su u konstantnoj predanosti inovacijama i visokokvalitetnim tehnologijama, kao i u bliskoj suradnji s korisnicima iz znanstvenih i proizvodnih krugova te tržištima diljem svijeta.

Top klasu instrumenata i rješenja potvrđuju brojne svjetske premijere i nagrade, dok svoje trenutne napore Shimadzu ulaže u razvoj i stvaranje novih, još osjetljivijih aplikacija.

Shimadzu I&R rješenja sutrašnjice stvara – danas!

Nekada

AA-646

GC-8A

GCMS-QP1000

UV-260

Danas

GCMS-QP2010 Ultra

Nexera

LCMS-8030

Bilo u kromatografiji, spektroskopiji, masenoj spektrometriji, mjerenju težine, ispitivanju materijala ili biološkim znanostima – Shimadzu neprestano pomiče granice tehnologije otvarajuće nove vidike u molekularnom svijetu.

SHIMADZU d.o.o.
Zavrtnica 17
HR – 10 000 Zagreb
Tel. +385 1 61 85 777
Fax. +385 1 61 85 207
shimadzu@shimadzu.hr
www.shimadzu.hr

SHIMADZU

Soak up mycotoxins

with the #1 mycotoxin sequestering agent

Feeding Mycosorb, from Alltech, reduces mycotoxin absorption within the animal, thereby negating the damaging effects of mycotoxins on the health of your animals.*

MYCOSORB®

gets the job done!

Key Facts About Mycotoxins

- There are approximately 500 known mycotoxins.
- Animals are consistently exposed to multiple toxins.
- Interaction between toxins makes diagnosis difficult.
- Long term exposure to low levels of mycotoxins in the diet can reduce production and reproductive performance.

*Article 13, Regulation (EC) No. 767/2009
Mycosorb is not available for sale in the US or Canada.

Alltech® | Alltech.com | facebook.com/AlltechNaturally | @AlltechTweets