

13th INTERNATIONAL
CONGRESS
OF THE SERBIAN SOCIETY
OF TOXICOLOGY



1st TOXSEE
REGIONAL
CONFERENCE

Present and Future of toxicology: Challenges and opportunities



10 - 12 May, 2023 Belgrade

electronic

ABSTRACT
BOOK

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13th INTERNATIONAL
CONGRESS
OF THE SERBIAN SOCIETY
OF TOXICOLOGY

&

1st TOXSEE
REGIONAL
Conference

10 - 12 May,
2023 Belgrade



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živi bezbedno",
Erasmus+ projekt
u oblasti obrazovanja odraslih

DEAR COLLEAGUES, DEAR FRIENDS,

We are delighted to greet you on the **13th International Congress of the Serbian Society of Toxicology & 1. TOXSEE Regional Conference - Present and Future of toxicology: challenges and opportunities**, organized in Belgrade from 10-12 May 2023.

Five years after our last international Congress we gathered in Belgrade, to further promote contemporary toxicology, in the broadest sense of meaning, as a response to the new challenges requiring innovative approaches and solutions, as it is understood in the third decade of the XXI century.

Initial concept, to blend the top scientific level in toxicology with the potentials of its' use in broad array of clinical and other domains, proved to be right. Line-up of more than 70 first class international and regional faculties as well as best Serbian scientists and toxicology professionals in all related domains fully justify the approach. Moreover, interest and presence of more than 250 colleagues from Serbia and region witness that our professional community has recognized the approach taken and shown vast interest.

The Serbian Society of Toxicology is committed to innovation and creativity in research and education, in cooperation with collegial associations and institutions in Serbia and abroad. As a regional leader, we developed and inaugurated the regional brand TOXSEE, with the idea to gather as much as possible expertise and know-how from the region and Europe, to capture knowledge, share experience and exchange practical skills with colleagues who deal with toxicology problems daily.

Time imposes on us the need to integrate science, top knowledge and daily practice in a quality and efficient way, to contribute to the better health of the society as a whole in the most purposeful manner. Therefore, a thematic and functional connections with domains of emergency medicine, general medicine, paediatrics, ecology, in addition to already standard toxicological disciplines i.e. clinical, forensic, occupational, and experimental toxicology have been enhanced.

We are glad to host you in a pleasant atmosphere of Belgrade in mid-May, to benefit from the attractive and dynamic program, exchange knowledge, and, equally important, to refresh existing and establish new contacts with colleagues and friends, while enjoying our hospitality and cherish the moment in one of the best partying cities of Europe.

YOU ARE MOST WELCOME!!!



Prof. dr Petar Bulat

- President of the STC
- President of the 13th STC Congress

Petar Bulat



Prof. dr Biljana Antonijević

- President of the CSC
- of the 13th STC Congress

B. Antonijević



Prof. dr Predrag Vukomanović

- President of the COC
- of the 13th STC Congress

P. Vukomanović

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Present and Future of toxicology: Challenges and opportunities



10 - 12 May, 2023 Belgrade

CONGRESS
PROGRAM



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Bojana Petrović Serbia

Andrea Pirković Serbia

Branislava Srdenović Čonić ... Serbia

Milena Stošić Serbia

WEDNESDAY / 10th MAY

PRE-CONGRESS MINI-SYMPOSIUM



HALL 1 ~ DONJI DORČOL

16:00-17:30

• **Danijela Đukić-Čosić**

Education of the general population about toxicity: MeeTox project

OPENING CEREMONY



HALL 1 ~ DONJI DORČOL

18:00

~

18:15

WELCOME ADDRESS

- **Prof. dr Petar Bulat**, *President of the Serbian Society of Toxicology*
- **Prof. dr Biljana Antonijević**, *President of the Scientific Committee*



PLENARY SESSION

18.15-19:00

• **Akademician Prof. dr Vladimir Kostić**

MPTP-Benefits of one toxin

19:00-19:45

• **José E. Manautou**

Multidrug resistance protein-4 (Mrp4) at the intersection of the liver- adipose tissue axis

19:45 - 22:00

WELCOME RECEPTION

THURSDAY / 11th MAY



PLENARY LECTURE



HALL 1 ~ DONJI DORČOL

Chairs: : Slavica Vučinić, Danijela Đukić-Ćosić

9:00-9:40

• **Leonardo Trasande**

Endocrine disrupting chemicals: mainstream recognition of health effects and policy implications

9:40-9:45



DISCUSSION

9:45-10:00



Coffee break

SCIENTIFIC SESSION

10:00

-

12:00

**IMPACT OF ENVIRONMENTAL
POLLUTANTS ON HUMAN HEALTH:
NEW CHALLENGES**

Chairs: Slavica Vučinić,
Danijela Đukić-Ćosić

**IS OCCUPATIONAL TOXICOLOGY
TODAY PROFESSIONAL ONCOLOGY**

Chairs:
Petar Bulat, Metoda Dodič Fikfak

HALL 1 ~ DONJI DORČOL

HALL 2 ~ SHONDA 4

10:00-10:20

• **Emanuela Corsini**

New insights on PFAS immunotoxicity

• **Petar Bulat**

Occupational toxicology in Serbia
future directions of development

10:20-10:40

• **Pinar Uysal-Onganer**

Role of cadmium and nickel on
selected microRNA expression
in pancreatic ductal adenocarcinoma

• **Metoda Dodič Fikfak**

Exposure to asbestos and its effect
on the health of workers in the
Slovenian cement-asbestos industry

HALL 1 ~ DONJI DORČOL

HALL 2 ~ SHONDA 4

10:40-11:00

• **Aleksandra Buha Đorđević**
Investing the role of exposome
in human endocrine health:
DecodExpo project results

• **Cveta Georgieva**

Aerosols and their organic extracts
as a result of the utilization
of alternative energy carriers -
benefits and toxicological risk

11:00-11:20

• **Milena Kataranovski**
Immunotoxicological
effects of cadmium

• **Roberto Madeddu**

Cadmium and gemcitabine
relationship in human pancreatic
cancer cells

11:20-11:40

• **Kamil Musilek**
Challenges and promises in reactivation
of cholinesterases inhibited by
organophosphorus agents

• **Stefan Mandić Rajčević**

Occupational exposure to nanomaterials:
a new public health concern?

11.40-12.00

 DISCUSSION

 DISCUSSION

12:00-13:30

 LUNCH



PLENARY LECTURE



HALL 1 ~ DONJI DORČOL

Chairs: : **Slobodan Nikolić, Zorica Bulat**

13:30-14:10

• **Predrag Minić**

Ambient air pollution and respiratory morbidity in children

14:10-14:15

 DISCUSSION

14:20-16:20

SCIENTIFIC SESSION

FORENSIC TOXICOLOGY IN THE SERVICE OF LIFE

Chairs:

Slobodan Nikolić,
Zorica Bulat

CHILDREN IN THE WORLD OF CHEMICALS

Chairs:

Vladislav Vukomanović,
Aleksandra Buha Đorđević

YOUNG RESEARCHERS

Chairs:

Evica Antonijević Miljković,
Dubravka Rašić

HALL 1 ~ DONJI DORČOL

HALL 2 ~ SHONDA 4

HALL 3 ~ SHONDA 2

14:20
-
14:40

• **Tomaž Zupanc**

Organization of the forensic toxicology service in Slovenia - disadvantages and advantages

• **Snežana Ristić**

Child poisoning in Serbia – 20 years of experience of the Institute for mother and child health care of Serbia "Dr. Vukan Čupić"

14:20
-
14:30

• **Katarina Baralić**

Three lines of evidence of the hepatotoxicity of a mixture containing phthalates and bisphenol A: in silico and two in vivo models

14:30
-
14:40

• **Dragana Vukelić**

Oxidative stress induced by low doses of lead: a subacute toxicity study in Wistar rats and benchmark modeling

14:40
-
15:00

• **Đorđe Alempijević**

Clinical forensic toxicology

• **Vladislav Vukomanović**

Verapamil poisoning

14:40
-
14:50

• **Katarina Živančević**

Neurotoxicity of lead, cadmium, mercury and arsenic low dosed mixtures relevant to environmental exposure

14:50
-
15:00

• **Dragica Božić**

Predicting toxic effects of an immunomodulator, Sulforaphane: bioinformatic analysis

HALL 1 ~ DONJI DORČOL	HALL 2 ~ SHONDA 4	HALL 3 ~ SHONDA 2
<p>15:00 - 15:20</p> <p>• Aleksandra Repić Psychoactive substances in wastewater</p>	<p>• Vojislav Parezanović Poisonings by psychoactive substances in children</p>	<p>15:00 - 15:10</p> <p>• Đurđica Marić The relationship between nickel exposure and thyroid dysfunction - results of a human biomonitoring study</p>
<p>15:20 - 15:40</p> <p>• Vera Lukić Paraffin molds of liver tissue as an alternative sample for toxicological analysis</p>	<p>• Ivana Kitić Poisoning of children with corrosive agents</p>	<p>15:10 - 15:20</p> <p>• Nikola Stojilković Effect of lead and polychlorinated- biphenyls mixture on sperm cells and serum level of testosterone in rats</p>
		<p>15:20 - 15:30</p> <p>• Biljana Radović Assessment of lead and polychlorinated biphenyls mixture toxic effects on redox status in rat kidneys</p>
		<p>15:30 - 15:40</p> <p>• Nina Umičević Oxidative stress induced by low doses of lead: a subacute toxicity study in Wistar rats and benchmark modeling</p>
<p>15:40 - 16:00</p> <p> DISCUSSION</p>	<p>15:40 - 16:00</p> <p> DISCUSSION</p>	<p>15:40 - 16:00</p> <p> DISCUSSION</p>
<p>16:00 - 16:20</p> <p> Coffee break</p>	<p>16:00 - 16:20</p> <p> Coffee break</p>	

16:20

-

18:00

SCIENTIFIC SESSIONS

DEBATE

Analytical toxicology is a powerful tool and a great challenge

Moderators:

Vesna Kilibarda, Dijana Đurović

HALL 1 ~ DONJI DORČOL

HALL 2 ~ SHONDA 4

HALL 3 ~ SHONDA 2

16:20

16:40

• **Danica Đukić**

Toxicological analysis in forensic medicine: the importance of alternative samples

16:40

17:00

• **Aleksa Leković**

Chirality of psychoactive substance

17:00

17:20

• **Vladimir Živković**

Sodium-nitrite poisoning – forensic significance and interpretation of the results

17:20

17:40

• **Slobodan Nikolić**

Toxicological analysis of subdural hematoma – interpretation of results and forensic implications

17:40

18:00

DISCUSSION

• **Jovana Putnik**

Acute kidney damage caused by bismuth succinate intoxication

• **Gordana Kovačević**

Botulism in children - diagnostic challenges

• **Miloš Kuzmanović**

Von Munchausen syndrome - warfarin poisoning

• **Borko Gobeljić**

Benzodiazepine poisoning in children - a five-year experience

DISCUSSION

16:20

18:00

• **Dijana Đurović**

Advantages and limitations of liquid and gas chromatography in toxicological analysis

• **Snežana Đorđević**

Liquid chromatography - a powerful weapon in the screening of drugs and psychoactive substances

• **Maja Vujović**

Gas Chromatography – The Application in Forensic Toxicology

9:00 ~ 18:00

POSTER SESSION 1

HALL ~ GALERIJA

18:00 - 19:30

SST Assembly

HALL 1 ~ DONJI DORČOL

20:30 - 23:00 GALA DINNER

RESTAURANT TRI ŠEŠIRA, SKADARLIJA

FRIDAY / 12th MAY



PLENARY LECTURE



HALL 1 ~ DONJI DORČOL

Chairs: : Biljana Antonijević, Miloš Stojiljković

9:00-9:40

• **Richard Brown**

Chemical risk assessment: change, challenges and controversy

9:40-9:45



DISCUSSION

9:45-10:00



Coffee break

SCIENTIFIC SESSION

10:00

-

12:00

NEW TRENDS IN TOXICOLOGICAL RESEARCH

Chairs: Biljana Antonijević,
Miloš Stojiljković

HALL 1 ~ DONJI DORČOL

CHALLENGES AND MODERN APPROACHES IN CLINICAL TOXICOLOGY

Chairs:
Slavica Vučinić, Nataša Perković-Vukčević

HALL 2 ~ SHONDA 4

10:00-10:20

• **Miloš Stojiljković**

Novel experimental achievements in therapy of organophosphate intoxications

10:20-10:40

• **Danijela Đukić-Čosić**

Opportunities and limitations of toxicogenomic data analysis – our experiences

• **Niko Bekjarovski**

Six years after legalization of cannabis for medical use. Did we make a mistake?

• **Jasmina Jović-Stošić**

Toxicity of household products: experience of National Poison Control Centre

11:00-11:20

• **Ksenija Durgo**

Equivocal effect of bioactive compounds from plants

• **Hulya Turkan**

Latest knowledge on opioids polymorphism in pain management: The way forward

11:00-11:20

• **Marijana Ćurčić**

Quantifying interactions in the mixture toxicity studies - BDE-209 and cadmium

• **Nataša Perković-Vukčević**

Factors affecting benzodiazepines elimination, clinical features and outcome in elderly people acutely poisoned with benzodiazepines

11:00-11:20

• **Evica Antonijević Miljaković**

Enzyme immobilization as a strategy towards efficient and sustainable determination of cholinesterase inhibitors and reactivators

• **Marko Antunović**

Gabapentinoid abuse in Serbia - are we following the global trend?

11:40-12:00

 **DISCUSSION**

 **DISCUSSION**

12:00-13:30



LUNCH



PLENARY LECTURE



HALL 1 ~ DONJI DORČOL

Chairs: : **Milena Kataranovski, Aleksandra Buha Đorđević**

13:30-14:10

• **Demetrios Kouretas**

Emerging concepts and challenges in nanotoxicology: the role of oxidative stress in nanomaterial - induced toxicity

14:10-14:15

 **DISCUSSION**

SCIENTIFIC SESSION

14:20

-

16:20

CHEMICALS ARE ALL AROUND US

Chairs: Milena Kataranovski,
Aleksandra Buha Đorđević

EMERGENCY/GENERAL MEDICINE

Chairs: Marko Ercegovac,
Jasmina Jović Stošić

HALL 1 ~ DONJI DORČOL

HALL 2 ~ SHONDA 4

14:20-14:40

• **Nursen Basaran**

Adverse consequences of adulteration of herbal products

• **Marko Ercegovac**

Endogenous and exogenous intoxications in emergency neurology

14:40-15:00

• **Doris Marko**

Alternaria mycotoxins as a challenge for risk assessment

• **Dušica Gujaničić**

Poisoning by psychoactive substances - early hospital treatment in an emergency center

15:00-15:20

• **Dragica Brkić**

The status of pesticide active substances in the European Union and further perspectives of their withdrawal

• **Bojana Uzelac**

ECG changes in poisoning with drugs and other xenobiotics

15:20-15:40

• **Emilija Brdarić**

Gut microbiota as a target of toxic compounds

• **Marina Đikić**

Early hospital treatment of overdose with internist therapy: What is urgent and what is more urgent?

15:40-16:00

• **Melita Vidaković**

The various aspects of epigenetics in toxicology research

• **Dragana Trifunović Balanović,**
• **Marija Lazarević**

General principles of emergency treatment of poisoning in a general medicine doctor's practice - challenges and impediments

16:00-16:20

 **DISCUSSION**

 **DISCUSSION**

16:20-16:40

 **Coffee break**

 **Coffee break**

SCIENTIFIC SESSION

16:40

-

18:20

VETERINARY/EXPERIMENTAL TOXICOLOGY

Chairs:

Vitomir Ćupić, Dragica Brkić

ROUND TABLE - DISSEMINATION OF SCIENCE IN TOXICOLOGY

Moderators:

Marijana Ćurčić, Javier Esteban

HALL 1 ~ DONJI DORĆOL

HALL 2 ~ SHONDA 4

16:40

-

17:00

• Vitomir Ćupić

For and against the use of marijuana (cannabis) in veterinary medicine

17:00

-

17:20

• Paul Ziegler

Progress in ecotoxicity testing with duckweeds

17:20

-

17:40

• Saša Ivanović

Neurotoxic effect of Vipera ammodytes venom on the rat isolated diaphragm model

17:40

-

18:00

• Sunčica Borozan

Biomarkers of oxidative stress in the liver of rats treated with diazinon

18:00

-

18:20

• Dejana Ćupić Miladinović

Vitamin B1 as a potential antidote in the treatment of the Japanese quail intoxicated with chlorpyrifos

16:40

-

18:20

• Javier Esteban, Gonca Cakmak, Ksenija Durgo, Marijana Ćurčić

Topic 1 -

Toxicology dissemination by talks/radio/TV/Instagram

Topic 2 -

Internationalisation of the higher education in Toxicology

9:00 ~ 18:00

POSTER SESSION 2



HALL ~ GALERIJA

18.20-18.30 CLOSING CEREMONY



HALL 1 ~ DONJI DORĆOL



INVITED LECTURERS abstracts

**13th INTERNATIONAL
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Present and Future of toxicology: Challenges and opportunities



PLENARNA PREDAVANJA / PLENARY LECTURES

RMPTP: KORISTI OD JEDNOG TOKSINA

Vladimir S. Kostić¹

1 Srpska akademija nauka i umetnosti, Beograd, Srbija

MPTP (1-metil-4-fenil-1,2,3,6-tetrahidropiridin), protoksin, je prekursor neurotoksina MPP+, koji može da izazove trajne simptome Parkinsonove bolesti (PB) time što uzrokuje smrt dopaminergičkih (DA) neurona u supstanciji nigri mozga. MPTP je stoga korišćen i za razvoj životinjskih modela PB sa ciljem da se stekne bolji uvid u patomehanizme bolesti, ali i da se testiraju nove terapijske mogućnosti. Sam MPTP nema psihoaktivne efekte, ali se može akscidentalno oslobadati tokom sinteze MPPP, sintetskog opioida sa efektima sličnim morfinu ili petidinu (meperidinu). Da ovo jedinjenje može da indukuje parkinsonizam je prvi put pokazano 1976. godine. Tokom 1983. godine grupa mladih zavisnika iz Kalifornije dijagnostikovana je kao parkinsonizam nakon uzimanja MPPP koji je bio kontaminiran sa MPTP.

Zanimljivo je da je MPTP izazvao većinu biohemijskih, patoloških i kliničkih osobina PB kod ljudi i drugih primata. Obzirom da je liposolubilna, MPTP lako prolazi u mozak preko krvno-moždane barijere. On sam nije toksičan, ali se u astrocitima i serotonergičkim neuronima koji sadrže MAO-B, enzim neophodan za konverziju MPTP u MPP+, konvertuje u svoj oksidirani i toksični produkt MPP+. Ovaj toksin se oslobađa u vanćelijski prostor i potom putem DA transportera (DAT) prenosi u DA nerve završetke (inhibicija MAO-B ili DAT prevenira toksičnost MPTP). Toksičnost MPP+ nastupa po njegovom nagomilavanju u mitohondrijama putem selektivnog preuzimanja, gde inhibira kompleks I (dejstvo slično efektima rotenona i nekih drugih jedinjenja).

Zbog toga je smanjeno stvaranje ATP, uz inhibiciju od energije-zavisnih procesa (npr. transport jona), ali je uporedo poremećena homeostaza kalcijuma sa posledičnom aktivacijom Ca²⁺-zavisnih enzima, tipa protein kinaze i kalpaina I i II. MPP+ takođe pospešuje oksidativni stres i, konačno, svoju toksičnost može da ostvaruje i moduliranjem alfa-sinukleinskih mehanizama. "Priča o MPTP" pokrenula je potragu za drugim endogenim i egzogenim neurotoksinima koji bi mogli da budu uključeni u pokretanje za PB karakteristično izumiranje nigrálnih DA ćelija.

KLJUČNE REČI: 1-metil-4-fenil-1,2,3,6-tetrahidropiridin, MPTP, toksin, dopaminergički neuroni



MPTP: BENEFITS OF ONE TOXIN

PLENARY LECTURES

Vladimir S. Kostić¹

1 Serbian academy of sciences and arts, Belgrade, Serbia

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a precursor to the neurotoxin MPP⁺, which may cause permanent symptoms of Parkinson's disease (PD) by destroying brain dopaminergic neurons in the substantia nigra (SN). MPTP has been used to develop animal models of PD in order to provide insights into the possible pathogenesis and for testing possible new therapies. MPTP itself has no psychoactive effects, but this compound may be accidentally produced during the manufacture of MPPP, a synthetic opioid drug with morphine- and pethidine (meperidine)-like effects.

The Parkinson-inducing effects of MPTP were first discovered in 1976 following accidental contaminated MPPP injection. In 1983, several young people in California were diagnosed with Parkinsonism after having used MPPP contaminated with MPTP. Interestingly enough, MPTP induced most of the biochemical, pathological and clinical features akin to PD in nonhuman primates. Lipid-soluble MPTP, easily penetrates the blood-brain barrier and enters the astrocytes. MPTP itself does not appear to be toxic, while its oxidized product, 1-methyl-4-phenylpyridinium (MPP⁺) is. Astrocytes and serotonergic neurons contain MAO-B, enzyme necessary for conversion of MPTP to MPP⁺, which reaches the extracellular fluid and then is transported by the DA transporter (DAT) into DA nerve terminals (inhibition of both, MAO-B and DAT prevents MPTP-generated MPP⁺ toxicity). It has been suggested that the toxicity of MPP⁺ is dependent on a mitochondrial concentrating mechanism via selective uptake, where it inhibits complex I (effect similar to the effect of rotenone and several other compounds), and blocks mitochondrial oxidation.

Therefore, ATP formation is decreased, resulting in the inhibition of energy-dependent processes (i.e. ion transport). In parallel, disruption of calcium ion homeostasis occurs leading to the activation of Ca²⁺-dependent enzymes, such as protein kinase and calpains I and II. MPP⁺ also appears to support the occurrence of oxidative stress, and finally it may toxically act through the alpha-synuclein-mediated mechanism. "MPTP story" triggered the search for other endogenous or exogenous neurotoxins which may be involved in eliciting the PD-characteristic nigral cell death.

KEYWORDS: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, MPTP, toxin, dopaminergic neurons



A NOVEL ROLE FOR THE MULTIDRUG RESISTANCE PROTEIN 4 (ABCC4) IN PROMOTING HEPATIC STEATOSIS, ADIPOSITY AND METABOLIC SYNDROME

José E. Manautou¹, Hangyu Wu¹, Ankit Laddha¹

1 Department of pharmaceutical sciences, School of pharmacy, University of Connecticut, Connecticut, USA

Liver transporters are not only determinants of drug pharmacokinetics and pharmacodynamics, but also important contributors of drug-induced liver injury (DILI). Our laboratory has shown that paracetamol-induced DILI confers resistance to subsequent paracetamol toxic insult. Furthermore, this acquired tolerance to paracetamol hepatotoxicity is associated with hepatocellular regeneration and enhanced expression of the hepatobiliary transporter multidrug resistance protein 4 (Mrp4 or ABCC4). To begin deciphering this relationship, we performed partial hepatectomy in mice that are genetically deficient in Mrp4. Unexpectedly, lack of Mrp4 function did not alter hepatocyte compensatory proliferation rates following partial hepatectomy, but instead led to regeneration of liver tissue with a persistent steatotic phenotype. Additional studies aimed at characterizing the phenotype of Mrp4 knockout showed never reported functions for this drug transporter, such as regulation of adipogenesis, insulin resistance and promotion of an overall state of metabolic syndrome. In conclusion, what began as a drug-induced liver toxicity led to novel studies establishing for the first time a role for Mrp4 as a genetic factor that contributes to the development of hepatic steatosis and metabolic syndrome.

KEYWORDS: drug-induced liver injury, paracetamol, Mrp4, ABCC4



ENDOCRINE DISRUPTING CHEMICALS: MAINSTREAM RECOGNITION OF HEALTH EFFECTS AND POLICY IMPLICATIONS

Leonardo Trasande¹

1 NYU Grossman school of medicine, New York, USA

Since reports published in 2015 and 2016 identified 15 probable exposure-outcome associations due to endocrine-disrupting chemicals (EDCs), new studies in humans has now deepened understanding of their effects on human health. Particularly strong has emerged for relations between perfluoroalkyl substances and child and adult obesity, impaired glucose tolerance, gestational diabetes, reduced birthweight, reduced semen quality, polycystic ovarian syndrome, endometriosis, and breast cancer. Evidence also exists for relations between bisphenols and adult diabetes, reduced semen quality, and polycystic ovarian syndrome; phthalates and prematurity, reduced anogenital distance in boys, childhood obesity, and impaired glucose tolerance; organophosphate pesticides and reduced semen quality; and occupational exposure to pesticides and prostate cancer.

EDCs substantially cost society as a result of increases in disease and disability but-unlike other toxicant classes such as carcinogens-have yet to be codified into regulations as a hazard category. This presentation examines economic, regulatory, and policy approaches to limit human EDC exposures and describes potential improvements. In the EU, general principles for EDCs call for minimisation of human exposure, identification as substances of very high concern, and ban on use in pesticides. In the USA, screening and testing programmes are focused on estrogenic EDCs exclusively, and regulation is strictly risk-based. Minimisation of human exposure is unlikely without a clear overarching definition for EDCs and relevant pre-marketing test requirements. We call for a multifaceted international programme (eg, modelled on the International Agency for Research in Cancer) to address the effects of EDCs on human health-an approach that would proactively identify hazards for subsequent regulation.

KEYWORDS: endocrine disrupting chemicals, disease burden, costs



UTICAJ AEROZAGAĐENJA NA POJAVU RESPIRATORNIH POREMEĆAJA KOD DECE

PLENARY
LECTURES

Predrag Minić¹

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Zagađenje vazduha dolazi iz različitih izvora koji obuhvataju izduvne gasove iz vozila, termoelektrane na fosilna goriva, rafinerije nafte, gasova iz uzgoja stoke i šumske požare. Aerozagađenje dovodi do promene klime, a one pogoršavaju efekte zagađenja. Odojčad i deca su posebno osetljiva na zagađenje vazduha, jer su njihovi organi u razvoju, a potrošnja vazduha veća kada se izrazi na jedinicu telesne mase.

Sadašnji nivoi zagađenja vazduha su udruženi sa povećanjem brojnih značajnih uzroka morbiditeta kod dece – incidencije i prevalencije astme, pretermijskim rađanjem, porođajnom masom malom za gestacijsku starost, poremećajima kognitivnog i bihejvioralnog razvoja, malignim bolestima i povećanim rizikom od razvoja hroničnih bolesti u uzrastu odraslog. Ove uticaje proizvodi oksidativni stres, hronična inflamacija, endokrina disfunkcija, genetski i epigenetski mehanizmi koji postoje tokom celog života.

Dobro je dokumentovano da je respiratorni sistem, posebno kod dece sa astmom, osetljiv na delovanje aerozagađenja. Izloženost ozonu, azotnim oksidima, aerosolnim česticama i izduvnim gasovima je jasno povezana sa lošijom kontrolom astme, pojavom vizinga, smanjenjem funkcije pluća i povećanim apsentizmom dece i roditelja. Dve najčešće infekcije u ranom detinjstvu – bronhiolitis i otitis medija se povezuju sa aerozagađenjem. Zagađenje vazduha može da kod osoba sa cističnom fibrozom poveća rizik od egzacerbacija plućnih infekcija i upotrebu antibiotika. Postoji sve više dokaza koji ukazuju na ulogu ranog izlaganja zagađenju (uključujući i ono in utero) u razvoju astme i drugih alergijskih bolesti kod dece, kao i na smanjenje funkcije pluća. Ovi nalazi su osnov za napore koji se ulažu u razvoj intervencija koje imaju za cilj da se smanji uticaj aerozagađenja kod trudnica i dece širom sveta.

KLJUČNE REČI: aerozagađenje, zdravstveni rizici, deca



AMBIENT AIR POLLUTION AND RESPIRATORY MORBIDITY IN CHILDREN

PLENARY
LECTURES

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Ambient air pollution is produced by sources including vehicular traffic, coal-fired power plants, hydraulic fracturing, agricultural production, and forest fires. Air pollution causes and exacerbates climate change, and climate change worsens health effects of air pollution. Infants and children are uniquely sensitive to air pollution, because their organs are developing and they have higher air per body weight intake. Current levels of air pollutants are associated with many of the most important pediatric morbidities, including asthma incidence and prevalence, adverse birth outcomes, behavioral and cognitive development, and pediatric cancers, as well as with increased risk for a range of chronic diseases in adult life.

These effects are mediated by oxidative stress, chronic inflammation, endocrine dysfunction, and genetic and epigenetic mechanisms across the life span. The respiratory system has been recognized and the particular vulnerability of children is well established. Children with asthma are highly vulnerable to the respiratory effects of air pollutants. Ozone, nitrogen oxide, particulate matters, and traffic-related air pollution have been consistently associated with reduced asthma control manifested as increased symptoms, such as wheezing, rescue medication use, and decreased lung function, as well as increased use of medical services and school absences. In addition, two of the most common infectious disease problems of early childhood, bronchiolitis and otitis media, have been linked with ambient air pollution.

Air pollution may adversely affect individuals with cystic fibrosis by increasing the risk of pulmonary exacerbations and related antibiotic use as well as by increasing the risk for a decline in lung function. There is a rapidly developing evidence base supporting the role of early-life exposures (including exposures in utero) in the development of asthma and allergic disease in childhood and in reduced lung function. These consistent findings support ongoing efforts and targeted interventions to reduce air pollution exposures among pregnant women and children in various locations.

KEYWORDS: ambient pollution, health hazards, children



CHEMICAL RISK ASSESSMENT: CHANGE, CHALLENGES AND CONTROVERSY

PLENARY
LECTURES

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Chemical risk assessment plays a key role in managing chemicals to protect public health. The science underpinning many areas of chemical risk assessment is rapidly developing and as a result changes are taking place in toxicology. There are new methods to test chemicals and investigate toxicological mechanisms. Other approaches have developed such as human biomonitoring. At the same time, risk managers and wider society are demanding greater understanding of how risk assessments were undertaken and the uncertainties around conclusions, while toxicological assessments are becoming more complex and more difficult to explain. This complexity and uncertainty can lead to controversy, in particular where there is absence of trust and where credibility is questioned. This takes place against a background where the burden of disease from exposure to chemicals is already substantial and is increasing, and where the production and use of chemicals is set to increase significantly over the coming years. This talk will describe some of the ways that the practise of toxicology and risk assessment is evolving. Some progress - such as incorporating problem formulation, systematic approaches, methods for assessing combined exposures and characterising uncertainties - will be highlighted.

Also - some of the challenges - increasing complexity, inadequate surveillance, misinformation and challenges to credibility. This talk will provide an overview of these issues and challenges, drawn from a global context and with reference to where the WHO can play a role.

KEYWORDS: chemical risk assessment



EMERGING CONCEPTS AND CHALLENGES IN NANOTOXICOLOGY: THE ROLE OF OXIDATIVE STRESS IN NANOMATERIAL-INDUCED TOXICITY

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Towards the development of effective and safe nanoscale materials, the deep understanding of the interactions at the nano-bio interface and of the corresponding biological outcomes is of the utmost importance. Oxidative stress has been reported as a primary mechanism of nanoscale materials to exert their toxic effects on biological systems. Therefore, methodological approaches designed to thoroughly assess this toxicological endpoint can provide valuable insights. In the view of the foregoing, the purpose of this presentation is two-fold; firstly to report the antioxidant activities of novel hybrid composites based on carbon nanotubes (CNTs) and inorganic particles using a complete set of cell-free screening techniques; and secondly to report the biological effects of newly synthesized submicron polymers, with potential applications in materials industry, in cell-based systems, on the basis of a simplified concept of an occupational exposure scenario. According to our results, the novel CNT-based materials possess potent antiradical, reducing, and antigenotoxic properties in cell-free systems, that might render them as promising candidates for broad-spectrum antioxidants.

However, we strongly promote the significance of validating these findings and of characterizing the toxicological impact of the novel CNT-based materials at higher levels of complexity. Contrariwise, our findings derived from the investigation of the biological effects of submicron polymer materials in cell-based systems suggest that the 8-h exposure of EA.hy926 endothelial cells and of RAW264.7 macrophages to various concentrations (12.5–200 µg/ml) does not cause any acute toxicity on cell viability in this experimental setup. Nevertheless, the thorough evaluation of a panel of translational redox biomarkers, including biomarkers of antioxidant capacity, the reactive oxygen species (ROS) per se, and biomarkers of oxidative damage reveals that the submicron polymer materials induce alterations in the intracellular redox state in a cell-specific manner, hence causing harmful outcomes.

KEYWORDS: nanoscale materials, oxidative stress, biological outcomes



UTICAJ ZAGAĐIVAČA ŽIVOTNE SREDINE NA ZDRAVLJE LJUDI- NOVI IZAZOVI / IMPACT OF ENVIROMENTAL POLLUTANTS ON HUMAN HEALT - NEW CHALLENGERS

NEW INSIGHTS ON PFAS IMMUNOTOXICITY

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Per- and polyfluoroalkyl substances or perfluorinated alkylated substances (PFAS), such as perfluorooctane sulfonate (PFOS) and perfluorooctane acid (PFOA), have been used extensively in commercial/industrial applications for the last 70 years. They possess a strong carbon-fluorine bond, which leads to their environmental persistence. Due to their widespread use, environmental persistence, they are an important class of environmental contaminants and are of major toxicological concern.

There is evidence from both epidemiology and laboratory studies that PFAS are immunotoxic, affecting both cell-mediated and humoral immunity. Based on available studies in animals and humans, effects on the immune system were considered the most critical for the risk assessment. The decrease in antibody response at vaccination in children was identified as the critical effect. PFAS can directly affect immune cells functionality with different molecular underlying mechanisms, including NF- κ B activation, PPAR α and glucocorticoid receptor. Published data and data recently obtained in our laboratory will be presented and discussed.

KEYWORDS: PFAS, PFOS, PFOA, immunotoxicity



ROLE OF CADMIUM AND NICKEL ON SELECTED MICRORNA EXPRESSION IN PANCREATIC DUCTAL ADENOCARCINOMA

IMPACT OF ENVIRONMENTAL
POLLUTANTS ON HUMAN
HEALTH - NEW CHALLENGERS

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy, with a 5-year overall survival of less than 8%. Early detection is particularly difficult due to the lack of symptoms even in advanced stages. microRNAs (miRs/miRNAs) are small (~18–24 nucleotides), endogenous, non-coding RNAs, which are involved in the pathogenesis of several malignancies including PDAC. Alterations of miR expressions can lead to apoptosis, angiogenesis, and metastasis. The role of environmental pollutants such as cadmium (Cd) and Nickel (Ni) in PDAC has been suggested but not fully understood. This study aimed to investigate miR expressions in response to Cd and Ni in metastatic PDAC cells. We used two different PDAC cells lines to investigate the effects of Cadmium and Nickel.

EMT markers could be proven a novel target for anticancer therapy, we also examined the expression levels of Wnt-11, E-cadherin, Snail, and Zeb1 following Cd exposure in PDAC in vitro. Following the treatment with CdCl₂ and NiCl₂, oncomiRs such as miR-221 and miR-155 were significantly overexpressed, whereas miR-126 was downregulated. CdCl₂ led to an increase in epithelial–mesenchymal transition (EMT) via the dysregulation of mesenchymal markers such as Wnt-11, E-cadherin, Snail, and Zeb1. Conclusion Our study has provided evidence to suggest that the environmental pollutant Cd and Ni can have a significant role in the development of PDAC. Further studies are needed to investigate the precise role of miRs in PDAC progression as well as the role of Cd and Ni as well as other environmental pollutants. Furthermore, epigenetic data should be incorporated into risk assessments for Cd and/or Ni exposures improving our ability to predict outcomes and define more efficient prevention measures.

KEYWORDS: PDAC, cadmium, nickel, microRNA



ISPITIVANJE ULOGE EKSPOZOMA U ENDOKRINOM ZDRAVLJU LJUDI: REZULTATI DECODEXPO PROJEKTA

IMPACT OF ENVIRONMENTAL
POLLUTANTS ON HUMAN
HEALTH - NEW CHALLENGERS

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Ekspozom predstavlja sredinski ekvivalent ljudskom genomu. Istražujući ulogu toksičnih metala u različitim endokrinim poremećajima, projekat DecodExpo je nastojao da istraži kompleksne uticaje poreklom iz životne sredine na ljudsko endokrino zdravlje. Prvi deo projekta sastojao se iz studije humanog biomonitoringa (HBM), koja je nastojala da utvrdi nivoe odabranih šest toksičnih meta(oid)a (Pb, Cd, Hg, As, Cr i Ni), kojima su ljudi izloženi u svakodnevnom životu, kao i da ispita vezu između metala i različitih nivoa hormona u organizmu.

Tokom studije humanog biomonitoringa prikupljeno je ukupno 435 uzoraka krvi i to 299 uzoraka pacijenata obolelih od karcinoma prostate, testisa, dojke i pankreasa, kao različitih poremećaja štitaste žlezde i metabolizma, te 136 uzoraka sakupljenih od zdravih dobrovoljaca. Rezultati studije ukazuju na moguću vezu između nivoa ispitivanih metal(oida) u nivoima izmerenim u opštoj populaciji Srbije i većeg rizika za nastanak promena u reproduktivnoj, tiroidnoj i metaboličkoj funkciji u oba pola. Na osnovu nivoa metala određenih u studiji humanog biomonitoringa i na osnovu nivoa izračunatih primenom Benčmark doza (BMD) metodologije, kao novog alata predloženog za analizu podataka prikupljenih iz HBM studija. Istraživanje na životinjama je trajalo 90 dana, a u eksperimentu su korišćene životinje oba pola. Studija na životinjama otkrila je da smeša metal(oida) koja odslikava realne nivoe izloženosti ljudi iz životne sredine, pod kontrolisanim eksperimentalnim uslovima, ispoljava znake sistematske toksičnosti, endokrine toksičnosti kao i efekte na mikrobiom. Ovi nalazi zajedno sa nalazima dobijenim iz HBM studija pružaju dve linije dokaza o značajnoj ulozi smeša metala(oida) kojima smo izloženi iz životne sredine, na ljudski endokrini sistem.

KLJUČNE REČI: ekspozom, endokrino zdravlje, smeše, BMD koncept, DecodExpo



INVESTING THE ROLE OF EXPOSOME IN HUMAN ENDOCRINE HEALTH: DECODEXPO PROJECT RESULTS

IMPACT OF ENVIRONMENTAL
POLLUTANTS ON HUMAN
HEALTH - NEW CHALLENGERS

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The exposome represents the environmental equivalent of the human genome. The DecodExpo project aimed to explore the relationship between toxic metals present in the environment and various endocrine disorders. The first part of the project involved a human biomonitoring (HBM) study, which measured the levels of six toxic metal(oid)s (Pb, Cd, Hg, As, Cr, and Ni) in the general population and examined their connection to hormone levels in the body. The study involved 435 blood samples, with 299 from patients with various cancers and disorders and 136 from healthy volunteers.

The study found that the median levels of these metal(oid)s in the Serbian general population could be linked with the higher risk for substantial alterations of reproductive, thyroid, and metabolic function in both sexes. To further investigate the effect of toxic metals in animals, doses were chosen based on the levels found in the human biomonitoring study using the Benchmark dose (BMD) methodology. The animal study lasted for 90 days, and both male and female animals were used to consider gender differences in endocrine diseases. The study found that the "real-life" mixture of toxic metals had systematic toxicity and endocrine toxicity and affected the microbiome. These results together with the ones obtained from HBM study provide two lines of evidence pointing to the significant role of exposure to toxic metal(oid)s on the function of the human endocrine system.

KEYWORDS: exposome, endocrine health, mixtures, BMD methodology, DecodExpo



IMUNOTOKSIKOLOŠKI EFEKTI KADMIJUMA

IMPACT OF ENVIRONMENTAL
POLLUTANTS ON HUMAN
HEALTH - NEW CHALLENGERS

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Kadmijum (Cd) je među najtoksičnijim supstancama koja pogađaju mnoga tkiva/organe uključujući imunski sistem. Zbog kompleksne prirode imunskog sistema i njegove aktivnosti (koja se zasniva na mnogobrojnim interakcijama), kao i zbog razlika u studijama koje ispituju efekte Cd, mehanizmi koji su u osnovi imunotoksičnosti ovog metala još nisu dovoljno jasni. Čelije imunskog sistema se nalaze svuda u telu, organizovane u limfnim organima i kao mobilne ćelije u cirkulaciji. One se takođe nalaze u anatomskim barijerama (tkiva spoljašnjih i unutrašnjih površina tela kao što su koža, pluća, creva) gde sa rezidentnim ćelijama stvaraju lokalne interakcije koje regulišu odbranu i imunski-posredovanu homeostazu ovih tkiva. Izloženost Cd dovodi do disfunkcije limfnih organa, uglavnom zbog supresije T ćelija i ćelija urođene imunosti, što ugrožava odbranu organizma od patogena.

Sa druge strane, mobilne ćelije urođene imunosti infiltriraju tkiva u kojima je deponovan Cd (jetru, bubrege, anatomske barijere) izazivajući inflamaciju. Infiltrirani leukociti, kao i tkivne rezidentne imunске ćelije, proizvode različite solubilne medijatore inflamacije, citokine u prvom redu, koji organizuju inflamatorni proces i oslobađanje efektorskih molekula (reaktivnih vrsta kiseonika i azota, proteolitičke enzime) koji mogu da oštete tkivo. U barijernim tkivima Cd može da izazove odgovore koji ugrožavaju održanje imunski-posredovane homeostaze. Nepoželjni imunski efekti Cd u kombinaciji sa izmenjenim homeostatskim reparativnim aktivnostima mogu da doprinesu tkivnoj disfunkciji, što je potencijalno značajno za promenu osetljivost na razvoj bolesti, promociju bolesti ili potenciranje razvoja postojećih patoloških stanja.

KLJUČNE REČI: kadmijum, imunski efekti, osetljivost na bolest



IMMUNOTOXICOLOGICAL EFFECTS OF CADMIUM

IMPACT OF ENVIRONMENTAL
POLLUTANTS ON HUMAN
HEALTH - NEW CHALLENGERS

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Cadmium (Cd) is one of the most toxic substances affecting numerous tissues/organs, including the immune system. Due to the complex nature of the immune system and its activities (which are determined by multiple interactions) as well as variations in studies examining Cd effects, the underlying mechanisms of immunotoxicity of this metal are still vague. Cells of the immune system are found throughout the body, organized in lymphoid organs and as mobile cells in the circulation. Immune system cells also colocalize and interact with resident cells of anatomical barrier tissues (outer and inner body surfaces such as skin, lungs, gut) forming local circuits that control their defense and establish immune-mediated homeostasis.

Exposure to Cd causes malfunctioning of the lymphoid organs, mainly because of the suppression of T cells and innate lymphoid cells function compromising host protection against pathogens. On the other hand, mobile innate immune cells infiltrate Cd-deposited tissues such as the liver and the kidneys, as well anatomical barriers, facilitating inflammation. Infiltrated and tissue-resident leukocytes produce a variety of soluble mediators, in the first instance cytokines, which orchestrate the inflammatory response and release of effector molecules (reactive oxygen and nitrogen species, proteolytic enzymes) which might cause tissue injury. In anatomical barrier tissues Cd can induce responses that may exert an impact on the maintenance of immune-mediated homeostasis. Cd-induced adverse immune effects in combination with Cd-induced alterations in homeostatic reparative activities, may contribute to tissue dysfunction. This is potentially relevant for disease susceptibility, disease promotion, or facilitating development of pre-existing pathologies.

KEYWORDS: cadmium, immune effects, disease susceptibility



CHALLENGES AND PROMISES IN REACTIVATION OF CHOLINESTERASES INHIBITED BY ORGANOPHOSPHORUS AGENTS

IMPACT OF ENVIRONMENTAL
POLLUTANTS ON HUMAN
HEALTH - NEW CHALLENGERS

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The cholinesterase reactivators (so called “oximes”) are used as causal antidotes in the case of organophosphorus intoxications due to their ability to restore function of phosphorylated cholinesterases. Their research and development originated in the second half of 20th century, when pralidoxime, methoxime, trimedoxime, obidoxime and asoxime (HI-6) were developed and they are commercially available to date.

These standard oximes are able to reactivate organophosphorus (OP) G- and V-agents or insecticides to only certain extent and the most of them lack broad-spectrum effects. In addition, such charged reactivators have only minimal CNS bioavailability to counteract brain exposure by OP agents. Thus, the more recent studies were concerned on addressing issues of standard molecules. To enhance the broad-spectrum efficacy, the mixtures of charged reactivators were used. The uncharged acetylcholinesterase reactivators were tailored to overcome CNS bioavailability issues. And recently, charged reactivators with modified oximate forming properties (e.g. halogens) were introduced as more broad-spectrum and rapid reactivators of multiple OPs. Their particular instability and minimal CNS penetration can be handled by encapsulation strategies e.g. using modified human ferritins with oxime cargo [6]. However, there are also new challenges arising from e.g. A-agent human exposure. In this particular case, butyrylcholinesterase (BChE) reactivators seems to be way forward as well as simultaneous AChE and BChE reactivators.

KEYWORDS: organophosphate, cholinesterase, reactivator

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DA LI JE PROFESIONALNA TOKSIKOLOGIJA DANAS PROFESIONALNA ONKOLOGIJA / IS PROFESSIONAL TOXICOLOGY TODAY PROFESSIONAL ONCOLOGY

BUDUĆI PRAVCI RAZVOJA PROFESIONALNE TOKSIKOLOGIJE U SRBIJI

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Tokom poslednjih 30 godina došlo je do značajnog smanjenja izloženosti hemikalijama u proizvodnim procesima usled uvođenja novih tehnoloških rešenja ali i unapređenja u oblasti bezbednosti i zdravlja na radu. U tom periodu, iz ekonomskih razloga, smanjen je i broj preduzeća u Srbiji kojima je osnovna delatnost u oblasti hemijske industrije. Treba istaći da je u ovom periodu i sprovedena reorganizacija medicine rada. Sve ovo je rezultovalo smanjenjem broja zainteresovanih za profesionalnu toksikologiju. Najbolja ilustracija ovog pada interesa je da je u periodu 2010. do kraja 2022. upisan samo jedan kandidat užu specijalizaciju iz oblasti profesionalne toksikologije.

Takođe, postoji konstantan pad broja laboratorija koje pružaju usluge iz oblasti biološkog monitoringa profesionalno izloženih osoba, tako da 2023. godine samo jedna laboratorija pruža širi program usluga određivanja bioloških markera. Pored nje, još dve laboratorije pružaju usluge određivanja najosnovnijih bioloških markera koji se koriste za procenu profesionalne ekspozicije. Nažalost, iako je Pravilnik o prethodnim i periodičnim lekarskim pregledima zaposlenih na radnim mestima sa povećanim rizikom donet 2007. godine još uvek ne postoji mogućnost određivanja svih bioloških markera predviđenih ovim pravilnikom. Sve navedeno, između ostalog, rezultovalo je drastičnim padom broja profesionalnih bolesti izazvanih hemijskim štetnostima. U periodu 2003-2012 utvrđena su 62 profesionalna oboljenja a u periodu 2013-2022 samo jedno, profesionalno trovanje olovom. Imajući u vidu trend smanjenja nivoa ekspozicije kao sve više saznanja o toksičnim efektima dugotrajne niske izloženosti hemijskim štetnostima u narednom periodu treba usmeriti razvoj profesionalne toksikologije u Srbiji ka karcinogenim, mutagenim i reproduktivnim efektima profesionalne izloženosti hemikalijama zašta je neophodno značajno unapređenje toksikoloških laboratorija i proširivanje programa određivanja bioloških markera.

KLJUČNE REČI: Profesionalna toksikologija, biološki markeri, profesionalni rak



OCCUPATIONAL TOXICOLOGY IN SERBIA: FUTURE DIRECTIONS OF DEVELOPMENT

IS PROFESSIONAL
TOXICOLOGY TODAY
PROFESSIONAL ONCOLOGY

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Over the last 30 years, there has been a significant reduction in exposure to chemicals in production processes due to the introduction of new technological solutions and improvements in the field of occupational safety and health. In that period, due to economic reasons, the number of companies in Serbia whose main activity is in the field of chemical industry also decreased. It should be noted that the reorganization of occupational health in Serbia was carried out in this period. All this resulted in a decrease in the number of people interested in occupational toxicology. The best illustration of this decline in interest is that in the period from 2010 to the end of 2022, only one candidate was enrolled in the super-specialization in the field of occupational toxicology.

Also, there is a constant decrease in the number of laboratories that provide services in the field of biological monitoring of occupationally exposed persons, so that in 2023 only one laboratory offers a service of relatively wider program of biological marker determination. In addition to it, two more laboratories provide services for the determination of the most basic biological markers used to assess occupational exposure. Unfortunately, although the Rulebook on previous and periodic medical examinations of employees at workplaces with increased risk was adopted in 2007, it is still not possible to determine all the biological markers requested by this rulebook. All of the above, among other things, resulted in a drastic drop in the number of occupational diseases caused by chemical hazards. In the period 2003-2012, 62 occupational diseases were identified, and in the period 2013-2022, only one, occupational lead poisoning. Bearing in mind the trend of reducing the level of exposure as more and more knowledge about the toxic effects of long-term low exposure to chemical hazards, in the coming period, the development of occupational toxicology in Serbia should be directed towards the carcinogenic, mutagenic and reproductive effects of occupational exposure to chemicals, and consequently it is necessary to significantly improve toxicological laboratories and expand the program of biological markers determination.

KEYWORDS: Occupational toxicology, biological markers, occupational cancer



EXPOSURE TO ASBESTOS AND THE EFFECT ON THE HEALTH OF WORKERS IN THE SLOVENIAN CEMENT-ASBESTOS INDUSTRY

IS PROFESSIONAL
TOXICOLOGY TODAY
PROFESSIONAL ONCOLOGY

Metoda Dodič Fikfak

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In Slovenia, the use of asbestos was banned by law in 1996. Until then we imported 670 000 t of asbestos, we had around 1500 directly and 22500 potentially exposed workers, 596 workers developed asbestosis, 1927 pleural diseases, 84 lung cancer and 237 malignant mesothelioma.

In a nested case control study the cumulative exposure was calculated in f/cm³-years using conversion factors which took into account the type of exposure, the processing method, and the process type. The highest mean cumulative exposure of asbestos was calculated for asbestosis (31.8 fibers/cm³-yr). There was week evidence that exposure to chrysotile more than 35 years prior to diagnosis was associated with an increased risk of lung cancer (OR =2,2; CI = 0,4-11,9).

Our next aim was to investigate the role of genetic factors in development of asbestos related diseases. GSTP1 genotype increases the risk of developing asbestosis (OR = 1.49; CI = 1.06–2.10), the risk of asbestosis was 1.50 (CI = 1.01-2.24) for the MnSOD Ala/Ala genotype versus Ala/Val and Val/Val genotypes, and 1.63 (CI = 0.62-4.27) for the ECSOD Arg/Gly compared to Arg/Arg genotype.

We also investigated the influence of GCLC, GCLM, GSTM1 and GSTT1 polymorphisms, as well as the influence of interactions between polymorphism and interactions between polymorphisms and asbestos exposure, on the risk of developing asbestos related diseases. GSTT1 null genotype was associated with the decreased risk for pleural plaques (OR = 0.63; CI = 0.40-0.98) and asbestosis (OR = 0.51; CI = 0.28-0.93). A positive association was found between GSTP1 vrs 1695 AG + GG vs. AA genotypes for mesothelioma when compared to pleural plaques (OR = 1.39; CI = 1.00-1.94).



AEROSOLS AND THEIR ORGANIC EXTRACTS AS A RESULT OF THE UTILIZATION OF ALTERNATIVE ENERGY CARRIERS - BENEFITS AND TOXICOLOGICAL RISK

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PROFESSIONAL ONCOLOGY

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Residential heating, as well as uncontrolled combustion or partial oxidation of solid biofuels is the major sector causing particulate matter (PM) exceedance in many European cities. Initially, biomass combustion was accepted as zero emission alternative biofuel, thus gaining significant interest during the last decades. As a matter of fact, most of the solid biofuels are produced from lignocellulose biomass of different origin. However, recent findings confirmed the hazardous effects of the exhaust of biomass conversion. Assessment of the toxic impact of key pollutants, such as PMs and their organic extracts, resulting from the utilization of fossil and modern biofuels is essential at national, regional and international level. In 2016, according to the WHO, air pollution caused 4.2 million deaths. It is estimated that air pollution is responsible for a large proportion of deaths due to diseases, mainly of the respiratory and cardiovascular systems: lung cancer - 29%, chronic obstructive pulmonary disease - 43%, ischemic heart disease - 25%, strokes - 24%. It is known that local and systemic inflammatory processes, as well as decompensated oxidative stress, play a major role among the main factors involved in the pathogenesis of the listed diseases. An effective approach for evaluating their direct impact on the cellular system is determining the presence of cytotoxic effects, as well as analysing their mechanisms by determining direct structural damage due to oxidative modification of cellular proteins and indirectly - by changing gene expression and subsequent adaptive processes. The present work introduces a contemporary review on methods used to evaluate cytotoxic effects on cellular systems as a result of the direct impact of PMs and their organic extracts obtained in the process of thermal conversion of solid fossils and alternative fuels.

KEY WORDS: aerosols, biomass conversion, PMs, oxidative stress

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CADMIUM AND GEMCITABINE RELATIONSHIP IN HUMAN PANCREATIC CANCER CELLS

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Cadmium is one of the more abundant toxicants to which humans are exposed. It can be absorbed by inhalation, ingestion, and direct contact with skin. Excluding occupational exposure, exposure to Cd occurs mostly through cigarette smoking, consumption of contaminated food, water and air.

Its toxicological properties are due to its chemical similarity to and ability to compete with zinc, an essential micronutrient for plants, animals, and humans. Cadmium have been classified as carcinogenic by the IARC and many studies have found a correlation with several types of cancer including pancreatic cancer. Gemcitabine is the first-line chemotherapy used in the treatment of pancreatic cancer.

The principal pharmacological activity of the drug is competitive inhibition of DNA synthesis, leading to chain termination, DNA fragmentation and cell death but the development of chemoresistance still leads to poor clinical outcomes. In order to investigate whether the presence of cadmium in pancreatic cancer tissue could influence the effect of gemcitabine, we used two cell lines: BxPC-3, representative of primary pancreatic tumor, and AsPC-1, representative of metastatic cancer. These two cell lines were treated with cadmium for 7 and 14 days, simulating a chronic exposure. Then, we treated the same cells with gemcitabine for 72h. After treatments, cells were analyzed, by flow cytometry, for levels of apoptosis, cell cycle, presence of EMT and stemness markers, and in progress, the expression of miRNAs of interest. Our preliminary studies, currently underway, seem to highlight the fact that cadmium could influence the cytotoxic effects of gemcitabine in the cell lines studied, altering the characteristic effects of the drug. Further studies are needed to better see how cadmium alters the efficacy of gemcitabine.

KEYWORDS: toxic metal, pancreatic cancer cells, in vitro



IZLOŽENOST NANOČESTICAMA NA RADNOM MESTU: NOVI IZAZOV JAVNOG ZDRAVLJA?

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Broj proizvoda široke potrošnje i komercijalnih proizvoda koji sadrže nanomaterijale u porastu je decenijama, a mnogi autori su upotrebu i proizvodnju nanomaterijala identifikovali kao toksikološki rizik. Povećana upotreba nanomaterijala može se pripisati njihovim prednostima, kao što su mala veličina i pridružene karakteristike, ali te iste karakteristike povećavaju njihov toksični potencijal.

Dodatni izazov predstavljaju različiti scenariji izloženosti i putevi ekspozicije, uključujući respiratorni, oralni ili dermalni. Cilj ovog rada je da se da pregled i predstave toksični efekti, istraživački izazovi i potencijalni uticaj na javno zdravlje nanomaterijala i njihove široke upotrebe. Nanomaterijali i nanočestice se koriste u raznim industrijama širom sveta. Najčešće proučavani nanomaterijali uključuju metale, kao što su aluminijum, volfram karbid kobalt, cink oksid, platina, titanijum dioksid, dok najčešće proučavani (potencijalni) toksični efekti uključuju profesionalne alergije respiratornog trakta i kože, "hard metal disease", karcinom pluća i hepatotoksičnost. Glavne izazove predstavlja određivanje granica izloženosti, trajanje izloženosti, nedostatak biomarkera i proučavanje toksičnih efekta. Nanomaterijali predstavljaju potencijalni novi izazov za javno zdravlje i neophodno je istražiti njihove toksične efekte.

KLJUČNE REČI: nanočestice, profesionalna toksikologija, javno zdravlje



OCCUPATIONAL EXPOSURE TO NANOMATERIALS: A NEW PUBLIC HEALTH CONCERN?

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The number of consumer products and commercial products containing engineered nanomaterials has been on the rise for decades, and many authors have identified the use and production of nanomaterials as a toxicological concern. Nanomaterials' increased use can be attributed to their advantages, such as small size and associated characteristics, but those same characteristics increase their toxic potentials.

An additional challenge is the varying exposure settings and routes, including inhalation, ingestion or dermal. The aim of this work is to give an overview and present the potential toxic effects, research challenges, and impact on public health of nanomaterials. Nanomaterials and nanoparticles are used in various industries throughout the world. Most commonly studied nanomaterials include nanoscale metals, such as aluminum, tungsten carbide cobalt, zinc oxide, platinum, and titanium dioxide, while the most commonly studied (potential) toxic effects include occupational allergy in the respiratory tract and in the skin, „hard metal lung disease“, lung cancer, hepatotoxicity, nephrotoxicity, and various other diseases. The main challenges are posed by the difficulty of setting occupational exposure limits, estimating the duration of exposure, lack of adequate biomarkers and toxicological endpoints. Nanomaterials present a potential public health concern and it is necessary to further study their toxic effects.

KEYWORDS: nanoparticles, occupational toxicology, public health



FORENZIČKA TOKSIKOLOGIJA U SLUŽBI ŽIVOTA / FORENSIC TOXICOLOGY IN THE SERVICE OF LIFE

ORGANIZACIJA FORENZIČKE TOKSIKOLOŠKE SLUŽBE U SLOVENIJI – NEDOSTACI I PREDNOSTI

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Godine 1946. počela je sa radom Laboratorija za toksikologiju na Institutu za sudsku medicinu u Ljubljani: prvo su rađeni alkoholometrijski testovi po Vidmarkovoj proceduri i kvalitativna detekcija fosfolipida, lizola, ugljen-monoksida, nitrata i arsena u biološkim uzorcima. Prva instrumentalna metoda koja je korišćena bila je spektrofotometrija za kvantitativno određivanje karboksihemoglobina, barbiturata i metala u biološkim uzorcima. Laboratorija je tokom svog postojanja pratila razvoj analitičkih metoda i instrumentacije. Tako je uvedena ADH metoda za određivanje koncentracije alkohola, a 1978. i metoda gasne hromatografije: ove se metode, osavremenjene, koriste i danas.

U Laboratoriji vršimo toksikološka ispitivanja bioloških uzoraka za zdravstvene ustanove, sudove, policiju i dr. U radu koristimo tehnike instrumentalne separacije, tj. gasna hromatografija sa jednostavnim detektorima, gasna hromatografija u kombinaciji sa masenim spektrometrom, tečna hromatografija u kombinaciji sa tandem masenom spektrometrijom i imunohemijski analizator za skrining analize urina na najčešće lekove i psihoaktivne lekove, kao i UV, VIS spektrofotometar za brzo određivanje nekih jednostavnih i poznatih ili očekivanih analita. Redovno i uspešno učestvujemo u međunarodnim kružnim testovima za određivanje lekova (Skrining lekova u urinu (DS); Sistematska toksikološka analiza (SKS); Toksikološka analiza (TKS)) i kvantitativnom testu etanola (ET), koje sprovedi Referenzinstitut fur Bioanalitik u Bonu. Uključeni smo i u Sistem za rano upozoravanje na pojavu novih psihoaktivnih supstanci u Republici Sloveniji.

KLJUČNE REČI: forenzička toksikologija, sudska medicina, organizacija, Slovenija



ORGANIZATION OF THE FORENSIC TOXICOLOGY SERVICE IN SLOVENIA – DISADVANTAGES AND ADVANTAGES

FORENSIC TOXICOLOGY
IN THE SERVICE OF LIFE

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In 1946, the Toxicology Laboratory started operating, first with alcoholometric tests according to Widmark's procedure and with the qualitative detection of phospholipids, lysol, carbon monoxide, nitrates and arsenic in biological samples. The first instrumental method introduced in the laboratory was spectrophotometry for the quantitative determination of carboxyhemoglobin, barbiturates and metals in biological samples. Throughout its existence, the laboratory followed the development of analytical methods and instrumentation.

Thus, the ADH method for determining alcohol concentration was introduced and the gas chromatography method in 1978, the successors of which are still used today in an updated form. In the laboratory, we perform toxicological tests of biological samples for medical institutions, courts, police, etc., in the Republic of Slovenia. In our work, we use instrumental separation techniques, i.e. gas chromatography with simple detectors, gas chromatography coupled with a mass spectrometer, liquid chromatography coupled with tandem mass spectrometry and an immunochemical analyzer for screening analyzes of urine samples for the most common drugs and psychoactive drugs, as well as a UV-VIS spectrophotometer for some simple and quick determinations of known or expected analytes. We regularly and successfully participate in international round-robin tests for drug determination (Drug screening in urine (DS); Systematic toxicologic analysis (SX); Toxicologic analysis (TX)) and the quantitative Ethanol test (ET) conducted by the Referenzinstitut für Bioanalytik in Bonn. We are also included in the System for early warning of the appearance of new psychoactive substances in the Republic of Slovenia.

KEYWORDS: toxicology, forensic medicine, organisation, Slovenia



KLINIČKA FORENZIČKA TOKSIKOLOGIJA

FORENSIC TOXICOLOGY
IN THE SERVICE OF LIFE

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Forenzička toksikologija predstavlja proučavanje i praktičnu primenu toksikologije u sudskim i drugim postupcima. Forenzička toksikologija se neprestano razvija ne samo zbog ljudske fascinacije otrovima, već zbog sve veće potrebe za toksikološkim dokazima u pravosuđu i postupcima, kao što su, na primer, postupci pred organima socijalnog staranja, u vezi sa poslom pred poslodavcima itd. Cilj forenzičkog toksikologa je da utvrdi odsustvo ili prisustvo isparljivih supstanci, lekova i drugih hemikalija u raznim biološkim uzorcima. Uzorci prikupljeni u kliničkoj praksi, dakle od živih osoba, mogu služiti u različite svrhe u forenzičkoj toksikologiji. Najčešće se očekuje da forenzički toksikolozi procene prisustvo lekova i drugih hemikalija koje mogu dovesti do izmene ljudskog ponašanja.

Primena ovih analiza obuhvata širok spektar slučajeva kao što su, na primer, bezbednost saobraćaja i saobraćajne nezgode na putevima, međuljudsko nasilje i nezgode na radu. Važno je za pravedno sprovođenje zakona i donošenje drugih odluka da se povežu rezultati toksikoloških ispitivanja i skrininga i njihovo tumačenje što uključuje toksikologe i lekare, posebno specijaliste za sudsku medicinu.

KLJUČNE REČI: forenzička toksikologija, klinička toksikologija, sudska medicina



CLINICAL FORENSIC TOXICOLOGY

FORENSIC TOXICOLOGY
IN THE SERVICE OF LIFE

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Forensic toxicology is the study and practice of the application of toxicology to the purposes of the law. The discipline is constantly developing not only because of human fascination with poisons, but due to the increasing need for toxicological evidence in judicial and proceedings, such as for example with social welfare, work related, etc. Aim of the forensic toxicologist is to reveal the absence or presence of volatiles and other drugs and chemicals in a variety of biological specimens. Specimens collected in clinical set-up, i.e., from the living subjects, may serve different purposes in forensic toxicology. Most often, forensic toxicologists are expected to assess presence of drugs and other chemicals capable for modification of human behaviour. Application of these analysis includes a wide range of cases such as for example traffic safety and road traffic accidents, interpersonal violence, and workplace accidents. It is important for fair administration of justice, and other decision-making processes to link results of toxicological screening and its interpretation, namely, to foster close co-operation of toxicologists and medical practitioners, in particular medico-legal specialists.

KEYWORDS: forensic toxicology, clinical toxicology, legal medicine



PSIHOAKTIVNE SUPSTANCE U OTPADNIM VODAMA

FORENSIC TOXICOLOGY
IN THE SERVICE OF LIFE

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Upotreba zabranjenih psihoaktivnih supstanci je složena, prikrivena i stigmatizovana pojava u društvu koju je teško pratiti i nadzirati i ima negativne zdravstvene, socijalne i ekonomske posledice. Otpadne komunalne vode predstavljaju adekvatan analitički uzorak pomoću kojeg se može proceniti konzumiranje psihoaktivnih supstanci u društvu. Analiziranje droga u otpadnim komunalnim vodama predstavlja novu naučnu disciplinu sa potencijalom da se u realnom vremenu vrši praćenje geografskih i vremenskih trendova upotrebe zabranjenih supstanci. Analiza se sprovodi korišćenjem hromatografskih tehnika, najčešće tačne hromatografije sa tandem masenom spektrometrijom. Uzorci otpadnih voda se mogu sakupiti iz sekundarnih ili glavnih kolektora kanalizacionih voda čime se vrši monitoring naselja ili manjih lokacija kao što su radna mesta, škole, muzički festivali, zatvori. Izmerene koncentracije droga ili njihovih metabolita, ekskretovanih urinom ili fecesom, npr. kanabis, kokain, MDMA, MDA, amfetamin, metamfetamin, ketamin, nove psihoaktivne supstance (sintetički kanabinoidi i katinoni) i dr, reflektovaće količinu supstance koja je konzumirana u određenom vremenu i u određenoj populaciji. Određivanjem psihoaktivnih supstanci u otpadnim vodama mogu se izvesti procene o vrsti i stepenu korišćenja supstanci, zastupljenosti tržišta ali i pratiti trend upotrebe i navika konzumenata u toku vremena. Mogućnost da se brzo otkriju novi trendovi konzumiranja doprinosi razvoju strategija za redukciju mogućih štetnih posledica zloupotrebe droga.

KLJUČNE REČI: forenzička toksikologija, psihoaktivne supstance, otpadne vode



PSYCHOACTIVE SUBSTANCES IN WASTEWATER

FORENSIC TOXICOLOGY
IN THE SERVICE OF LIFE

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The use of illicit psychoactive substances is a complex, covert and stigmatized phenomenon in a society that is difficult to surveil and monitor, with its negative health, social and economic consequences. Municipal wastewater represents an adequate analytical sample for assessment of the consumption of psychoactive substances in society. Drug analysis in municipal wastewater is a new scientific discipline with the potential for real-time assessment of the geographical and temporal trends of the use of illicit substances. The analysis is carried out using chromatographic techniques, usually liquid chromatography with tandem mass spectrometry. For monitoring the settlements or smaller locations such as workplaces, schools, music festivals, prisons, wastewater samples can be collected from the main and secondary sewage collectors.

The measured concentrations of drugs or their metabolites, excreted in urine or feces, e.g., cannabis, cocaine, MDMA, MDA, amphetamine, methamphetamine, ketamine, new psychoactive substances (synthetic cannabinoids and cathinones) and others, will reflect the amount of substance consumed at a given time in a particular population. Monitoring and estimations of drug consumption, drug markets, consumption trends and changes in consumer habits over time could be established using the drugs determination in wastewater. The ability to quickly detect new consumption trends contributes to the development of strategies to reduce the possible harmful consequences of drug abuse.

KEYWORDS: forensic toxicology, psychoactive substances, wastewater



PARAFINSKI KALUPI TKIVA JETRE KAO ALTERNATIVNI UZORAK ZA TOKSIKOLOŠKU ANALIZU

FORENSIC TOXICOLOGY
IN THE SERVICE OF LIFE

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Postmortalni uzorci za toksikološku analizu mogu biti brojni i promenljivi. Upotreba telesnih tečnosti ili uzoraka tkiva organa jeste uobičajena praksa u forenzičkoj toksikološkoj analizi. Međutim, ponekad uzorci nisu uzeti blagovremeno tokom obdukcije: npr. tokom obdukcije nije postojala sumnja na upotrebu droga ili izlaganje hemikalijama pa uzorci biološkog materijala nisu izdvojeni za toksikološku analizu, ili pak nisu dostupni iz nekog drugog razloga. U ovim slučajevima, može da se izvrši naknadna toksikološka analiza uzoraka uzetih posle ekshumacije tela, što je opet nemoguće u slučajevima kremacije.

Međutim, delovi organa i tkiva uzetih i fiksiranih formalinom, uzoraka koji se prikupljaju tokom većine kliničkih i sudsko-medicinskih obdukcija za histopatološke preglede, može biti i uzorak za toksikološku analizu. Rastvor formalina koji sadrži najčešće 2-20% formaldehida koristi se za fiksaciju delova organa ili tkiva, pa i jetre, glavnog metaboličkog organa. Formaldehid je veoma reaktivna hemijska supstanca koja može reagovati sa analitom u postupku fiksacije tkiva. Kako deo tkiva stoji određeno vreme u rastvoru formalina dolazi do razblaživanja uzorka jer deo analita prelazi u rastvor. Pored toga priprema tkiva fiksiranog formaldehidom za prebacivanje u tzv. parafinske kalupe podrazumeva i postupak dehidracije alkoholom što dodatno utiče na smanjenje koncentracije analita u tretiranom tkivu. Parafin iz patohistološkog kalupa posebno otežava ekstrakciju. Sve navedeno čini da je tkivo jetre fiksirano formalinom jedan od najizazovnijih uzoraka za analizu, a kasnije i za interpretaciju dobijenih rezultata.

KLJUČNE REČI: forenzička toksikologija, formalin, parafinski kalupi, toksikološka analiza, postmortalna dijagnostika



PARAFFIN MOLDS OF LIVER TISSUE AS AN ALTERNATIVE SAMPLE FOR TOXICOLOGICAL ANALYSIS

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Postmortal samples for toxicological analysis can be numerous and variable. The use of body fluids or tissue samples of organs is a common practice in forensic toxicological analysis. However, sometimes samples were not taken in a timely manner, during the autopsy: e.g., during the autopsy there was no suspicion of drug use or exposure to chemicals, so samples of biological material were not separated for toxicological analysis or are not available for some other reason. In these cases, a subsequent toxicological analysis of samples taken after the exhumation of the body can be performed, which is impossible in cases of cremation.

However, parts of organs and tissues taken and fixed with formalin, samples that are collected during most clinical and forensic autopsies for histopathological examination, may also be a sample for toxicological analysis. A formalin solution containing 2-20% formaldehyde is used to fix parts of organs or tissues, including the liver, the main metabolic organ. Formaldehyde is a highly reactive substance that can react with analyte in the tissue fixation process. As part of the tissue stands for a certain time in the formalin solution, the sample is diluted because part of the analyte passes into the solution. In addition, the preparation of tissue fixed with formaldehyde for transfer to so-called paraffin molds involves the procedure of dehydration with alcohol, which additionally affects the reduction of the concentration of analytes in the treated tissue. Paraffin from the pathohistological mold is particularly difficult to extract. All the above makes formalin-fixed liver tissue one of the most challenging samples for analysis and later for interpretation of the results.

KEYWORDS: forensic toxicology, formalin, paraffin molds, toxicological analysis, postmortal diagnostics



ZNAČAJ TOKSIKOLOŠKE ANALIZE ALTERNATIVNIH UZORAKA U FORENZIČKOJ MEDICINI

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Tokom obdukcije, za postmortalnu hemijsko-toksikološku analizu najčešće se uzimaju krv, urin i tečnost staklastog tela oka. Alternativni uzorci uzimaju se u slučajevima kada iz nekog razloga drugi, standardni uzorci nisu pogodni ili su nedostupni. Ovu grupu uzoraka čine delovi unutrašnjih organa i tkiva, kosa, nokti, kost i koštana srž, sinovijalna tečnost, posteljica i mekonijum, ali i uzorci intrakranijalnih krvnih izliva. U ovim se uzorcima najčešće određuje koncentracija alkohola, prisustvo i koncentracija lekova i psihoaktivnih supstanci, koncentracija karboksimioglobina i hronična izloženost drogama i/ili teškim metalima.

Za procenu izloženosti nekoj supstanci u nekom vremenskom periodu radi se tzv. segmentna analiza, koja se zasniva na prethodno poznatoj brzini rasta kose/noktiju (kosa u proseku raste oko 1 cm, a nokti oko 0,3 cm mesečno). Značajna je analiza alkoholemije iz krvi subduralnog/epiduralnog hematoma u slučajevima kada je osoba nadživljavala povredu neko vreme posle kritičnog događaja: u momentu smrti alkohola nema u perifernoj krvi jer je kroz metabolizam već potpuno razložen, ali ga ima u koagulisanom krvi intrakranijalnog izliva. Nalaženje hemijsko-toksikološkim analizama različitih otrova u alternativnim uzorcima ne mogu se uvek dovesti u nesumnjivu uzročno-posledičnu vezu sa smrtnim ishodom, te se prilikom interpretacije dobijenih rezultata uvek moraju uzeti u obzir obdukcioni nalaz i okolnosti umiranja u svakom konkretnom slučaju. Posebno treba biti oprezan u tumačenju alkoholemije.

KLJUČNE REČI: forenzička toksikologija, postmortalna dijagnostika, alternativni uzorci



TOXICOLOGICAL ANALYSIS IN FORENSIC MEDICINE: THE IMPORTANCE OF ALTERNATIVE SAMPLES

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Samples that are most commonly taken during autopsy and used for postmortem toxicological analysis are blood, urine and vitreous humor. Alternative samples are taken in cases where for some other reason, standard samples are not suitable or are unavailable. This group of samples consists of parts of internal organs and tissues, hair, nails, bone and bone marrow, synovial fluid, placenta, and meconium, as well as samples of intracranial haematomas. Alcohol concentration, presence and concentration of drugs and psychoactive substances, carboxymyoglobin concentration and chronic exposure to drugs and/or heavy metals are most often determined in these samples.

To assess the exposure to a substance in a certain period, the so-called segmental analysis is used, which is based on the previously known growth rate of hair/nails (hair grows on average about 1 cm and nails about 0.3 cm per month). The analysis of alcoholaemia from the blood of a subdural/epidural haematoma is significant in cases when the person has survived the injury for some time after the critical event: at the time of death, alcohol is not present in the peripheral blood because it has already been completely metabolized, but it is present in the coagulated blood of the intracranial haematomas. The finding of various substances in alternative samples cannot always be brought into an undoubted cause-and-effect relationship with the cause of death. When interpreting the obtained results, the autopsy findings, and the circumstances of death in each specific case must always be considered. One should be especially careful when interpreting alcoholaemia.

KEYWORDS: forensic toxicology, postmortal diagnostics, alternative samples



HIRALNOST MOLEKULA PSIHOAKTIVNIH SUPSTANCI

FORENSIC TOXICOLOGY
IN THE SERVICE OF LIFE

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Psihoaktivne supstance najčešće jesu hiralna jedinjenja i postoje u najmanje dve stereoizomerske, enantiomerske forme. Enantiomeri poseduju identične hemijske i fizičko-hemijske odlike, ali zbog stereoselektivnosti transportera, enzima i receptora u organizmu pokazuju veoma značajne razlike u farmakokinetici – apsorpciji, distribuciji, metabolizmu, i eliminaciji, kao i farmakodinamici – jačini, efikasnosti, a nekada čak i u vrsti farmakološkog efekta. Savremene analitičke metode u forenzičkoj praksi omogućile su pouzdanu identifikaciju i kvantifikaciju lekova i droga u biološkom materijalu. Međutim, najčešće se rezultati tumače na osnovu ukupne koncentracije supstance i/ili njenih metabolita, pritom ne uzimajući u obzir odnos enantiomera.

Značaj ovoga je dvojak. Prvo, metabolizam pojedinih neuroloških i psihijatrijskih lekova ukršta se sa metabolizmom zakonom zabranjenih supstanci iz grupe amfetamina npr., te poreklo metabolita ne može da se nedvosmisleno utvrdi. Drugo, još važnije, jeste otežano tumačenje dobijenih rezultata analize postmortalnih uzoraka pri utvrđivanju uzroka i porekla smrti, naročito kada utvrđena koncentracija psihoaktivne supstance ne ukazuje na očigledan, dozno-zavisan, toksičan efekat. Takođe, zbog tzv. „hiralne zamene“ (chiral switch), odnosno prelaska sa proizvodnje racemskih smeša psihotropnih lekova na one koji sadrže samo jedan, biološki mnogo potentniji enantiomer (npr. R(-)-metadon), toksični efekti mogu da se jave i pri tzv. ranijim uobičajenim dozama. Pored svega navedenog, od forenzičkog značaja jeste određivanje izomera aktivnih principa i adulteranasa pri analizi zaplenjenih supstanci, što može da ukaže na hemijski put njihove sinteze i tako pomogne u istrazi i otkrivanju nelegalnih laboratorija za proizvodnju psihoaktivnih supstanci. Poznavanje i analiza izomera psihoaktivnih supstanci jedan je od uslova za ispravno tumačenje dobijenih rezultata.

KLJUČNE REČI: forenzička toksikologija, hiralnost, enantiomer, psihoaktivne supstance, postmortalna dijagnostika



CHIRALITY OF PSYCHOACTIVE SUBSTANCES

FORENSIC TOXICOLOGY
IN THE SERVICE OF LIFE

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Psychoactive substances are mostly chiral chemical compounds and exist in at least two stereoisomeric, enantiomer forms. Enantiomers have identical chemical and physic-chemical properties, but due to the stereoselectivity of transporters, enzymes, and receptors in human body they show significant differences in pharmacokinetics – absorption, distribution, metabolism, and elimination, as well as in pharmacodynamics – potency, efficacy, and sometimes even in pharmacological effect. Modern analytical methods in forensics enabled reliable identification and quantification of drugs and illicit substances in biologic samples. However, the results are often interpreted based on reported total concentrations of detected substance and/or metabolites, not considering the ratio between the enantiomers. This is important for two reasons.

Firstly, some neurologic and psychiatric drugs metabolism overlaps with, for example, that of amphetamine-like substances, and the origin of the metabolites cannot be unambiguously determined. Secondly, more important, the interpretation of results of postmortal sample analysis in terms of cause and manner of death can be challenging, particularly if reported concentration of psychoactive substance does not point to an overt dose-dependent toxic effect. Moreover, due to the chiral switch, synthesis of more potent single-enantiomer pharmaceutical drugs instead of racemic mixture (e.g., R(-)-methadone), the toxic effects can occur upon intake of previously “regular” therapeutic dose. Finally, the enantiomer analysis of active compounds and impurities in seized substances can point to chemical synthesis reactions and thus aid in investigation of illegal laboratories for synthesis of illicit psychoactive substances.

KEYWORDS: forensic toxicology, chirality, enantiomer, psychoactive substance, sample analysis, postmortal diagnostics



TROVANJE NITRITIMA – FORENZIČKI ZNAČAJ I INTERPRETACIJA REZULTATA

FORENSIC TOXICOLOGY
IN THE SERVICE OF LIFE

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Natrijum nitrit se koristi kao konzervans, ali i za postizanje dobijanje odgovarajuće (crvene) boje mesa, ribe i nekih vrsta sira. Kontaminacija hrane toksičnim dozama natrijum nitrita najčešće je zadesna i dešava se usled nemara, neiskustva ili nepažnje, budući da je sličnog izgleda kao kuhinjska so ili drugi dodaci koji se koriste prilikom pripreme mesnih prerađevina.

Trovanje nitritima iako retko, može da dovede do smrtnog ishoda, zbog oksidacije hemoglobina u met-hemoglobin, dovodeći do akutne methemoglobinemije. Akutna methemoglobinemija je metabolički poremećaj u kojem je sa jedne strane, otežano vezivanje i transport kiseonika za hemoglobin, a sa druge, otežano je otpuštanje kiseonika od hemoglobina. Ovo stanje može da dovede do teških posledica bez adekvatnog lečenja. Antidot je metilensko plavo. Simptomi i znaci trovanja nitritima zavise od koncentracije methemoglobina u krvi – koncentracija oko 10% manifestuje se cijanozom, a preko 20% glavoboljom, vrtoglavicom, otežanim disanjem, tahikardijom i malaksalošću. Pri koncentracijama od oko 60% javlja se gubitak svesti, a koncentracija methemoglobina preko 70%, najčešće dovodi do smrtnog ishoda.

Prisustvo anemije, acidoze, poremećaja respiracije ili srčanih bolesti potencira toksični efekat met-hemoglobina. Obdukcioni nalaz je nespecifičan, osim karakterističnog spoljašnjeg znaka – smeđih („čokoladnih“) mrtvačkih mrlja. Dijagnoza trovanja nitritima zasniva na određivanju nivoa methemoglobinemije, kao i hemijsko-toksikološke analize sa određivanjem nivoa nitrata u krvi ili organima, uz isključenje drugih uzroka smrti obdukcijom.

KLJUČNE REČI: forenzička toksikologija, postmortalna dijagnostika, trovanje nitritima



SODIUM-NITRITE POISONING - FORENSIC SIGNIFICANCE AND INTERPRETATION OF THE RESULTS

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Sodium nitrite is used as a coloring agent or preservative in food and an antimicrobial agent in meat, fish, and some types of cheese. Contamination of food with toxic doses of nitrites is usually accidental, and it may occur through negligence, inexperience, or lack of caution, such as the use of nitrites in the preparation of salt substitutes or other ingredients that have a similar appearance. Sodium nitrite poisoning is an unusual and potentially fatal condition in which hemoglobin is oxidized to methemoglobin, causing acute methemoglobinemia. Acute methemoglobinemia is a metabolic disorder in which methemoglobin cannot bind and transport oxygen, and also with a reduced amount of oxygen released from hemoglobin. The condition can be severe if not adequately treated (with methylene blue).

Symptoms and signs of nitrite poisoning include cyanosis, with a methemoglobin level of about 10%, while with concentrations over 20%, headaches, dizziness, panting, tachycardia, and general weakness may appear. Loss of consciousness occurs with concentrations of about 60%, while methemoglobin levels of 70% are generally lethal, but the existence of underlying anemia, acidosis, respiratory compromise, and cardiac disease may exacerbate the toxicity of methemoglobin. Autopsy findings are usually non-specific, apart from a characteristic sign – brownish appearance of postmortem hypostasis (livores mortis). Diagnosis of nitrite poisoning is based on the level of methemoglobinemia, as well as toxicological analyses and determination of the level of nitrates in the blood or organs, with the exclusion of other potential causes of death.

KEYWORDS: forensic toxicology, postmortal diagnostics, sodium-nitrite poisoning



TOKSIKOLOŠKA ANALIZA SUBDURALNOG HEMATOMA - INTERPRETACIJA REZULTATA I FORENZIČKI ZNAČAJ

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Upotreba alkohola jeste jedan od faktora rizika u slučajevima nasilnih smrti. Kvalitativno i kvantitativno određivanje etanola u postmortalnim uzorcima postalo je relativno jednostavna analitička procedura i mogući su tačni, precizni i specifični rezultati. Međutim, tumačenje dobijenih rezultata toksikološkom analizom postmortalnog uzorka krvi ponekad nije tako jednostavno, a pogotovu zaključak o nivou alkohola u krvi za života, u trenutku povređivanja ili smrti. Krvni ugrušak jeste potencijalni izvor novih podataka u istrazi nasilnih smrti. Uzimanje uzorka krvi iz subduralnog ili epiduralnog hematoma jeste korisno u slučajevima smrti u vezi sa povredama glave i to posebno onda kada je žrtva nadživljavala povedu neko vreme posle pada ili udarca u glavu od strane napadača. Zbog smanjene ili potpuno odsutne cirkulacije u intrakranijalnom krvnom izlivu, alkohol se u krvnom ugrušku ne metaboliše u istoj meri kao krv koja cirkuliše kroz jetru, pa će krvni ugrušak sadržavati veću koncentraciju alkohola u poredenju sa uzorkom periferne venske krvi dobijenim na obdukciji. Uzimanje uzoraka i analizaintrakranijalnih krvnih ugrušaka može da pružiti korisne, komplementarne podatke o koncentraciji alkohola u krvi neke osobe nekoliko vreme pre smrti, odnosno u vreme kada je ugrušak formiran, tj. kada je žrtva povređena.

KLJUČNE REČI: forenzička toksikologija, postmortalna dijagnostika, subduralni izliv



TOXICOLOGICAL ANALYSIS OF SUBDURAL HEMATOMA- INTERPRETATION OF RESULTS AND FORENSIC IMPLICATIONS

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Over-consumption of alcohol plays a major role in trauma deaths. The qualitative and quantitative determination of ethanol in postmortem specimens has become a relatively simple analytical procedure and obtaining accurate, precise, and specific results is possible. However, interpreting postmortem blood alcohol concentration results could be challenge: the conclusions about antemortem blood alcohol level and the degree of person's behavioral impairment at the time of death is fraught with difficulties.

The blood clot is another potential source of information in an unnatural death investigation if intoxication is suspected or indicated by the behavior of the victim. Obtaining a blood specimen from a subdural or epidural hematoma is a useful strategy in deaths caused by a blow to the head. A victim could survive for a period of time after a fall or blunt trauma to the head with circulation remaining intact until the time of death. Owing to the reduced circulation in the damaged region of the brain, alcohol in the blood clot is not metabolized to the same extent as blood circulating through the liver. Accordingly, the blood clot will contain a higher concentration of alcohol compared to a specimen of peripheral venous blood obtained at autopsy. The sampling and analysis of intracranial blood clots might therefore provide useful complementary information about the person's blood alcohol concentration several hours before death, such as, when the trauma occurred.

KEYWORDS: forensic toxicology, postmortal diagnostics, subdural hematoma



DECA U SVETU HEMIKALIJA / CHILDREN IN THE WORLD OF CHEMICALS

TROVANJA DECE U SRBIJI - 20 GODINA ISKUSTVA INSTITUTA ZA ZDRAVSTVENU ZAŠTITU MAJKE I DETETA SRBIJE „DR VUKAN ČUPIĆ”

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Akutna trovanja, bez obzira na stepen ispoljenosti simptoma, predstavljaju urgentna stanja. Tačna incidencija trovanja dece nije poznata. U odnosu na vrstu, dominiraju materije koje se nalaze u okruženju u kom deca borave, a među njima trovanja lekovima su daleko najbrojnija. Dijagnozu trovanja je teško postaviti, jer mala deca ne znaju da govore, a veća su sklona da svesno prećute ili daju lažne podatke. Analiza interne baze podataka (protokola i istorija bolesti) u periodu 2002-2022. godine dece uzrasta 0-14 godina koja su lečena u Institutu zbog trovanja.

Cilj ovog rada je predstavljanje poslednjih dvadeset godina našeg iskustva. Trovanja u dečjem uzrastu predstavljaju treće akutno stanje po učestalosti. Najčešće akutna, zadesna, nenamerna i peropralna. U proseku je pregledano jedno dete dnevno zbog sumnje na trovanje. Među uzročnicima dominiraju lekovi i sredstva koja se koriste u domaćinstvu. Češća su u uzrastu do 4 godine, bez razlike u odnosu na pol i prezentovala su se uglavnom kliničkom slikom lakog stepena. Trovanja sa smrtnim ishodom nije bilo. Morbiditet i mortalitet usled trovanja kod dece poslednjih godina je u značajnom padu.

Zabrinjava porast zloupotrebe psihoaktivnih supstancija (cigareta, alkohola, legalnih i ilegalnih psihoaktivnih supstancija), kao i konzumacija novih »droga« u periodu rane adolescencije.

KLJUČNE REČI: trovanje, deca, incidencija



CHILD POISONING IN SERBIA - 20 YEARS OF EXPERIENCE OF THE INSTITUTE FOR MOTHER AND CHILD HEALTH CARE OF SERBIA "DR. VUKAN ČUPIĆ"

CHILDREN IN THE WORLD
OF CHEMICALS

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Acute poisonings, regardless of the degree of manifestation of symptoms, represent urgent conditions. The exact incidence of poisoning in children is not known. In relation to the type, substances found in the environment where children live dominate, and among them, drug poisoning is the most numerous. It is difficult to make a diagnosis of poisoning, because young children do not know how to speak, and older children tend to keep silent or give false information.

Analysis of the internal database (protocols and medical history) in the period 2002-2022. of children aged 0-14 who were treated at the Institute for poisoning. Aim of this work is to represent the last twenty years of our experience. Poisoning in children is the third most common acute condition. Most often, acute, unintentional and exposed through the oral route. On average, one child per day was examined for suspected poisoning. Medicines and household products dominate among the causative agents. They are more common in the age group of up to 4 years, with no difference in relation to gender. They presented mostly with a mild clinical picture. There was no fatal poisoning. Morbidity and mortality due to poisoning in children has decreased significantly in recent years. The increase in abuse of psychoactive substances (cigarettes, alcohol, legal and illegal psychoactive substances), as well as the consumption of new "drugs" in the period of early adolescence, is of concern.

KEYWORDS: poisoning, children, incidence



TROVANJE VERAPAMILOM

CHILDREN IN THE WORLD
OF CHEMICALS

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Blokatori kalcijumskih kanala (BCaK) su često propisivani lekovi u lečenju kardiovaskularnih bolesti uključujući hipertenziju, koronarnu bolest i pretkomorske aritmije. Verapamil selektivno blokira kalcijumove kanale L-tipa u miokardu koji su odgovorni za kontraktilnost kardiomiocita i vaskularnih glatkih mišića. Nenamerno izlaganje ili namerno predoziranje BCaK izaziva perifernu vazodilataciju, hipotenziju, bradikardiju, metaboličku acidozu, hiperglikemiju, kongestivnu srčanu insuficijenciju, edem pluća i srčani zastoj. S obzirom da ne postoji dovoljno podataka u literaturi o lečenju pedijatrijskih pacijenata sa teškim trovanjem BCaK, strategije lečenja pedijatrijskih pacijenata uglavnom su ograničene na slučajeve odraslih i konsenzusne preporuke stručnjaka za odrasle. Ne postoji specifičan antidot za predoziranje verapamilom, a ekstrakorporalno uklanjanje hemodijalizom nije efikasno, dok je orogastrična lavaža korisna jedino ako se izvodi u prva 2 h nakon uzimanja leka, ali vagalna stimulacija može pogoršati bradikardiju i hipotenziju izazvanu verapamilom.

Kod pacijenata koji su bez znakova trovanja mogu se primeniti nespecifične mere u lečenju trovanja, kao što je primena aktivnog uglja u roku od sat vremena nakon uzimanja tableta. U slučaju da deca razviju znake trovanja potrebno je sprovesti kardiovaskularni monitoring i primeniti simptomatsku terapiju u cilju postizanja i održavanja hemodinamske stabilnosti. U terapiju prve linije spadaju bolusi kristaloida, rastvora kalcijuma i vazopresori. Takode, kod trovanja verapamilom mogu se koristiti glukagon i insulin, jer glukagon ima pozitivne hronotropne i inotropne efekte, mada nije efikasan u oporavku bradikardije, dok je insulin uspešan u tretmanu hipotenzije, hiperglikemije i metaboličke acidoze, ali se nije pokazalo da ima dovoljan efekat u lečenju bradikardije, srčanog bloka i sprovodljivosti. Uprkos tome, insulin se preporučuje kao terapija prve linije za odrasle. Intravenska terapija lipidnom emulzijom (ILE) se koristi kod trovanja lipofilnim lekovima, kao što su BCaK, beta blokatori, neuroleptici, antidepresivi i antokonvulzivi i može se razmotriti kod pacijenata koji su refraktorni na prvu liniju terapije trovanja verapamilom. U in vitro studijama, pokazano je da tradicionalni lekovi kao što su suplementi kalcijuma, glukagon i insulin koji se koriste u toksičnosti verapamila ne utiču na kalcijumske kanale L-tipa, ali ILE terapija direktno utiče na L-tip kalcijumskih kanala, te vraćajući kontraktilnost miocita. Iako najveći broj pedijatrijskih pacijenata ne razvije klinički sliku trovanja verapamilom, trebalo je sprovesti kardiovaskularni monitoring u cilju što ranijeg prepoznavanja i lečenja potencijalno fatalnih komplikacija.

KLJUČNE REČI: blokatori kalcijumskih kanala, kardiovaskularne bolesti, varfarin



VERAPAMIL POISONING

CHILDREN IN THE WORLD
OF CHEMICALS

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Calcium channel blockers (CCB) are commonly prescribed drugs in treating cardiovascular diseases, including hypertension, coronary heart disease and atrial arrhythmias. Verapamil selectively blocks L-type calcium channels in the myocardium, which are responsible for the contractility of cardiomyocytes and vascular smooth muscles. Accidental exposure or intentional overdose of CCB causes peripheral vasodilatation, hypotension, bradycardia, metabolic acidosis, hyperglycemia, congestive heart failure, pulmonary oedema, and cardiac arrest. Given the insufficient data in the literature on treating pediatric patients with severe CCB toxicity, treatment strategies for pediatric patients are generally limited to adult cases and expert consensus recommendations for adults. There is no specific antidote for verapamil overdose, and extracorporeal hemodialysis removal is ineffective. At the same time, orogastric lavage is only useful if performed within the first two hours after drug administration, but vagal stimulation may exacerbate verapamil-induced bradycardia and hypotension. In patients without signs of poisoning, non-specific measures can be used to treat poisoning, such as administering activated charcoal within an hour after taking the tablets.

If children develop signs of poisoning, it is necessary to conduct cardiovascular monitoring and apply symptomatic therapy to achieve and maintain hemodynamic stability. First-line therapy includes boluses of crystalloids, calcium solutions, and vasopressors. Also, glucagon and insulin can be used in verapamil poisoning because glucagon has positive chronotropic and inotropic effects. However, it is not effective in bradycardia recovery. At the same time, insulin successfully treats hypotension, hyperglycemia and metabolic acidosis; it has not been shown to have a sufficient effect in treating bradycardia, heart block and viability. Despite this, insulin is recommended as first-line therapy for adults. Intravenous lipid emulsion (ILE) therapy could be given in lipophilic drug poisonings, such as CCB, beta-blockers, neuroleptics, antidepressants, and anticonvulsants. It may be considered in patients refractory to first-line therapy for verapamil poisoning. In vitro studies have shown that traditional drugs such as calcium supplements, glucagon, and insulin used in verapamil toxicity do not affect L-type calcium channels. Still, ILE therapy directly affects L-type calcium channels, restoring myocyte contractility. Although most pediatric patients do not develop a clinical picture of verapamil poisoning, it is necessary to carry out cardiovascular monitoring to recognise and treat potentially fatal complications as early as possible.

KEYWORDS: calcium channel blockers, cardiovascular diseases, warfarin



ZLOUPOTREBA LEKOVA U CILJU POKUŠAJA SUICIDA KOD DECE I MLADIH

CHILDREN IN THE WORLD
OF CHEMICALS

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Samoubistvo i samoubilačko ponašanje mladih predstavlja ozbiljno zdravstveno i socijalno pitanje i značajan problem svakog društva. Pokušaji samoubistva glavni su uzrok hospitalizacije među adolescentima i mladima uzrasta od 15 do 25 godina. Svetska zdravstvena organizacija procenila je da oko milion ljudi godišnje izvrši samoubistvo, što predstavlja globalnu stopu samoubistva od 16 na 100.000 stanovnika.

Cilj ovog istraživanja je pregled slučajeva namerne intoksikacije mladih koji su zbrinuti na Univerzitetnoj dečjoj klinici u poslednje dve godine i utvrđivanje najčešćih razloga intoksikacije, demografskih karakteristika populacije, prevalence recidiva, kao i koraka koji su preduzeti i koje je potrebno preduzeti radi prevencije budućeg suicidalnog ponašanja. Korišćene su osnovne kvantitativne statističke analize za opis uzorka, kao i kvalitativne analize izveštaja i razgovora sa hospitalizovanim mladima. Ukupno je 18 dece primljeno zbog intoksikacije tokom 2021. i 2022. godine, od toga 17 su bile devojčice, uzrasta od 12 do 18 godina i jedan dečak. Od njih 14 je hospitalizovano, dok su 4 odmah prosleđeni u ustanovu mentalnog zdravlja. Od onih koji su hospitalizovani za njih 6 su potraživane konsultacije psihologa. Potrebno je dalje praćenje ovih tendencija i posebno obraćanje pažnje na mlade sa ponovljenim pokušajima. Važno je postojanje protokola postupaka i upućivanja koji bi podrazumevao smernice za komunikaciju sa pacijentom i timski pristup pedijataru, medicinskih saradnika i kolega sa klinika za mentalno zdravlje.

KLJUČNE REČI: samoubilačko trovanje, adolescenti, trovanje lekovima



DRUG ABUSE FOR SUICIDAL ATTEMPTS IN CHILDREN AND ADOLESCENT

CHILDREN IN THE WORLD
OF CHEMICALS

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Suicide attempts are the leading cause of hospitalization among adolescents and young people aged 15 to 25 (1). The World Health Organization has estimated that around one million people commit suicide each year, representing a global suicide rate of 16 per 100,000 population. The objective of this research is to review cases of intoxication among young people who have been cared for at the University Children's Clinic in the last two years and to determine the most common reasons for intoxication, demographic characteristics of the population, the prevalence of relapse, as well as the steps that have been taken to prevent future suicidal behavior.

Basic quantitative statistical analyses were used to describe the sample and qualitative analyses of reports and conversations with hospitalized youth. Eighteen children were admitted due to intoxication during 2021 and 2022, of which 17 were girls aged 12 to 18 and one boy. Of them, 14 were hospitalized, while four were immediately referred to a mental health institution. Psychologist consultations were requested for 6 of those who were hospitalized. Further monitoring of these trends and exceptional attention to youth with repeated attempts are needed. It is essential to have a procedure and referral protocol that would include guidelines for communication with the patient and a team approach of pediatricians, medical associates, and colleagues from mental health clinics.

KEY WORDS: suicidal poisoning, adolescents, drug poisoning



TROVANJE DECE KOROZIVNIM SREDSTVIMA

CHILDREN IN THE WORLD
OF CHEMICALS

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Korozivne povrede organa za varenje nastaju zbog kontaktnog delovanja korozivnih supstanci nakon ingestije. Korozivne supstance mogu biti baze, kiseline i hemijski aktivne supstance. Baze su slatkastog ukusa dok su kiseline kiselo-gorkog ukusa. Baze dovode do kolikvacione nekroze a kiseline do koagulacione nekroze. Nakon ingestije mogu se javiti promene na koži lica, usana i usne duplje u vidu opekotina, otežano disanje i kašalj. Ređe se javljaju sistemske manifestacije. Međutim ono što je značajno napomenuti je da težina kliničke slike nije u korelaciji sa stepenom i ekstenzivnošću lezije organa za varenje. Pri svakoj sumnji na korozivnu povredu indikovano je bolničko ispitivanje i lečenje.

Neophodno je dobro uzeti anamnestičke podatke (gde, kada, kako, koliko?), obavezno je donošenje originalnog pakovanja korozivnog sredstva na uvid, klinički pregled, ORL pregled, radiografska ispitivanja (radiografija grudnog koša i abdomena), laboratorijske analize i endoskopsko ispitivanje.

Terapijske mere zavise od ekstenzivnosti lezija. Najčešće komplikacije su stenoze jednjaka i pilorusa, a najteže perforacije. U proteklih nekoliko godina susrećemo se sa još jednim oblikom korozivnih supstanci kao što je dugmetasta baterija. Zbog sve učestalijeg trovanja dece korozivnim supstancama prikazaćemo desetogodišnje iskustvo u lečenju korozivnih povreda u Institutu za zdravstvenu zaštitu majke i deteta Srbije.

KLJUČNE REČI: baze, kiseline, hemijski aktivne supstance, trovanje dece



POISONING OF CHILDREN WITH CORROSIVE AGENTS

CHILDREN IN THE WORLD
OF CHEMICALS

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Corrosive injuries of the gastrointestinal tract occur due to the contact action of corrosive substances after ingestion. Corrosive agents can be alkalis, acids and chemically active substances. Alkalis have a sweet taste, while acids have a sour-bitter taste. Alkalis lead to liquefactive necrosis and acids to coagulation necrosis. After ingestion, changes may occur on the skin of the face, lips and oral cavity in the form of burns, difficulty breathing and coughing.

Systemic manifestations occur less often. However, what is important to note is that the severity of the clinical presentation does not correlate with the degree and extensiveness of the lesion of the gastrointestinal tract. Hospital examination and treatment are indicated for any suspected corrosive injury. It is necessary to take good history (where, when, how, how much?), it is of utmost importance to deliver the original packaging of the corrosive agent to identify the ingested agent regarding the type, concentration and pH, clinical examination, ORL examination, radiographic examinations (radiography of the chest and abdomen), laboratory analyzes and GI endoscopy.

Therapeutic measures depend on the extent of the lesions. The most common complications are stenosis of the esophagus and pylorus. In the past few years we have encountered another form of corrosive substances such as the button cell battery. Due to the increasingly frequent poisoning of children with corrosive substances, we will present a ten-year experience in the treatment of corrosive injuries at the Mother and Child Health care Institute of Serbia

KEYWORDS: alkalis, acids, chemically active substances, poisoning of children



AKUTNO OŠTEĆENJE BUBREGA UZROKOVANO TROVANJEM BIZMUT SUBCITRATOM

CHILDREN IN THE WORLD
OF CHEMICALS

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Soli bizmuta, posebno koloidni bizmut subcitrati i bizmut subsalicilat, koriste se za lečenje peptičkog ulkusa i hroničnog gastritisa. Prijavljeni toksični efekti uzrokovani predoziranjem ovim lekovima uključuju encefalopatiju, nefropatiju, osteoartropatiju, gingivostomatitis i kolitis. Koncentracija bizmuta u bubrezima i vreme njegovog zadržavanja je duže nego u drugim organima, a akutno oštećenje bubrega je najčešća manifestacija intoksikacije bizmutom. Toksičnost bizmuta može da dovede do smanjenja glomerulske filtracije, smanjenog protoka krvi u bubregu i tubulske nekroze sa posledičnim razvojem akutne bubrežne insuficijencije.

U cilju prepoznavanja i procene težine akutnog oštećenja bubrega, primenjuju se dva dijagnostička kriterijuma (RIFLE i AKIN) koji su zasnovani na vrednostima serumskog kreatinina i diurezi. Zbog toga se kod svih pacijenata kod kojih postoji rizik za nastanak renalne disfunkcije, savetuje redovna kontrola serumskog kreatinina i praćenje diureze. Prikazujemo adolescentkinju sa akutnim oštećenjem bubrega uzrokovanim intoksikacijom bizmut subcitratom u toku pokušaja samoubistva. Klinički ishod je bio povoljan, a bubrežna funkcija se oporavila nakon dve nedelje zamene bubrežne funkcije. Trovanje bizmutom je redak uzrok akutne bubrežne insuficijencije i obično je reverzibilno ako se na vreme dijagnostikuje i pravilno leči. Kliničari treba da budu svesni da intoksikacija bizmutom može da bude uzrok akutne bubrežne insuficijencije.

KLJUČNE REČI: akutno oštećenje bubrega, bizmut subcitrati



ACUTE KIDNEY INJURY DUE TO BISMUTH SUBCITRATE INTOXICATION

CHILDREN IN THE WORLD
OF CHEMICALS

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Bismuth salts, especially colloidal bismuth subcitrate and bismuth subsalicylate, are widely used to treat peptic ulcers and chronic gastritis. The reported toxic effects caused by overdose of bismuth salts include encephalopathy, nephropathy, osteoarthropathy, gingivostomatitis and colitis. The concentration of bismuth in the kidney, and its retention time is higher than in other organs and nephrotoxicity is the most frequent serious manifestation. Bismuth toxicity may cause decreased glomerular filtration, reduced renal blood flow and tubular necrosis leading to acute renal failure. In order to recognize and assess the severity of acute kidney injury, two diagnostic criteria based on serum creatinine values and diuresis are developed (RIFLE and AKIN). Therefore, regular control of serum creatinine and monitoring of diuresis is advised in all patients who are at risk of developing renal dysfunction. We report a female adolescent with acute kidney injury due to bismuth subcitrate intoxication during suicide attempt. Clinical outcome was favorable and renal function recovered after two weeks of hemodialysis.

Bismuth intoxication is a rare cause of acute renal failure and is usually reversible if early diagnosed and properly treated. Clinicians should be aware that acute renal failure could occur after bismuth intoxication.

KEYWORDS: acute kidney injury, bismuth subcitrate



BOTULIZAM KOD DECE - DIJAGNOSTIČKI IZAZOVI

CHILDREN IN THE WORLD
OF CHEMICALS

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Botulizam je retko, potencijalno životno ugrožavajuće oboljenje koje se karakteriše bilateralnom, flakcidnom paralizom kranijalnih i perifernih nerava. Dijagnoza botulizma se zasniva na kliničkom pregledu i laboratorijskoj potvrdi bolesti. U diferencijalnoj dijagnozi treba razmatrati brojna neurološka oboljenja. Cilj ovog rada je prikazati slučaj adolescenta sa kliničkom slikom koja bi mogla odgovarati botulizmu a kod kojeg su ekstenzivnim ispitivanjem isključeni drugi potencijalni uzroci tegoba. Bolest je počela povraćanjem, bolom u trbuhu i dijarejom, nastalim par sati nakon konzumacije hamburgera. Hospitalizovan je u regionalnoj bolnici, odakle se, nakon 13 dana prevodi u našu ustanovu zbog razvoja slabosti mišića ekstremiteta i lica, ptoze, mutnog vida. Iz anamnestičkih podataka i dostupne dokumentacije se nije moglo utvrditi vreme nastanka i evolucija neuroloških tegoba.

Na prijemu je registrovana bilateralna ptoza, oftalmoplegija, midrijaza, facijalna diplegija, disfonija, disfagija, kvadriplegija uz očuvane tetivne reflekse kao i autonomni poremećaji-bradikardija, suvoća usana, opstipacija. Rezultati ekstenzivnog ispitivanja nisu potvrdili dijagnozu Guillain Barre-ovog, Miller Fisher-ovog sindroma kao ni mijastenije gravis. Laboratorijska dijagnostika za botulizam je bila negativna. S obzirom na težinu kliničke slike, lečen je intravenskim imunoglobulinima a potom i anti-botulinskim serumom. Neurološki oporavak je bio nekompletan, otpušten je na zahtev roditelja nakon 18 dana hospitalizacije. Botulizam, iako retka bolest, i dalje predstavlja dijagnostički izazov.

Treba ga razmatrati u diferencijalnoj dijagnozi akutne descendantne paralize. Ovim prikazom smo želeli da istaknemo činjenicu da su odsustvo anamnestičkih podataka o vremenu javljanja i progresije neuroloških tegoba, kao i zakasnelo uzimanje uzoraka za analizu, faktori koji značajno otežavaju dijagnozu botulizma.

KLJUČNE REČI: botulizam, descendantna slabost, oftalmoplegija



BOTULISM IN CHILDREN - A DIAGNOSTIC CHALLENGES

CHILDREN IN THE WORLD
OF CHEMICALS

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Botulism is a rare, life-threatening disease characterized by bilateral flaccid, descending paralysis affecting cranial and peripheral nerves. The diagnosis is based on clinical examination and confirmed by laboratory testing. Differential diagnosis is challenging, many neurological diseases should be considered. The aim of this work is to present the case of an adolescent with neurological symptoms which may be related to food born botulism, and in whom other potential causes were excluded by extensive examination. The disease began with vomiting, abdominal pain and diarrhea, which occurred a few hours following ingestion a hamburger. He was admitted in a regional hospital, and transferred to our institution after 13 days due to the development of generalized muscle weakness, ptosis, and blurred vision. From the available documentation, it was not possible to determine the time of onset and evolution of neurological symptoms.

On admission, bilateral ptosis, ophthalmoplegia, mydriasis, facial diplegia, dysphonia, dysphagia, quadriplegia with preserved tendon reflexes and autonomic disturbances – bradycardia, dry mouth, constipation, were registered. Extensive investigation did not confirm Guillain Barre syndrome, Miller Fisher syndrome and myasthenia gravis. Laboratory testing for botulism was negative. Considering the severity of the neurological symptoms, he was treated with intravenous immunoglobulins, and antitobulinum serum. Recovery was incomplete, discharged at parents' request after 18 days of hospitalization. Botulism remains a diagnostic challenge. It should always be considered in differential diagnosis of descendent paralysis. Our case highlights the fact that the absence of anamnestic data on the time of onset and progression of neurological symptoms, as well as late collection of samples for analysis, are factors that significantly complicate the diagnosis of botulism.

KEYWORDS: botulism, descendent paralysis, ophtalmoplegia



SINDROM VON MINHAUZEN - TROVANJE VARFARINOM

CHILDREN IN THE WORLD
OF CHEMICALS

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Sindrom zlostavljanja preko posrednika je definisan kao okolnost kada osoba koja brine o detetu namerno narušava njegov fizički integritet ili izaziva simptome i znake koji su povod za nepotrebne invazivne dijagnostičke i terapijske postupke. Prikaz toka hospitalizacije dečaka upućenog u IMD radi ispitivanja poremećaja hemostaze. Dečak uzrasta od 8 godina primljen je u Institut za zdravstvenu zaštitu majke i deteta Srbije „Dr Vukan Čupić“ posle nekoliko višenedeljnih hospitalizacija u tercijernim pedijatrijskim ustanovama u Beogradu i Novom Sadu. Kliničkom slikom dominirale su nekonzistentne polimorfne tegobe koje se nisu mogle objektivizovati (bol u zglobovima, bol u stomaku, mučnina i sl.), kao i poremećaji skrininga za hemostazu.

Na prijemu u Institut dečak je bio normalnog fizikalnog nalaza, a laboratorijski nalazi su bili normalni. Narednog dana majka ukazuje na pojavu hematoma, zbog čega je ponovljen skrining za hemostazu koji je ukazao na značajno patološke vrednosti protrombinskog vremena. S obzirom na to da se ovakav nalaz hemostaze održavao i narednih dana, dečak je svakodnevno dobijao vitamin K intravenski. Istovremeno, poslat je i uzorak krvi u dve referentne toksikološke laboratorije. Izveštaj u obe laboratorije je pokazao prisustvo metabolita varfarina, koji dečak nije mogao da ingestira zadesno jer mu nije bio dostupan. Dečak je odvojen od majke, nakon čega se laboratorijski rezultati normalizuju, a navedene subjektivne tegobe (bol u zglobovima, bol u stomaku, mučnina) nestaju. Hemoragijski sindrom može da bude deo sindroma zlostavljanja preko posrednika. Za pravovremeno prepoznavanje problema neophodno je izmenjene laboratorijske analize tumačiti u kontekstu kliničke slike i paramedicinskih okolnosti.

KLJUČNE REČI: Sindrom zlostavljanja preko posrednika, varfarin, prikaz slučaja



SYNDROME VON MINCHAUSEN - VARFARIN POISONING

CHILDREN IN THE WORLD
OF CHEMICALS

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Munchausen by proxy syndrome is defined as a circumstance when a person who takes care of a child deliberately violates his physical integrity or causes symptoms and signs that lead to unnecessary invasive diagnostic and therapeutic procedures. Presentation of the course of hospitalization of a boy referred to the IMD for examination of hemostasis disorders. An 8-year-old boy was admitted to the Institute after several weeks of hospitalization in tertiary pediatric institutions in Belgrade and Novi Sad. The clinical picture was dominated by inconsistent polymorphic complaints that could not be easily objectified (pain in the joints, stomach pain, nausea, etc.), as well as disorders of screening for hemostasis. On admission to the Institute, the boy had a normal physical appearance, and laboratory findings were normal.

The next day, the mother indicated the appearance of a hematoma, which is why the screening for hemostasis was repeated, which indicated significantly abnormal prothrombin time values. Considering that this finding of hemostasis was maintained in the following days, the boy received vitamin K intravenously every day. At the same time, a blood sample was sent to two reference toxicology laboratories. The report in both laboratories showed the presence of warfarin metabolites, which the boy could not ingest accidentally because it was not available to him. The boy was separated from his mother, after which the laboratory results normalized, and the mentioned subjective complaints (pain in the joints, pain in the stomach, nausea) disappeared. Hemorrhagic syndrome can be part of Munchausen by proxy syndrome. For the timely recognition of problems, it is necessary to interpret the pathological laboratory results in the context of the clinical picture and paramedical circumstances.

KEYWORDS: Munchausen by proxy syndrome, warfarin, case report



TROVANJE DECE BENZODIAZEPINIMA - PETOGODIŠNJE ISKUSTVO

CHILDREN IN THE WORLD
OF CHEMICALS

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Među najčešće prepisivanim lekovima u svetu lekova nalaze se benzodiazepini zbog njihovog povoljnog bezbednosnog profila kao i njihove visoke efikasnosti. Izolovano predoziranje benzodiazepinima, bilo namerno ili akcidentalno, retko izaziva značajan toksidrom i često dovodi od blage do umerene depresije centralnog nervnog sistema, a retko do duboke kome koja zahteva asistiranu ventilaciju. Uobičajeni simptomi trovanja/predožiranja uključuju pospanost ili letargiju, konfuziju ili dezorijentaciju, nejasan i usporen govor, ataksiju, tremor, mučninu/povraćanje i retko respiratornu depresiju, konvulzije i komu, a u slučajevima ekstremnih predožiranja i do kardiovaskularnih i pulmonalnih oštećenja. Standardna terapija akutnog trovanja benzodiazepinima je suportivna, premda se i specifični antidot – flumazenil može primeniti budući da efikasno vrši reverziju sedacije. Institut za zdravstvenu zaštitu majke i deteta Srbije „Dr Vukan Čupić” ima višedecenijsko iskustvo u zbrinjavanju i lečenju trovanja dece mlađe od 14 godina.

Cilj ovog rada je predstavljanje poslednjih pet godina našeg iskustva. Podaci o trovanjima - polu, vrsti i broju supstanci, težini kliničke slike, terapiji i ishodu lečenja preuzeti su iz dostupne medicinske dokumentacije, poštujući zaštitu i tajnost podataka o ličnosti, a zatim statistički obrađeni koristeći deskriptivne statističke metode. Tokom poslednjih pet godina u Institutu je u proseku, godišnje, zbrinjavano oko 200 dece kao posledica trovanja različitim agensima. Polna distribucija je bila ravnomerna, dok je najčešći razlog trovanja bilo zadesno trovanje (oko 80%) dok je ostatak bilo samotrovanje ili zloupotreba rekreativnih droga. Za više od trećine hospitalizacija bila su odgovorna trovanja lekovima, od čega su najčešći bili benzodiazepini i njihovi derivati. Najčešći put trovanja bila je ingestija. Uzrasno su više od polovine hospitalizacija činila deca do 5 godina, sa blagom, odnosno asimptomatskom kliničkom slikom.

KLJUČNE REČI: anksiolitici, trovanje dece, benzodiazepini



BENZODIAZEPINE POISONING IN CHILDREN - A TEN YEARS OF EXPERIENCE

CHILDREN IN THE WORLD
OF CHEMICALS

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Most commonly prescribed medications are benzodiazepines due to their favorable safety profile and their efficacy. Isolated benzodiazepine overdose, being purposeful or accidental without other coingestants rarely causes a significant toxidrome and often result in a mild to moderate central nervous system depression and rarely leads to a profound coma requiring assisted ventilation. Common symptoms include drowsiness or lethargy, confusion or disorientation, slurred speech, ataxia, tremors, nausea/vomiting and, rarely, respiratory depression, seizures and coma.

The severity of CNS depression is the result of the drug type, dose, the patient's age and clinical status prior to consumption, as well as the concomitant use of other CNS depressants and rarely leads to death. The standard treatment for acute benzodiazepine toxicity is supportive care but flumazenil, a specific receptor antagonist, although not routinely recommended, can successfully reverse benzodiazepine-induced sedation. Our Institute has decades long experience in treating children under 14 years of age due to poisoning. The goal of this paper is to present the last five years of our experience. Patient data - gender, type and number of substances, severity of the clinical picture, therapy and treatment outcome were obtained from medical records and then statistically processed using descriptive statistical methods. During the last five years, on average, around 200 children were treated annually at the Institute as a result of poisoning. Gender distribution was even and the most common were accidental poisonings (about 80%) while the rest were self-poisoning or abuse of recreational drugs. Medications were responsible for more than a third of hospitalizations, the most common of which were benzodiazepines and their derivatives. The most common route of poisoning was oral ingestion. More than half of the hospitalizations were comprised of children up to 5 years old with mild or asymptomatic clinical picture.

KEYWORDS: anxiolytics, poisoning in children, benzodiazepines



MLADI ISTRAŽIVAČI / YOUNG RESEARCHERS

TRI NIVOA DOKAZA HEPATOTOKSIČNOSTI SMEŠE FTALATA I BISFENOLA A: *IN SILICO* I DVA *IN VIVO* MODELA

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Široka upotreba bis(2-etilheksil) ftalata (DEHP), dibutil ftalata (DBP) i bisfenola A (BPA) otvara niz mogućnosti za kombinovanu izloženost ljudi ovim toksičnim jedinjenjima u svakodnevnom životu i brojne štetne ishode, uključujući hepatotoksičnost. *In silico* istraživanje i dva *in vivo* modela su korišćena da se ispita povezanost smeše DEHP, DBP i BPA i oštećenja jetre. Bioinformatička analiza urađena je pomoću Komparativne toksikogenomske baze podataka (engl. Comparative Tokicogenomics Database, CTD), ShinyGO portala, ToppCluster alata i Cytoscape softvera. *In vivo* subakutna studija uključila je pet grupa pacova (n = 6): (1) Kontrola: kukuruzno ulje, (2) DEHP: 50 mg/kg t.m./dan, (3) DBP: 50 mg/kg t.m./dan, (4) BPA: 25 mg/kg t.m./dan, (5) MIX: DEHP + DBP + BPA. Embrioni zebriце bili su izloženi ispitivanim supstancama u više doza, pojedinačno i u kombinaciji (binarne i tercijarne smeše). Oštećenje jetre moglo je da se dovede u vezu sa 75 gena sa kojima DEHP, DBP i BPA intereaguju, od kojih je većina bila povezana sa inflamacijom/oksidativnim stresom, identifikovanim kao najrelevantniji molekularni mehanizmi. Kod pacova, značajne promene u redoks statusu/nivou bioelemenata i patohistologiji tkiva bile su izraženije ili prisutne samo u MIX grupi, ukazujući na potencijalnu aditivnost. BPA je smanjio indeks površine jetre (engl. liver area index, LA) na dozno zavisan način. Vrednosti LA bile su smanjene i nakon izloženosti DEHP (2 µg/mL) i DBP (5 µg/mL), dok je LA indeks bio povišen pri njihovim višim koncentracijama. U binarnim smešama, DBP je doveo do letalnog efekta pri dve najviše koncentracije, dok je BPA usmeravao hepatotoksičnost smeše DEHP/DBP/BPA.

KLJUČNE REČI: plastifikatori, hepatotoksičnost, toksikogenomska analiza podataka, pacov, zebrića



THREE LINES OF EVIDENCE OF THE HEPATOTOXICITY OF A MIXTURE CONTAINING PHTHALATES AND BISPHENOL A: *IN SILICO* AND TWO *IN VIVO* MODELS

YOUNG RESEARCHERS

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The extensive usage of bis(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), and bisphenol A (BPA) creates a lot of opportunities for combined human exposure to these hazardous compounds in everyday life and a variety of negative outcomes, including hepatotoxicity. *In silico* research and two *in vivo* models were used to investigate the links between a mixture including DEHP, DBP and BPA and liver injury. Bioinformatic analysis was performed by Comparative Toxicogenomics Database, ShinyGO, TopCluster, and Cytoscape. *In vivo* subacute study included five groups of rats (n = 6): (1) Control: corn oil, (2) DEHP: 50 mg/kg b.w./day, (3) DBP: 50 mg/kg b.w./day, (4) BPA: 25 mg/kg b.w./day, (5) MIX: DEHP + DBP + BPA. Zebrafish embryos were exposed to the investigated substances in multiple dosages, both alone and in combination (binary and ternary mixtures). Liver damage was linked to 75 DEHP, DBP, and BPA genes, the majority of which were associated with inflammation/oxidative stress, identified as the most relevant molecular pathways. In rats, significant changes in redox status/bioelements' level and pathohistology were more pronounced or evident only in MIX group, suggesting probable additivity. In a dose-dependent manner, BPA reduced the liver area (LA) index. LA values were decreased by DEHP (2 µg/mL) and DBP (5 µg/mL), whereas LA index was raised by their higher concentrations. In binary mixtures, DBP had a lethal effect at the two highest concentrations, whereas BPA directed hepatotoxicity of the DEHP/DBP/BPA mixture.

KEYWORDS: plasticizers, hepatotoxicity, toxicogenomic data mining, rat, zebrafish



OKSIDATIVNI STRES IZAZVAN NISKIM DOZAMA OLOVA: SUBAKUTNA STUDIJA TOKSIČNOSTI NA WISTAR PACOVIMA I BENCHMARK MODELOVANJE

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Novija saznanja ukazuju da niska izloženost olovu (Pb), koja se u današnje vreme sreće, predstavlja rizik po zdravlje ljudi. Jedan od najznačajnijih mehanizama toksičnosti Pb je oksidativni stres koji nastaje usled osobine Pb da indukuje stvaranje slobodnih kiseoničnih i azotovih radikala i suprimira antioksidativnu zaštitu. Cilj ove studije bio je da se detektuju mehanizmi kojima Pb izaziva oksidativni stres na animalnom modelu pri izloženosti niskim dozama. Odrasli mužjaci Wistar pacova su podeljeni u sedam grupa (n=6), kontrola i šest grupa tretiranih per os sa 0,1; 0,5; 1; 3; 7 i 15 mg Pb/kg telesne mase (t.m.)/dan tokom 28 dana. Koncentracija olova i parametri oksidativnog statusa su određivani u krvi/serumu, jetri, bubrežima, srcu i mozgu. Modelovanje odnosa eksterne i interne doze (koncentracija Pb u krvi, µg/dL) – odgovor vršeno je pomoću PROAST70.1 softvera.

Modelovanjem odnosa eksterne doze-odgovor dobijena je dozno-zavisna inhibicija superoksid-dismutaze (SOD) u serumu i bubrežima, dok je nasuprot tome aktivnost SOD indukovana u jetri. Utvrđeno je i dozno-zavisno povećanje superoksidanjon radikala (O₂^{•-}) i uznapredovalih produkata proteina (AOPP) u mozgu, AOPP u jetri, i malondialdehida (MDA) u srcu. Modelovanjem odnosa interna doza-odgovor, potvrđena je dozno-zavisnost za povećanje O₂^{•-} u mozgu i MDA u srcu i dodatno utvrđena za rast AOPP u serumu. Najniže benchmark doze dobijene su za inhibiciju SOD u bubrežima (1,27e-06 mg Pb/kg t.m./dan) i indukciju stvaranja O₂^{•-} u krvi (2,1e-06 µg Pb/dL). Rezultati ovog istraživanja daju korisne podatke za procenu rizika po zdravlje ljudi i procenu polazne tačke pri određivanju referentnih doza Pb.

KLJUČNE REČI: olovo, niske doze, oksidativni status, odnos doza-odgovor



OXIDATIVE STRESS INDUCED BY LOW DOSES OF LEAD: A SUBACUTE TOXICITY STUDY IN WISTAR RATS AND BENCHMARK MODELING

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Recent findings indicate that low-dose lead (Pb) exposure, which is encountered today, represents a risk to human health. One of the most important mechanisms of Pb toxicity is oxidative stress, which occurs due to Pb ability to induce the formation of free oxygen and nitrogen radicals and suppress antioxidant protection. The aim of this study was to detect the mechanisms by which low-dose Pb exposure causes oxidative stress in an animal model. Adult male Wistar rats were divided into seven groups (n=6), a control group, and six groups treated per os with 0.1; 0.5; 1; 3; 7 and 15 mg Pb/kg body weight (b.w.)/day for 28 days. Lead concentration and oxidative status parameters were determined in blood/serum, liver, kidney, heart and brain. Modeling of external and internal dose (blood Pb concentration, µg/dL) – response was performed using PROAST70.1 software. By modeling the external dose-response relationship, dose-dependent inhibition of superoxide dismutase (SOD) was obtained in the serum and kidneys, while, on the other hand, SOD activity was induced in the liver. A dose-dependent increase of superoxide anion radical (O₂^{•-}) and advanced oxidant protein products (AOPP) in the brain, a dose-dependent increase of AOPP in the liver, and a dose-dependent increase of malondialdehyde (MDA) in the heart were obtained. In the case of internal dose-response modeling, dose-dependence was obtained for the increase of AOPP in the serum, the increase of MDA in the heart, and the increase of O₂^{•-} in the brain. The lowest benchmark doses were obtained for the inhibition of SOD in the kidneys (1.27e-06 mg Pb/kg bw/day) and the induction of O₂^{•-} production in the blood (2.1e-06 µg Pb/dL). The results of this study provide useful data for assessing the risk to human health and estimating the point of departure for determining reference doses of Pb.

KEYWORDS: lead, low doses, oxidative status, dose-response



NEUROTOKSIČNOST SMEŠE NISKIH DOZA OLOVA, KADMIJUMA, ŽIVE I ARSENA ZNAČAJNIH ZA IZLOŽENOST IZ ŽIVOTNE SREDINE

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Izloženost olovu (Pb), kadmijumu (Cd), živi (Hg) i arsenu (As) iz životne sredine, kako pojedinačno tako i njihovim smešama jedan je od faktora za razvoj brojnih zdravstvenih poremećaja, među kojima su i štetni efekti na nivou centralnog nervnog sistema. Cilj rada – ispitivanje neurotoksičnih efekata izazvanih smešom olova, kadmijuma, žive i arsena u dozama značajnim za izloženost iz životne sredine pri subakutnoj oralnoj primeni. Sprovedena je studija na eksperimentalnim životinjama: 30 pacova podeljenih u 6 grupa: kontrolnu, MIX1-MIX5 (mg/kg t.m./dan) (Pb: 0,003, 0,01, 0,1, 0,3, 1; Cd: 0,01, 0,03, 0,3, 0,9, 3; Hg: 0,0002, 0,0006, 0,006, 0,018, 0,06; As: 0,002, 0,006, 0,06, 0,18, 0,6). Bihevioralni eksperimenti sprovedeni su poslednjeg dana studije (test spontane lokomotorne aktivnosti – SLA; test prepoznavanja novih objekata – NORT), a nakon žrtvovanja životinja u uzorcima moždanog tkiva ispitani su parametri oksidativnog stresa (O2-, TOS, PAB, AOPP, MDA, IMA), parametri antioksidativne zaštite (TAS, SH grupe, SOD, GSH) i histopatološke promene. SLA test pokazao je da primenjene doze smeše metala nisu značajno uticale na ponašanje, dok je NORT test pokazao da su najizraženije efekte na zaboravljanje poznatog objekta imale životinje MIX5 grupe. Uočene promene u ponašanju životinja praćene su dozno-zavisnom indukcijom oksidativnog stresa u mozgu (najuzi Benchmark interval – BMDI (PROAST veb 70.1 soft- ver) utvrđen je za aktivnost superoksid-dismutaze: 1e-06 – 3.18e-05 mg As/kg t.m./dan) i histološkim promenama (hiperemija, edem, lakostepena gliozna, degeneracija neurona, satelitoza), najizraženijim u MIX5 grupi. U opsegu doza relevantnih za izloženost metalima iz životne sredine značajni neurotoksični efekti dobijeni su za najviše primenjene doze u studiji.

KLJUČNE REČI: neurotoksičnost, toksični metali, smeše, relevantne doze za izloženost iz životne sredine, benchmark metodologija



NEUROTOXICITY OF LEAD, CADMIUM, MERCURY AND ARSENIC LOW DOSED MIXTURES RELEVANT TO ENVIRONMENTAL EXPOSURE

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Lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As) are top ranked toxic metal(oid)s on the list of the most important environmental pollutants from the aspect of public health. Literature data show that exposure to individual metals, as well as their mixtures, is one of the factors for numerous health disorders development, including adverse effects on the central nervous system. Aim: to determine the neurotoxic effects caused by the aforementioned mixture in doses significant for environmental exposure. Experimental animals were divided into 6 groups: control, MIX1- MIX5 (mg/kg bw/day) (Pb: 0.003, 0.01, 0.1, 0.3, 1; Cd: 0.01, 0.03, 0.3, 0.9, 3; Hg: 0.0002, 0.0006, 0.006, 0.018, 0.06; As: 0.002, 0.006, 0.06, 0.18, 0.6). Behavioral experiments were conducted on the last day of exposure (SLA, NORT). After animal sacrifice, parameters of oxidative stress (O₂·-, TOS, PAB, AOPP, MDA, IMA), parameters of antioxidant protection (TAS, SH groups, SOD, GSH) were determined, along with the histopathological investigation. SLA test showed that the applied doses of the metal mixture did not significantly affect the behavior, while NORT test showed that MIX5 group had the most pronounced effects on familiar object forgetting. Changes in animal behavior were accompanied by dose-dependent induction of brain oxidative stress (the narrowest BMDI for superoxide dismutase activity: 1e-06 – 3.18e-05 mg As/kg b.w./day). Histological changes (hyperemia, edema, mild gliosis, neuron degeneration, satellitosis) were most pronounced in MIX5 group. In the range of environmentally relevant doses to metal exposure, significant neurotoxic effects were obtained for the highest administered doses.

KEYWORDS: neurotoxicity, toxic metals, mixtures, environmentally relevant doses, benchmark methodology



PREDVIĐANJE TOKSIČNIH EFEKATA IMUNOMODULATORA SULFORAFANA: BIOINFORMATIČKA ANALIZA

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Sulforafan (SFN) je imunomodulator poreklom iz kupusastog povrća čija su antioksidativna i antikan- cerogena svojstva prepoznata sredinom prošlog veka. Međutim, njegov toksikološki profil i potencijal da izazove štetne efekte su nedovoljno razjašnjeni. Cilj ove studije bio je predviđanje toksičnog po- tencijala SFN kombinovanjem bioinformatičkih analiza. Dodatno, in silico metode korišćenje su u cilju razotkrivanja molekularnih mehanizama koji vode nastanku toksičnih efekata SFN. Hemijske grupe SFN koje imaju potencijal da izazovu toksične efekte (‘toksifore’) identifikovane su korišćenjem Derek Nexus softvera. Podaci o interakcijama SFN sa humanim genomom, preuzeti su iz Komparativne toksikogenomske baze podataka (eng. Comparative Toxicogenomic Database) i ukršteni sa podacima iz REACTOME, NetworkAnalyst i ToppGene baza pri čemu su izdvojeni molekularni putevi koji pokreću niz reakcija vodeći ka potencijalno toksičnom efektu.

Dve hemijske strukture: izocijanat i izotiocijanat prepoznate su kao toksifore koje mogu izazvati oštećenje hromozoma i/ili oštećenje kože. Sulforafan interaguje sa ukupno 11 i 146 gena koji su takođe povezani sa oštećenjem hromozoma i oštećenjem kože, redom. Ovi geni uključeni su u regulaciju ćeli- jskog ciklusa, proces apoptoze i aktivaciju imunskog sistema. Paralelnom bioinformatičkom analizom utvrđeno je da SFN interaguje sa 86 gena čija je ekspresija izmenjena kod pacijenata obolelih od kol- orektalnog karcinoma. Ovi geni su od značaja za proces ateroskleroze, renalne fibroze, imunosupresije kao i diferencijacije adipocita. Sprovedena in silico studija ukazuje na potencijal SFN da utiče na ek- spresiju gena uključenih u procese kao što su apoptoza, aktivacija transkripcionih faktora i imunskog sistema i posledično izazivanje toksičnih efekata.

KLJUČNE REČI: sulforafan, in silico, toksikološki profil, apoptoza



PREDICTING TOXIC EFFECTS OF AN IMMUNOMODULATOR, SULFORAPHANE: BIOINFORMATIC ANALYSIS

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Sulforaphane (SFN) is an immunomodulator from cruciferous vegetables whose anti-oxidative and anti-carcinogenic properties were recognized in the middle of the last century. However, its toxicological profile remains insufficiently elucidated. The aim of the study was to predict the toxicity of SFN by combining bioinformatic analyses. Additionally, *in silico* methods unravel the molecular mechanisms that lead to the toxic effects. Structural alerts of SFNs with the potential to cause toxic effects ('toxophores') were identified using Derek Nexus software. Interactions of SFN with the human genome were taken from the Comparative Toxicogenomic Database and cross-referenced with data from the REACTOME, NetworkAnalyst, and ToppGene databases, to detect the molecular pathways that trigger a series of reactions leading to potentially toxic effects. Recognized toxophores (isocyanate and isothiocyanate) could cause chromosomal damage and/or skin diseases.

Sulforaphane interacts with 11 and 146 genes that are also associated with chromosome and skin damage, respectively. These genes are involved in the regulation of the cell cycle, the process of apoptosis and the activation of the immune system. Parallel bioinformatics analysis revealed that SFN interacts with 86 genes whose expression is altered in patients with colorectal cancer. These genes are important for atherosclerosis, renal fibrosis, immunosuppression as well as adipocyte differentiation. Conducted *in silico* studies indicate the potential of SFN to affect the expression of genes linked to processes such as apoptosis, activation of transcription factors, and the immune system, which consequently might cause toxic effects.

KEYWORDS: sulforaphane, *in silico*, toxicity profile, apoptosis



VEZA IZMEĐU IZLOŽENOSTI NIKLU I POREMEĆAJA FUNKCIJE ŠTITASTE ŽLEZDE - REZULTATI STUDIJE HUMANOG BIOMONITORINGA

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Uticaj toksičnog metala nikla (Ni) na funkciju štitaste žlezde još uvek nije dovoljno istražen. Stoga je cilj ove studije bio da se ispita potencijal Ni da poremeti funkciju štitaste žlezde ispitivanjem odnosa između koncentracije Ni u krvi i nivoa hormona u serumu (TSH, T4, T3, fT4, fT3), kao i parametara tiroidne homeostaze (SPINA-GT i SPINA-GD) korišćenjem korelacione analize i Benchmark (BMD) koncepta. U istraživanju je učestvovalo 435 ispitanika čiji su uzorci krvi sakupljeni u Kliničkom centru Srbije i Kliničko-bolničkom centru „Bežanijska kosa“. Koncentracija Ni je određena ICP-MS metodom, dok je CLIA korišćena za određivanje koncentracije hormona u serumu. SPINA Thyr softver je korišćen za izračunavanje SPINA-GT i SPINA-GD parametara.

Analiza odnosa doza-odgovor vršena je pomoću PROAST softvera (70.1). Najviša medijana koncentracije Ni zabeležena je kod muške populacije (8.278 µg/L). Utvrđeno je postojanje odnosa doza-odgovor između Ni i svih izmerenih parametara funkcije štitaste žlezde u celoj populaciji i kod oba pola.

Međutim, uski BMD intervali su dobijeni samo kod muškaraca, za parove Ni - SPINA-GT (1,36-60,9 µg/L) i Ni - fT3 (0,397-66,8 µg/L), što ukazuje da čak 78,68 i 83,25% muškaraca u ispitivanoj populaciji može imati 10% veći rizik od nastanka Ni-indukovanih promena SPINA-GT i fT3. Zbog uspostavljene veze između Ni i SPINA-GT parametra, može se zaključiti da Ni ima uticaj na sekretornu funkciju štitaste žlezde kod muškaraca. Iako su potrebna dalja istraživanja, ovi nalazi ukazuju na moguću ulogu izloženosti Ni u poremećajima funkcije štitaste žlezde.

KLJUČNE REČI: tiroidna funkcija, SPINA parametri, toksični metali, BMD koncept

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THE RELATIONSHIP BETWEEN NICKEL EXPOSURE AND THYROID DYSFUNCTION - RESULTS OF A HUMAN BIOMONITORING STUDY

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Nickel (Ni) is one of the well-known toxic metals whose effect on thyroid function has not been sufficiently investigated yet. The aim of this study was to analyse the potential of Ni to disrupt thyroid function by exploring the relationship between blood Ni concentration and serum hormone levels (TSH, T4, T3, fT4, fT3), as well as the parameters of thyroid homeostasis (SPINA-GT and SPINA-GD) by using correlation analysis and Benchmark (BMD) concept. Blood samples from 435 participants were collected at the Clinical Center of Serbia and the Clinical Hospital Center “Bežanijska kosa”. Ni concentration was measured by the ICP-MS method, while CLIA was used for serum hormone determination. SPINA Thy software was used to calculate SPINA-GT and SPINA-GD parameters.

BMD analysis was performed by PROAST software (70.1). The highest median value for Ni concentration was observed for the male population (8,278 µg/L). The existence of a dose-response relationship was established between Ni and all the measured parameters of thyroid functions in the entire population and in both sexes. However, the narrow BMD intervals were obtained only in men, for Ni - SPINA-GT pair (1.36-60.9 µg/L) and Ni - fT3 pair (0.397-66.8 µg/L), meaning that even 78.68 and 83.25% of men in our study might be in 10% higher risk of Ni-induced SPINA-GT and fT3 alterations, respectively. The relationship established between Ni and the SPINA-GT indicates the Ni influence on the secretory thyroid gland function in men. Although further research is required, these findings suggest a possible role of Ni in thyroid function disturbances.

KEYWORDS: thyroid function, SPINA parameters, toxic metals, BMD concept

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EFEKTI SMEŠE OLOVA I POLIHLOOROVANIH- BIFENILA NA SPERMATOZOIDE I NIVO TESTOSTERONA KOD PACOVA

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Jedan od najvećih izazova u toksikologiji je procena toksičnosti hemijskim smešama, odnosno procena rizika po zdravlje ljudi usled celoživotnog izlaganja složenim smešama različitih supstanci. Studije bio-monitoringa su nesumnjivo pokazale prisustvo preko stotinu hemikalija u ljudskom telu. Cilj ove studije bio je da se ispita da li mešavina olova (Pb) i polihlorovanih-bifenila (PCB) ima uticaj na spermatozoide i nivo testosterona (T) u serumu i da li su ovi efekti dozno zavisni. Sprovedeni eksperiment je odobrila Etička komisija (323-07-11822/2018-05). Mužjaci Wistar pacova uključeni su u tretman smešom Pb i PCB-a oralnom gavažom tokom 28 dana. Doze Pb u smeši bile su 0,1, 0,5 i 1 mg/kg/dan, dok su doze PCB-a bile 0,25, 0,5 i 1 mg/kg/dan. Pacovi su anestetizirani kombinacijom ketamin/ksilazin do gubitka refleksa. Kroz uzdužni presek skrotuma vađeni su testis i epididimis, dok su spermatozoidi uzeti iz repa epididimisa difuzijom u toplom fiziološkom rastvoru. Pokretljivost spermatozoida određivana je svetlosnim mikroskopom. Alikvot spermatozoida je fiksiran kombinacijom formalina u fosfatnom puferu i korišćen za analizu morfologije i koncentracije sperme. Donja granica pouzdanosti referentne doze (BMDL₂₀) od 0,0228 mg/kg/dan je izračunata za efekat Pb i PCB-a kao kovarijante za nivo T u serumu. Iz naših rezultata možemo zaključiti da je nivo T bio najosetljiviji parametar.

KLJUČNE REČI: Benčmark doza; olovo i PCB smeša; reproduktivna toksičnost; PROAST program;

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EFFECT OF LEAD AND POLYCHLORINATED-BIPHENYLS MIXTURE ON SPERM CELLS AND SERUM LEVEL OF TESTOSTERONE IN RATS

YOUNG RESEARCHERS

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One of the biggest challenges in toxicology is the evaluation of the toxicity of chemical mixtures, as well as the risk to human health due to lifelong exposure to complex mixtures of different substances. Biomonitoring studies have undoubtedly shown the presence of over a hundred chemicals in the human body. The objective of this study was to investigate whether a mixture of lead (Pb) and polychlorinated-biphenyls (PCBs) has any effect on sperm cells and the serum level of testosterone (T) and whether these effects are dose-dependent. The conducted experiment was approved by the Ethical Committee No. 323-07-11822/2018-05. Male Wistar rats were included in the treatment with the mixture of Pb and PCBs by oral gavage for 28 days. Doses of Pb in the mixture were 0.1, 0.5, and 1 mg/kg/day, while doses of PCBs in the mixture were 0.25, 0.5, and 1 mg/kg/day respectively. Rats were anesthetized by the ketamine/xylazine combination to the loss of reflexes. Through the scrotum longitudinal section, the testis and epididymis were harvested, while sperm cells were taken from the cauda of epididymis by diffusion in a warm saline solution. Sperm motility was determined by the light microscope. The aliquot of the sperm cells was fixated with the combination of formalin in the phosphate buffer and used for morphology and concentration sperm analysis. The benchmark dose lower confidence limit (BMDL₂₀) of 0.0228 mg/kg/day was derived for the effect of Pb and PCBs as co-variant on the serum level of T. From our results, we can conclude that the level of T was the most sensitive parameter.

KEYWORDS: benchmark dose; lead and PCBs mixture; reproductive toxicity; PROAST software;

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PROCENA TOKSIČNIH EFEKATA SMEŠE OLOVA I POLIHLOOROVANIH BIFENILA NA REDOKS STATUS U BUBREZIMA PACOVA

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S obzirom na to da su ljudi u svakodnevnom životu konstantno izloženi smešama hemikalija koje deluju putem zajedničkih mehanizama, važno je proceniti da li su ovi „koktel efekti“ izraženiji u poređenju sa dejstvom pojedinačnih hemikalija. Cilj ovog istraživanja bio je da se ispita kako smeša olova (Pb) i polihlorovanih-bifenila (PCBs) utiče na različite parametre oksidativnog stresa u bubrezima mužjaka Wistar pacova i da se na osnovu dobijenih rezultata uspostavi odnos između doze i toksičnog odgovora.

Životinje (6 po grupi) su primale različite smeše olova (0.1, 0.5 i 1 mg/kg telesne težine) i polihlorovanih-bifenila (0.25, 0.5 i 1 mg/kg telesne težine) putem oralne gavaže tokom 28 dana (3x3 dizajn), dok je kontrolna grupa dobijala samo kukuruzno ulje. Rezultati su pokazali da koekspozicija različitim smešama olova i organskih zagađivača može dovesti do izmenjenog redoks statusa u ispitivanom tkivu u poređenju sa kontrolom, što sugeriše da se oksidativni stres može smatrati jednim od glavnih mehanizama toksičnosti ispitivanih hemikalija. Dobijeni podaci mogu biti korisni u daljoj proceni štetnih efekata na zdravlje ljudi nakon kombinovanog izlaganja ovim toksičnim supstancama.

KLJUČNE REČI: toksični metali, perzistentni organski zagađivači, oksidativni stres, koekspozicija, interakcije



ASSESSMENT OF LEAD AND POLYCHLORINATED BIPHENYLS MIXTURE TOXIC EFFECTS ON REDOX STATUS IN RAT KIDNEYS

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Due to the fact that in daily life humans are constantly exposed to mixtures of chemicals acting by a common mechanism of action, it is important to assess whether these "cocktail effects" are more pronounced compared to the effects of single chemicals. The aim of this study was to investigate how a mixture of lead (Pb) and polychlorinated-biphenyls (PCBs) affects various parameters of oxidative stress in the kidneys of male Wistar rats and based on the obtained results to establish a dose-toxic response relationship. Animals (6 per group) were receiving different mixtures of Pb (0.1, 0.5 and 1 mg/kg body weight (b.w.)) and PCBs (0.25, 0.5 and 1 mg/kg b.w.) by oral gavage for 28 days (3x3 design), while the control group received corn oil only.

The results have shown that co-exposure to different mixtures of Pb and organic pollutants can result in altered redox status in investigated tissue compared to control, suggesting that oxidative stress can be considered as one of the major toxicity mechanisms of investigated chemicals. Obtained data may be useful in further evaluation of harmful effects on human health after combined exposure to these toxic substances.

KEYWORDS: toxic metals, persistent organic pollutants, oxidative stress, co-exposure, interactions



PARAMETRI OKSIDATIVNOG STRESA KOD RADNIKA IZLOŽENIH SMJEŠI NISKIH KONCENTRACIJA ORGANSKIH RASTVARAČA

YOUNG RESEARCHERS

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Zaposleni u industriji obuće su potencijalno izloženi smješi organskih rastvarača, što predstavlja povećan rizik od hepatotoksičnosti, neurotoksičnosti i hematotoksičnosti. Toksičnost smješe se često može previdjeti, a još uvek nema dovoljno podataka o mehanizmima koji se aktiviraju u ljudskom organizmu uslijed profesionalne izloženosti niskim koncentracijama rastvarača. Ova studija je imala za cilj da istraži vezu između inhalacione izloženosti organskim rastvaračima prisutnih u niskim koncentracijama u radnoj atmosferi i pojavi oksidativnog stresa kao mehanizma toksičnosti kod zaposlenih u industriji obuće. Studija je obuhvatila 16 radnika i 55 radnica zaposlenih u industriji obuće više od godinu dana. Kontrolnu grupu činilo je 30 zdravih muškaraca i 30 zdravih žena bez profesionalne izloženosti. Superoksidni anjon (O₂⁻), uznapredovali produkti oksidacije proteina (AOPP), ukupni oksidativni status (TOS), prooksidativno-antioksidativni balans (PAB), indeks oksidativnog stresa (OSI) i parametri antioksidativne odbrane (aktivnosti enzima superoksid dismutaze (SOD), vrijednosti SH grupa, aktivnosti paraoksonaze-1 (PON1) i ukupni antioksidativni status (TAS)) su određeni u plazmi kod svih ispitanika. Radnici oba pola su imali povišene vrijednosti parametara oksidativnog stresa i antioksidativne zaštite osim PON1, čija je vrijednost bila niža. Takođe, dobijene razlike u vrijednostima AOPP i TAS nisu bile statistički značajne kod muških ispitanika. Ovi rezultati sugerišu da profesionalna izloženost čak i dozvoljenim nivoima rastvarača u industriji obuće, može izazvati oksidativni stres kao mehanizam koji pozitivno doprinosi razvoju štetnih efekata kod radnika.

KLJUČNE REČI: smješa organskih rastvarača, profesionalna izloženost, oksidativni stres, antioksidativna zaštita



OXIDATIVE STRESS PARAMETERS IN WORKERS EXPOSED TO A MIXTURE OF LOW-LEVEL ORGANIC SOLVENTS

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Employees in the shoe industry are potentially exposed to a mixture of organic solvents, which poses an increased risk of hepatotoxicity, neurotoxicity and hematotoxicity. The toxicity of the mixture can often be overlooked, and there are still uncertainties in occupational exposure to low concentrations of solvents regarding the mechanisms triggered in the human body. This study aimed to investigate the relationship between inhalation exposure to low-level organic solvents present in the mixture and the occurrence of oxidative stress as a mechanism of toxicity in shoe industry workers. The study included 16 male and 55 female workers with at least one year of work experience. The control group consisted of 30 healthy males and 30 healthy females with no occupational exposure. The superoxide anion (O₂⁻), advanced oxidation protein products (AOPP), total oxidative status (TOS), prooxidative-antioxidative balance (PAB), oxidative stress index (OSI) and antioxidative defense parameters (superoxide dismutase (SOD) enzyme activities, values of SH groups, paraoxonase-1 (PON1) activities and total antioxidant status (TAS)) were determined in plasma in all subjects. Workers of both genders had increased values of oxidative stress and antioxidative defense parameters, except for PON, which was lower. Also, the obtained differences of AOPP and TAS were not statistically significant in males. These results suggest that occupational exposure to even permissible levels of solvents in the shoe industry may induce oxidative stress, which positively contributes to the development of adverse effects in workers.

KEYWORDS: organic solvents mixture, occupational exposure, oxidative stress, antioxidative defense



NOVE TENDENCIJE U TOKSIKOLOŠKOM ISTRAŽIVANJU / NEW TRENDS IN TOXICOLOGICAL RESEARCH CHAIRS

KVANTIFIKACIJA INTERAKCIJA U STUDIJAMA ISPITIVANJA TOKSIČNOSTI BINARNIH SMEŠA – BDE-209 I KADMIJUM

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Zagađujuće supstance kao što su BDE-209 i Cd (posebno sa deponija električnog i elektronskog otpada) su među najprisutnijim u životnoj sredini i potencijalno mogu da izazovu toksične efekte kod ljudi i u životnoj sredini. Toksikološki profili pojedinačnih supstanci su poznati, međutim toksični efekti smeše zavise od mnogih faktora, posebno od mogućih interakcija između zagađujućih supstanci. Cilj studije je bio da se identifikuju prednosti matematičkog modela korišćenog za kvantifikaciju mogućih interakcija između BDE-209 i Cd. Wistar pacovi su tretirani oralnom gavažom tokom 28 dana sa tri doze BDE-209 od 1000, 2000 i 4000 mg/kg/dan, tri doze Cd od 2,5, 7,5 i 15 mg/kg/dan, svih 9 kombinacija pojedinačnih doza test supstanci i kontrolne grupe. Praćeno je ukupno 84 različita hematološka, biohemijska, hormonska i drugih mogućih štetnih efekata.

Za kvantifikaciju interakcija za ovu binarnu smešu korišćena je analiza multiple faktorske regresije. Dosadašnji rezultati ukazuju da multifaktorska regresiona analiza i procena koeficijenta koji množi kvadratni član polinoma može smatrati pouzdanim pristupom. Model se može prikazati jednačinom: $Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{12}X_1X_2 + g$ gde je Y zavisna varijable tj. intenzitet efekta, X doza Cd ili BDE-209, β_0 je odsečak, β_1 , β_2 i β_{12} su regresioni koeficijenti procenjeni multiplom faktorskom regresionom analizom. Koeficijent β_{12} identifikuje tip interakcija: ukoliko je vrednost β_{12} statistički značajna i pozitivna, tip interakcije je sinergizam, kada je vrednost koeficijenta statistički signifikantna i negativna, ukazuje na antagonizam, dok nulta vrednost bez statističke značajnosti pokazuje odsustvo interakcija.

KLJUČNE REČI: interakcije, toksikologija smeša, metal, POPs



QUANTIFYING INTERACTIONS IN THE BINARY MIXTURE TOXICITY STUDIES - BDE-209 AND CADMIUM

NEW TRENDS
IN TOXICOLOGICAL
RESEARCH CHAIRS

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Highly toxic pollutants such as BDE -209 and Cd (particularly from the e-waste landfills) are among most present in the environment with potential to cause toxic effects in humans and environment. Toxicological profile of single chemicals are well known, however toxic effects of mixture depend on many factors specially on possible interactions between pollutants. Objective of the study was to identify advantages of the mathematical model used for the quantification of possible interactions between BDE-209 and Cd. Wistar rats were treated by oral gavage for 28 days with three doses of BDE-209 of 1000, 2000, and 4000 mg/bw/day, three doses of Cd of 2.5, 7.5, and 15 mg/bw/day, all 9 combinations of single chemicals doses and control group.

Totally 84 different hematological, biochemical, hormonal and other possible adverse effects were observed. Multiple factorial regression analysis was used for the quantification of interactions for this binary mixture. So far, results indicate that multiple factorial regression analysis and the coefficient that multiplying squared part of model could be considered as reliable approach. Model is given by equation: $Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{12}X_1X_2 + \dots$ where Y is dependent variable i.e. measured effect, X is the dose of Cd or BDE-209, β_0 is intercept, β_1 , β_2 and β_{12} are regression coefficients evaluated by multiple factorial regression. Coefficient β_{12} identify the type of interaction: if the value of β_{12} is statistically significant and positive, interaction is synergistic, while when it is statistically significant and negative, interaction is antagonism. Zero value of β_{12} implies absence of interaction.

KEYWORDS: interactions, mixture toxicity, metal, POPs



NOVEL EXPERIMENTAL ACHIEVEMENTS IN THERAPY OF ORGANOPHOSPHATE INTOXICATIONS

NEW TRENDS
IN TOXICOLOGICAL
RESEARCH CHAIRS

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Acetylcholinesterase (AChE) inhibitors – carbamates and organophosphates (including nerve agents tabun, sarin, soman, VX and Novichok agents), act by inhibiting AChE. The fundament of the treatment of poisonings induced by organophosphorus AChE inhibitors remains triple. It consists of the use of oximes that act by causal antidotes – AChE reactivators – and of anticholinergics and anticonvulsants, as symptomatic antidotes. Over the last eighty years, many other classes of antidotes were also tried, with various success. They can be roughly divided into the pre-exposure (prophylactic) and post-exposure antidotes.

Pre-exposure antidotes include large and small molecules. Large molecules are enzymatic scavengers of organophosphates that can be further divided in stoichiometric (eg, AChE, butyrylcholinesterase) and catalytic (eg, paraoxonase 1, phosphotriesterase), depending on the reversibility of their binding to organophosphates. Small molecules include primarily carbamates, the most explored ones being physostigmine and pyridostigmine. This concept is based on the fact that low doses of carbamates reversibly inhibit a small fraction of AChE that remains protected from the subsequent irreversible inhibition by organophosphates. The post-exposure antidotes usually include atropine or some other more liposoluble anticholinergic (eg, scopolamine, procyclidine), an oxime (most frequently pralidoxime or obidoxime) and an anticonvulsant (eg, diazepam, midazolam). Novel approach to the post-exposure therapy consists in development of new oximes, beyond the old H-oximes, such as the K-oximes. One of these oximes, K-870, assures higher potentiation of atropine protection in rats poisoned with paraoxon than obidoxime. Additionally, various N-methyl-D-aspartate (NMDA) receptor antagonists (memantine) were tried in order to prevent or treat organophosphate-induced convulsions.

KEYWORDS: organophosphates, antidotes, acute poisoning, prophylaxis, therapy



MOGUĆNOSTI I OGRANIČENJA ANALIZE TOKSIKOGENOMSKIH PODATAKA – NAŠA ISKUSTVA

NEW TRENDS
IN TOXICOLOGICAL
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Ispitivanja toksičnosti na eksperimentalnim životinjama predstavlja tradicionalni pristup u toksikologiji koji je ograničen dugim trajanjem i visokim troškovima ispitivanja. U novije vreme postoji sve veći interes za razvoj, validiranje i korišćenje brojnih metoda in silico analize koje mogu da pruže efikasniju procenu toksičnosti. Značajan doprinos ovim metodama pruža toksikogenomika, koja kombinacijom tradicionalne toksikologije i bioinformatike sa –omika tehnologijama, istražuje uticaj izloženosti različitim supstancama na gene, proteine i metabolite u okviru ćelija ili tkiva organizma. Rezultati toksikogenomskih ispitivanja, prikupljeni i sadržani u bazama podataka, kao i njihovo analiziranje, mogu da posluže kao osnova za razvoj novih naučnih hipoteza.

Analiza toksikogenomskih podataka omogućava: 1) razumevanje povezanosti između toksičnih supstanci iz životne sredine i bolesti ljudi, 2) pronalaženje relevantnih biomarkera bolesti pri izlaganju toksičnim supstancama i 3) razotkrivanje molekularnih mehanizama toksičnosti, naročito pri ispitivanju smeša toksičnih supstanci, uzimajući u obzir sve potencijalne interakcije na nivou gena, proteina i metabolita koje mogu biti značajne u ispoljavanju toksičnosti. Stoga bi se analiza toksikogenomskih podataka mogla posmatrati kao važan korak za dalja in vitro i in vivo ispitivanjima, omogućavajući smanjenje vremena i troškova celokupnih istraživanja. Nalazi primenu u razvoju lekova, predviđanju toksičnosti i proceni rizika po zdravlje ljudi. Analiza toksikogenomskih podataka biće predstavljena na primerima ispitivanja potencijalnih štetnih efekata kombinovane primene lekova i predviđanja toksičnosti, posebno nakon izloženosti mešama toksičnih supstanci, sa sažetim rezimeom potencijalno korisnih softvera i alata, prvenstveno Komparativne toksikogenomske baze podataka, uključujući njihove prednosti, ali i ograničenja.

KLJUČNE REČI: In silico, toksičnost, toksikogenomika, analiza podataka, mogućnosti, ograničenja
Srbija-Kina projekat: 451-03-1203/2021-09



OPPORTUNITIES AND LIMITATIONS OF TOXICOGENOMIC DATA ANALYSIS - OUR EXPERIENCES

NEW TRENDS
IN TOXICOLOGICAL
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Traditional toxicity testing on experimental animals is limited by long duration and high costs. Recently, there has been a growing interest in the development, validation and use of numerous methods of in silico analysis that can provide more efficient assessment of toxicity. A significant contribution to these methods is provided by toxicogenomics, which, by combining traditional toxicology and bioinformatics with -omics technologies, investigates the impact of exposure to various substances on genes, proteins and metabolites within cells or tissues. The results of toxicogenomic investigations, collected and contained in databases, and their analysis, can serve as a basis for development of new scientific hypotheses.

Toxicogenomic data analysis allows: 1) understanding the relationship between toxic substances from the environment and human diseases, 2) finding relevant disease biomarkers upon the exposure to toxic substances and 3) unravelling molecular mechanisms of toxicity, especially for mixtures of toxic substances, taking into account all the potential interactions on at the gene, protein and metabolite level. Therefore, toxicogenomic data analysis can be regarded as an important stepping stone for further in vitro and in vivo investigations, enabling the reduction of time and costs. It can be applied in drug development, toxicity prediction and human health risk assessment. Toxicogenomic data analysis will be presented by giving examples of investigating potential adverse effects of combined drug administration and toxicity prediction, especially in the case of mixtures of toxic substances, with a concise summary of potentially useful software and tools, primarily Comparative Toxicogenomics Databases, including their advantages, but also limitations.

KEYWORDS: in silico, toxicity, toxicogenomics, data analysis, opportunities, limitations
Serbia-China project: 451-03-1203/2021-09



DVOJAKO DJELOVANJE BIOLOŠKI AKTIVNIH TVARI IZ BILJAKA

NEW TRENDS
IN TOXICOLOGICAL
RESEARCH CHAIRS

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Biološki aktivni spojevi iz biljaka su sekundarni metaboliti, a glavni predstavnici su flavonoidi, terpenoidi i alkaloidi. Djeluju kao antioksidansi, antimikrobnici, atraktanti ili repelenti te su uključeni u brojne procese poput stanične signalizacije. U životinjskim stanicama ovi spojevi utječu na oksidacijski stres i antioksidacijske procese. Mogu inhibirati različite enzime ili modificiraju gene uključene u metabolizam ksenobiotika. Neki od ovih spojeva mogu izazvati epigenetičke promjene te utjecati na endokrnu regulaciju. Iako se tradicionalne ljekovite biljke koriste dulje vrijeme i često se pretpostavlja da su sigurne, postoji mnogo dokaza o smrtnim slučajevima, ozbiljnim i umjerenim do blagim nuspojavama uzrokovanim nepravilnom uporabom ljekovitih biljaka, dodataka prehrani i drugih tradicionalnih medicinskih proizvoda.

Nadalje, biljke i njihovi pripravci mogu biti toksične, mutagene ili čak kancerogene. Kako bismo ukazali na dvostruko djelovanje fitokemikalija, u ovom izlaganju bit će prikazani rezultati in vitro ispitivanja genotoksičnosti ekstrakata lista ružmarina i maslačka. Kao biološki test sustav korištene su stanice epitelnog karcinoma ljudskog jezika CAL 27 kao predstavnika usne šupljine. Istražene koncentracije oba ekstrakta odgovarale su onima prisutnima u hrani ili piću. Istražen je njihov genotoksični učinak te potencijalni zaštitni učinak na genetički materijal nakon oštećenja izazvanog vodikovim peroksidom. Ekstrakti maslačka i ružmarina povećali su intenzitet repa komete. Oba ekstrakta povećala su stvaranje mikronukleusa čak i pri koncentracijama nekoliko puta manjim od dnevnih preporučenih doza.

Veće koncentracije inducirale su apoptozu i nekrozu stanica. S druge strane, ekstrakt ružmarina pokazao je zaštitni učinak protiv oksidativnog oštećenja izazvanog vodikovim peroksidom smanjujući broj apoptotskih stanica.

Kako bi se regulirala i promicala racionalna i terapijski ispravna uporaba, ljekovite bilje trebalo bi integrirati kao politiku u nacionalne zdravstvene sustave te izraditi smjernice o njihovoj sigurnosti, učinkovitosti i kvaliteti.

KLJUČNE REČI: fitokemikalije, genotoksičnost, antioksidacijski učinak, oksidacijski stres, regulative



EQUIVOCAL EFFECT OF BIOACTIVE COMPOUNDS FROM PLANTS

NEW TRENDS
IN TOXICOLOGICAL
RESEARCH CHAIRS

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Biologically active compounds from plants are secondary metabolites and main representatives are flavonoids, terpenoids and alkaloids. They act as antioxidants, antimicrobials, attractants or repellents or are involved in numerous processes such as cell signaling. In animal cells, these compounds processes such as oxidative stress and antioxidative events. They inhibit different enzymes or are involved in modification of xenobiotic metabolism genes. Some of these compounds can cause epigenetic modification and change the endocrine regulation. Even though traditional medicinal plants are used for long periods of time and are often assumed to be safe there is lots of evidence of death cases, serious and moderate to mild adverse events caused by incorrect use of medicinal plants, dietary supplements and other traditional medicinal products.

Furthermore, plants that are used either as food ingredients or in traditional medicine can not only be toxic but also mutagenic or even carcinogenic. To point out the dual effects of phytochemicals, in this presentation the results of in vitro genotoxicity testing of rosemary and dandelion leaves extracts will be presented. As a biological test system human tongue epithelial carcinoma cells CAL 27 as a representative of oral cavity were used. Examined concentrations of both extracts were relevant to those present in food or beverage and were tested on their genotoxicity and H2O2 – induced oxidative damage protection. The results have revealed that both, dandelion and rosemary extracts increased comet tail intensity. Both extracts increased micronucleus formation even at concentrations several times lower than daily recommended doses. Higher concentrations induced cell apoptosis and necrosis. On the other hand, rosemary extract showed a protective effect against hydrogen peroxide – induced oxidative damage by decreasing the number of apoptotic cells. In order to regulate and promote rational and therapeutically sound usage, medicinal plants should be integrated as policy into national healthcare systems, and guidelines on their safety, efficacy, and quality should be conducted.

KEYWORDS: phytochemicals, genotoxicity, antioxidative effect, oxidative stress, regulatives



IMOBILIZACIJA ENZIMA KAO STRATEGIJA ZA EFIKASNO I ODRŽIVO ODREĐIVANJE INHIBITORA I REAKTIVATORA HOLINESTERAZA

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Imobilizacija enzima predstavlja značajnu naučnu metodologiju zbog odličnog funkcionalnog poboljšanja svojstava proteina kao što su ponovna upotreba, ekonomičnost i lako odvajanje od reakcionog medijuma. Po prvi put, prema našim najboljim saznanjima, primenili smo afinitetnu hromatografiju imobilisanih metalnih jona na magnetnim česticama na holinesteraze (ChEs), kao ciljne enzime za dejstvo antidota kod trovanja organofosforinim (OF) pesticidima i nervnim agensima, kao i lekova za lečenje Alchajmerove bolesti. Humana rekombinantna butirilholinesteraza (hrBChE) je imobilizovana pomoću Dinabeads His-Tag mikročestica i ispitana je njena biokatalitička aktivnost uključujući: stabilnost tokom vremena; kapacitet ponovne upotrebe/recikliranja; kinetiku enzima; mehanizam inhibicije; kapacitet inhibicije i reaktivacije. Sa tim ciljem korišćeni su odabrani standardni inhibitori holinesteraza: takrin, donepezil, karbofuran, bendiokarb, paraokson i surogati nervnih agenasa (sarina, somana i tabuna), kao i standardni reaktivatori holinesteraza inhibiranih organofosfatima: pralidoksim i obidoksim. Aktivnost enzima je određena modifikovanom Ellman-ovom metodom korišćenjem čitača mikroploča Tecan Spark (Tecan Trading AG, Švajcarska). Svi eksperimenti su urađeni sa slobodnom/neimobilizovanom hrBChE pod istim eksperimentalnim uslovima. Za sve ispitivane parametre, biokatalitička aktivnost imobilizovane hrBChE bila je uporediva sa aktivnošću slobodnog enzima, uz održavanje superiorne stabilnosti u vremenu. U zaključku, magnetnim česticama imobilizovana hrBChE se pokazala kao novi, moćni biokatalizator i alat za istraživanje inhibitora i reaktivatora holinesteraza. Ovo istraživanje finansirano je od strane Univerziteta Hradec Kralove, Republika Češka (broj CZ.02.2.69/0.0/0.0/18_053/00 17841) i Ministarstva nauke, tehnološkog razvoja i inovacija Republike Srbije kroz Ugovor sa Univerzitetom u Beogradu-Farmaceutskim fakultetom (broj 451-03-47/2023-01/ 200161).

KLJUČNE REČI: magnetne čestice, holinesteraze, histidin, afinitetna hromatografija



ENZYME IMMOBILIZATION AS A STRATEGY TOWARDS EFFICIENT AND SUSTAINABLE DETERMINATION OF CHOLINESTERASE INHIBITORS AND REACTIVATORS

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Enzyme immobilization presents substantial scientific methodology due to excellent functional improvement of protein properties such as reusability, cost-effectiveness and easy separation from the reaction medium. For the first time, according to our best knowledge, we have applied immobilized metal affinity chromatography (IMAC) magnetic beads on human cholinesterases (ChEs), being the crucial targets for antidotes in organophosphorus (OP) pesticide and nerve agent poisonings, as well as drugs for Alzheimer disease treatment. Human recombinant butyrylcholinesterase (hrBChE) was immobilized by Dynabeads His-Tag microparticles and evaluated for its biocatalytic performance including: stability in time; reuse/recycling capacity; enzyme kinetics; inhibition mechanism; inhibition and reactivation capacity.

With that aim, selected standard ChE inhibitors were tested: tacrine, donepezil, carbofuran, bendiocarb, paraoxon and nerve agent (sarin, soman and tabun) surrogates, along with standard OP-inhibited ChE reactivators: pralidoxime and obidoxime. Enzyme activity was determined by modified Ellman's method using Tecan Spark microplate reader (Tecan Trading AG, Switzerland). All the experiments were done with free/non-immobilized hrBChE under the same experimental conditions. For all determined parameters, biocatalytic activity of immobilized hrBChE was comparable with that of free enzyme, while maintaining superior stability in time. In conclusion, immobilized hrBChE was demonstrated as a new, powerful biocatalyst and tool for research concerning cholinesterase inhibition or reactivation processes. Supported by International mobilities for research activities of the University of Hradec Králové II (No. CZ.02.2.69/0.0/0.0/18_053/0017841) and the Ministry of Science, Technological Development and Innovation, Republic of Serbia through Grant Agreement with University of Belgrade-Faculty of Pharmacy (No. 451-03-47/2023-01/ 200161).

KEYWORDS: magnetic particles, cholinesterase, His-tag, IMAC



IZAZOVI I SAVREMENI PRISTUPI U KLINIČKOJ TOKSIKOLOGIJI / CHALLENGERS AND MODERN APPROACHES IN CLINICAL TOXICOLOGY CHAIRS

SIX YEARS AFTER LEGALIZATION OF CANNABIS FOR MEDICAL USE: DID WE MAKE A MISTAKE?

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The both form of Cannabis oil (THC and CBD) are approved for medical use in North Macedonia since February 2016. The aim of the study was to analyze the benefits and eventual troubles caused by legalization of cannabis for medical use. For that purpose we analyzed the Register of the Health Fund Insurance for prescript doses of both types of cannabis oil in last six years, The Annual Bulletins of car accidents caused by Cannabis abuse from the Ministry of Interior in last twelve years, the patients observed with ultrasound examination after cancers in our Ultrasound cabinet in last six years, and the number of patients registered after abuse of psychoactive substances in our Poisoning Information System in last twelve years.

The number of sold Cannabis oil have continues trend of growing in the last six years, from 14.327 doses in 2016 to 47.653 in 2022, More than 97% of the sold bottles are with pure CBD oil, mostly 12%. More than 65% of the patients examined in our ultrasound cabinet after different types of cancers, used Cannabis oil during their treatment with cytostatics. There weren't noticed any significant disturbances in the number of car accidents after abuse of cannabis, and abuse of PAS after legalization of cannabis for medical use. Legalization of cannabis for medical use was a brave but successful step forward that gave another opportunity for treatment of some medical conditions without any significant side effects for patients and our society.

KEYWORDS: cannabis oil, legalization, trends



TOKSIČNOST PROIZVODA KUĆNE HEMIJE: ISKUSTVA NACIONALNOG CENTRA ZA KONTROLU TROVANJA VMAKT VMA

CHALLENGERS AND MODERN
APPROACHES IN CLINICAL
TOXICOLOGY CHAIRS

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Upotreba potencijalno toksičnih hemikalija u domaćinstvima predstavlja uobičajeni način savremenog života, što rezultira velikim brojem zadesnih ili namernih trovanja. Cilj rada je procena karakteristika trovanja proizvodima kućne hemije kod pacijenata Nacionalnog centra za kontrolu trovanja VMA (NCKT). Retrospektivno su analizirani istorije bolesnika i podaci iz elektronske baze Centra za 10-godišnji period (2010-2019). U prijemno-trijažnom odeljenju NCKT se zbog akutnih trovanja godišnje zbrine više od 4000 bolesnika, od kojih trovanja proizvodima kućne hemije čine 2-3%. Zbog ozbiljnosti povreda, oko 2/3 ovih pacijenata se prima na hospitalno lečenje (48-92 godišnje). Najčešći razlog je ingestija hlorovodonične kiseline za čišćenje sanitarija (33%) ili konzervansa koji sadrži koncentrovanu sircetnu kiselinu (24%). Proizvodi na bazi NaOH su uzrok oko 9% trovanja, a izbeljivači oko 6%.

Druga sredstva za čišćenje i dezinfekciju koja sadrže kombinacije različitih kiselina i deterdženti čine preostalih 28%. Na osnovu endoskopski dijagnostikovanih oštećenja digestivnog trakta i kliničke slike, prema kriterijumima PSS skora, kod 1/3 hospitalizovanih pacijenata trovanja su procenjena kao laka, kod 20% kao srednje teška, a kod čak 42% kao teška. Letalitet je u odnosu na ukupan broj hospitalizovanih iznosio 14%, a u odnosu na podgrupu sa srednje teškim i teškim trovanjima čak 33%. Svi letalni ishodi su posledica namernih trovanja navedenim koncentrovanim kiselinama ili bazama, dok prilikom ingestije izbeljivača ili kombinovanih sredstava za čišćenje ne dolazi do najtežih oštećenja digestivnih sluznica i letalnih ishoda. Smanjenje koncentracije sircetne kiseline u konzervansima i zamena koncentrovanih kiselina sredstvima koja sadrže kombinacije kiselina i surfaktanata, može biti jedan od načina prevencije teških trovanja.

KLJUČNE REČI: kućna hemija, hlorovodonična kiselina, sircetna kiselina, izbeljivači, deterdženti



TOXICITY OF HOUSEHOLD PRODUCTS: EXPERIENCE OF NATIONAL POISON CONTROL CENTRE

CHALLENGERS AND MODERN
APPROACHES IN CLINICAL
TOXICOLOGY CHAIRS

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Extensive use of potentially toxic household products is a common way of modern life, resulting in a large number of accidental or intentional poisonings. The aim of the paper is to evaluate the characteristics of poisoning by household products in patients of the National Poison Control Centre VMA (NCKT). Patient's medical records and data from the Centre's electronic database for a 10-year period (2010-2019) were retrospectively analyzed. More than 4,000 patients are treated annually for acute poisoning in the emergency unit of NCKT, of which 2-3% are poisoned by household products. Due to the severity of the injuries, about 2/3 of these patients are admitted for hospital treatment (48-92 per year).

The most common reason was the ingestion of hydrochloric acid for cleaning toilets (33%) or a preservative containing concentrated acetic acid (24%). NaOH-based products was the cause of 9% of poisoning, and bleaches of 6%. Other cleaning and disinfecting products containing combinations of different acids and detergents made the remaining 28%. Based on endoscopically diagnosed lesions of the digestive tract and clinical picture, according to PSS criteria, in 1/3 of hospitalized patients, poisoning was assessed as mild, in 20% as moderate, and in as many as 42% as severe. The fatality rate in patients hospitalized for these agents was 14%, and in relation to the subgroup with moderate and severe poisoning, it was as much as 33%. All fatal outcomes are the result of deliberate poisoning with the above-mentioned concentrated acids or alkalis, while ingestion of bleach or combined cleaning agents does not result in severe damage to the digestive mucosa and fatal outcomes. Reducing the concentration of acetic acid in preservatives and replacing concentrated acids with products containing combinations of acids and surfactants could be one of the modalities to prevent severe poisoning.

KEYWORDS: household products, hydrochloric acid, acetic acid, bleachers, detergents



LATEST KNOWLEDGE ON OPIOIDS POLYMORPHISM IN PAIN MANAGEMENT: THE WAY FORWARD

CHALLENGERS AND MODERN
APPROACHES IN CLINICAL
TOXICOLOGY CHAIRS

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Opioids are cornerstone therapy for moderate to severe pain. However, significant increases in opioid use for pain relief in world in the last years increase concerns about opioid epidemic and emphasizes the importance of new therapeutic strategies for appropriate opioid pain management. Large inter-individual genetic variability in the efficacy is important problem for pain management in terms of both inadequate pain relief but also for adverse effects. Single nucleotide polymorphisms (SNPs) is the most common genetic variation affecting both pharmacokinetic and pharmacodynamic pain elements.

In my presentation, I will present our research data on μ -opioid gene polymorphism (OPRM1 A118G) affecting opioid response in the Turkish population, compare it with other population's frequencies and present the latest literature knowledge on pain polymorphism. Data on literature showed that the lowest opioid polymorphism allele frequency was found in African Americans. Asian populations have higher frequencies of it compared to American and European populations. Although conflicting results are seen in studies about polymorphism and drug effects, meta-analyses evaluating all research showed that opioid polymorphism has mostly impact on opioid response in Asian populations. Genetic polymorphism can be useful guide for the successful use of long-term opioid therapy especially for chronic pain. Although routine genetic tests are not routine for pain management, polymorphism screening should be considered in case of persisting pain, grade 3 side effects or more than 4 episodes of breakthrough pain per a day.

KEYWORDS: Single nucleotide polymorphisms, opioid, pain management



FAKTORI KOJI UTIČU NA ELIMINACIJU, KLINIČKU SLIKU I ISHOD AKUTNE INTOKSIKACIJE BENZODIAZEPINIMA KOD PACIJENATA STARIJE ŽIVOTNE DOBI

CHALLENGERS AND MODERN
APPROACHES IN CLINICAL
TOXICOLOGY CHAIRS

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Benzodiazepini su najčešće korišćeni lekovi u slučaju akutnih samotrovanja. Cilj ovog rada je analiza faktora koji utiču na proces eliminacije benzodiazepina, težinu kliničke slike, razvoj komplikacija i ishod akutne intoksikacije kod osoba starije životne dobi. Sprovedena je klinička opservaciona kohortna studija. Ukupno je analizirano 95 bolesnika hospitalno lečenih u Klinici za toksikologiju Vojnomedicinske akademije. Podeljeni su u tri grupe u odnosu na životnu dob (18-40 godina, 41-65 godina, stariji od 65) i praćeni su u skladu sa kliničkom slikom i detektovanim toksičnim koncentracijama minimalno 48 sati. Najčešće su intoksikacije bromazepamom, a poređenjem srednjih koncentracija bromazepama na prijemu među različitim dobnim grupama, u grupi starijih od 65 godina one su bile više, bez statističke značajnosti u odnosu na mlađe dobne grupe. Eliminacija benzodiazepina je analizirana poređenjem koncentracija lekova na prijemu i određivanjem procentulanog smanjenja koncentracije posle 24 i 48 sati.

Dominantan klinički znak u akutnim intoksikacijama benzodiazepinima je poremećaj stanja svesti različitog stepena procenjivan pomoću Glasgow Coma Scale (GCS). Procena težine trovanja vršena je u skladu sa Skalom težine trovanja (engl. Poisoning Severity Score, PSS). Pokazano je da je kod osoba starije životne dobi u poređenju sa drugim starosnim grupama metabolički kapacitet manji, a renalna eliminacija sporija. Kod starijih od 65 godina uočena je i češća hipoalbuminemija što doprinosi dužem održavanju toksičnih koncentracija benzodiazepina. Kod bolesnika starijih od 65 godina zabeležena je teža klinička slika (dugotrajniji poremećaji svesti, češći kardiovaskularni poremećaji), duža hospitalizacija, veća potreba za primenom antidota, češće komplikacije (aspiraciona pneumonija i rabdomioliza), oporavak sporiji, a letalitet veći.

KLJUČNE REČI: intoksikacije; benzodiazepini; stare osobe.



FACTORS AFFECTING BENZODIAZEPINES ELIMINATION, CLINICAL FEATURES AND OUTCOME IN ELDERLY PEOPLE ACUTELY POISONED WITH BENZODIAZEPINES

CHALLENGERS AND MODERN
APPROACHES IN CLINICAL
TOXICOLOGY CHAIRS

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Benzodiazepines are the most frequent drugs used in cases of deliberate self-poisoning. Aim of this study was analyzing the factors affecting the elimination process of benzodiazepines, severity of clinical features, complications and acute poisoning outcome in elderly patients. Clinical observation cohort study was made including 95 patients hospitalized in the Department of Clinical Toxicology in Belgrade. Patients were divided into three groups according to their age (18-40; 41-65; ≥65 years old).

Patients were monitored in accordance with the clinical features as well as the detected toxic concentrations of benzodiazepines in 48-hour period. The most common ingested benzodiazepine was bromazepam. Comparing the mean concentration of bromazepam among different age groups, drug concentration is slightly higher in group of elderly people, but not statistically important compared to younger age groups. Drug elimination was analyzed by comparing its concentration after 24 and 48 hours. Dominant clinical characteristic of an acute benzodiazepine intoxication is different level of consciousness disorder estimated by Glasgow Coma Scale (GCS). Evaluation of the poisoning was performed in accordance with the Poisoning Severity Score (PSS). The study shows that metabolic capacity decreases and renal elimination is slower in elderly people compared to other age groups. More common hypoalbuminemia is also observed in group of elderly people, contributing to extended maintenance of toxic concentrations of benzodiazepines. Severe clinical features is observed in elderly population- deeper and prolonged disorders of consciousness, more common cardiovascular disorder, longer hospitalization, increased need for antidotes, frequent complications (aspiration bronchopneumonia and rhabdomyolysis), slower recovery and higher lethality.

KEYWORDS: self-poisoning; benzodiazepines; elderly



ZLOUPOTREBA GABAPENTINOIDA U SRBIJI - DA LI PRATIMO GLOBALNE TRENDOVE?

CHALLENGERS AND MODERN
APPROACHES IN CLINICAL
TOXICOLOGY CHAIRS

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Zloupotreba lekova je veliki zdravstveno-društveni problem i predstavlja značajno opterećenje za zdravstvene sisteme. Pregabalin i gabapentin pripadaju gabapentinoidima, grupi lekova koji tradicionalno nisu bili korišćeni u cilju zloupotrebe. Sa značajnim porastom terapijske upotrebe tokom prethodne decenije, sve je više izveštaja o zloupotrebi i akutnim trovanjima ovim lekovima. Pretpostavlja se da je pregabalin češće zloupotreblijan zbog svojih farmakoloških karakteristika. U nekim zemljama potencijal za zloupotrebu gabapentinoida je prepoznat te su pregabalin i/ili gabapentin klasifikovani kao kontrolisane psihoaktivne supstance.

Cilj ovog rada je da prikaže i detaljno analizira akutna trovanja gabapentinoidima u Nacionalnom centru za kontrolu trovanja Srbije (NCKT) tokom desetogodišnjeg perioda (2012-2022), što može poslužiti kao indikator stepena zloupotrebe ove grupe lekova u našoj zemlji. Kako bi se bolje razumeli mehanizmi toksičnosti i zloupotrebe, analizirane su glavne karakteristike ispitivane populacije i klinički efekti trovanja. Tokom perioda studije bilo je 357 (95,5%) slučajeva trovanja pregabalinom i 17 (4,5%) gabapentinom, dok je zloupotreba detektovana kod skoro trećine pacijenata.

Prema podacima Agencije za lekove i medicinska sredstva Srbije, u istom periodu je registrovan porast potrošnje pregabalina preko 200 puta, dok nije bilo značajnijih promena u potrošnji gabapentina. Zloupotreba je detektovana najčešće kod mladih muškaraca i u visokom procentu u migrantskoj populaciji. Češća su bila kombinovana trovanja sa drugim psihoaktivnim supstancama, što je rezultiralo težom kliničkom slikom. Neophodan je oprez i bolja kontrola pri propisivanju gabapentinoida kao i edukacija pacijenata i zdravstvenih radnika. Pored lečenja akutno otrovanih pacijenata, NCKT će nastaviti da sprovodi toksikovigilancu, kako bi se skrenula pažnja relevantnim institucijama i preduzele adekvatne mere u cilju sprečavanja zloupotrebe u našoj zemlji.

KLJUČNE REČI: pregabalin, zloupotreba, akutna trovanja, psihoaktivne supstance



GABAPENTINOID ABUSE IN SERBIA - ARE WE FOLLOWING THE GLOBAL TREND?

CHALLENGERS AND MODERN
APPROACHES IN CLINICAL
TOXICOLOGY CHAIRS

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Drug abuse is a major problem and represents a significant burden on health care systems. Pregabalin and gabapentin belong to the gabapentinoids, a group of drugs that have not traditionally been abused. With the substantial increase in therapeutic use over the past decade, the number of reports of abuse and acute poisoning with these drugs is also increasing. It is assumed that pregabalin is more commonly abused due to its pharmacological properties. The abuse potential of gabapentinoids is recognized in some countries and pregabalin and/or gabapentin are classified as controlled psychoactive substances. The aim of this study was to analyze data on gabapentinoid-related attendances in the National Poison Control Centre of Serbia (NPCC) over the ten-year period (2012-2022), which can serve as an indicator of their abuse rate in our country. In order to better understand the mechanisms of toxicity and abuse, we aimed to analyze the main characteristics of the study population and to investigate the major clinical effects in poisoned patients.

During the study period, there were 357 (95.5%) cases of poisoning related to pregabalin and 17 (4.5%) related to gabapentin, with almost one-third of patients being found to have abused the drugs. According to the annual reports of the Serbian Agency for Medicines and Medical Devices, the consumption of pregabalin increased more than 200-fold during the same period while there were no significant changes in the prescription and consumption of gabapentin. Abuse was detected primarily in younger men and in a high percentage among the migrant population. Combined poisonings with other psychoactive substances occurred more frequently and resulted in more severe clinical symptoms. Caution and monitoring, as well as better patient and physician education, are necessary when prescribing gabapentinoids. In addition to treating acutely poisoned patients, the NPCC will continue to conduct toxicovigilance to raise awareness among relevant institutions and take appropriate measures to prevent abuse in our country.

KEYWORDS: pregabalin, abuse, acute poisoning, psychoactive substances



HEMIKALIJE SU SVUDA OKO NAS / CHEMICALS ARE ALL AROUND US

ADVERSE CONSEQUENCES OF ADULTERATION OF HERBAL PRODUCTS

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The market for herbal products taken to improve the health or well-being of the customer is enormous. Their use has been increasing because of the belief of their safety in the treatment or prevention of diseases. However, as the contents of herbal products have many bioactive components, the lack of sufficient study on their toxicity, inadequate controls of their availability, reduce their safety.

Adulterants found in the herbal products, is the most common malpractice in herbal raw-material trade. Adulteration is described as intentional substitution with another plant species or intentional addition of a foreign drug or a chemical to increase the potency of the herbal product or to decrease its cost. Chemical adulterants have been observed in many herbal products used to decrease appetite and to enhance sexual performance.

The primary adulterant in the herbal weight loss suppressants is sibutramine which was banned both in Europe and the United States in 2010 for its potential cardiovascular risks or even strokes. Anti-obesity drugs mainly sibutramine or analogs have been detected in many weight-loss herbal supplements from many countries. On the other hand, during the last few years, the demand for phosphodiesterase-5 (PDE-5) inhibitors such as sildenafil citrate, and tadalafil that can cause serious adverse effects even death, has also been increasing worldwide, not only for the treatment of patients with erectile dysfunction but also to enhance sexual performance. The unpredictable adverse effects of herbal products due to the adulteration with prohibited chemicals will be discussed in detail.

KEYWORDS: adulteration, herbal medicines, adverse effects



ALTERNARIA MYCOTOXINS AS A CHALLENGE FOR RISK ASSESSMENT

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Alternaria toxins are formed by black molds of the genus *Alternaria*, which occur ubiquitously and are able to grow under varying temperature and moisture condition as well as on a large diversity of substrates. Reports on the occurrence of *Alternaria* toxins comprise a broad spectrum of plant-based food commodities including grain and grain-based products, apples, oilseeds, sun flower oil and tomato products. Although *Alternaria* species are known to generate a spectrum of secondary metabolites, toxicological studies have focused so far predominantly on the commercially available toxins e.g. alternariol (AOH), its monomethyl ether AME, tenuazonic acid (TeA) and tentoxin (TEN) as single compounds. In 2011, EFSA performed the first risk assessment on *Alternaria* toxins, but could only evaluate these four toxins due to the limited amount of data.

In April 2022, the European Commission published a recommendation of indicative values for AOH, AME and TeA in certain food commodities. However, *Alternaria alternata* is able to generate a broad spectrum of secondary metabolites with different activity profile. Thus, in native toxin mixtures, a complex overlay of biological activities might occur including immunosuppressive, endocrine disruptive and genotoxic properties. Besides TeA, TEN and the two major dibenzo- α -pyrones AOH and AME, *Alternaria* spp. may produce significant amounts of perylene quinone derivatives, such as alterperlylenol (ALP), altertoxins (ATX) I-III and stemphytoxin III (STTX-III). ATX-II, exceeds by far the genotoxicity of AOH, and represents an important DNA-damaging component in complex mixtures. So far, the occurrence of perylene quinones in food is still unknown. Taken together, *Alternaria* toxins comprise a spectrum of mycotoxins with different molecular targets. Data are accumulating, arguing for relevance of these compounds in the food chain.

KEYWORDS: black molds, genus *Alternaria*, mycotoxins, health risk assessment



STATUS AKTIVNIH SUPSTANCI PESTICIDA U EVROPSKOJ UNIJI I DALJE PERSPEKTIVE POVLAČENJA IZ PRIMENE

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Trenutno se u prometu u zemljama Evropske unije (EU) nalaze 453 odobrene aktivne supstance (AS) pesticida, povučениh iz primene je ukupno 944, još neprocenjenih je 19, a onih čiji zahtev još uvek nije razmatran, u skladu sa propisima kojima se uređuju sredstva za zaštitu bilja, je ukupno 65. Među odobrenim AS ima 53 kandidata za zamenu. Po stupanju na snagu Regulative (EC) 1107/2009 definisani su kriterijumi za neprihvatljive efekte AS pesticida i značajan broj je povučen iz primene na osnovu tih kriterijuma.

Prema ovoj Regulativi AS ne mogu biti odobrene ako su, ili će biti, između ostalog, klasifikovane prema Regulativi (EC) 1272/2008, kao mutagenost germinativnih ćelija, kategorija 1, karcinogenost, kategorija 1 i toksičnost po reprodukciju, kategorija 1, zatim ako su perzistentne, bioakumulativne i toksične (PBT), veoma perzistentne i veoma bioakumulativne (vPvB), ako pripadaju grupu perzistentnih organskih zagađujućih supstanci (POPs), ako deluju kao ometači endokrinog sistema i dr. Među odobrenim AS nema klasifikovanih u kategoriju 1 po karcinogenim, mutagenim i reprotoksičnim svojstvima, 17 je klasifikovanih kao karcinogenost, kategorija 2, svi mutageni kategorije 2 su povučeni iz primene (poslednji je povučen tiofanat-metil 2021. godine), a toksičnih po reprodukciju kategorije 2 u prometu je ukupno 19. U procesu reevaluacije trenutno se nalaze neke od ekonomski, ali i sa aspekta primene, veoma značajnih AS, kao što su: kaptan, S-metolahlor, etefon, klofentezin, abamektin, oksamil, ditianon, dimoksistrobin, deltametrin, i dr. Svi oni koji se bave zaštitom bilja nestrpljivo očekuju odluke Stalnog komiteta za biljke, životinje, hranu i stočnu hranu vezane za dalji status ovih AS.

KLJUČNE REČI: aktivne supstance pesticida, mutagenost, karcinogenost, toksičnost po reprodukciju, neprihvatljivi efekti



THE STATUS OF PESTICIDE ACTIVE SUBSTANCES IN THE EUROPEAN UNION AND FURTHER PERSPECTIVES OF THEIR WITHDRAWAL

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Currently, there are 453 approved pesticide active substances (ASs) on the market in the countries of the European Union (EU), a total of 944 have been withdrawn, 19 are not yet assessed at EU level, and 65 are pending. Among the approved ASs there are 53 candidates for substitution. After the entry into force of Regulation 1107/2009, criteria for unacceptable effects of pesticide ASs were defined and a number were withdrawn from application based on them.

According to this Regulation, an AS shall only be approved, among other things, if it is not or has not to be classified, in accordance with Regulation 1272/2008, as mutagen category 1, carcinogen category 1, toxic for reproduction category 1, if it is not considered to be persistent, bioaccumulative and toxic (PBT), very persistent and very bioaccumulative (vPvB), persistent organic pollutant (POP), or have endocrine disrupting properties, etc. There are no approved ASs classified as carcinogen, mutagen or toxic for reproduction category 1, 17 are classified as carcinogen cat. 2, all mutagen cat. 2 are no longer approved (Thiophanate-methyl was the last withdrawn in 2021), and 19 are toxic for reproduction cat. 2.

In the process of reevaluation, there are currently some of the economically, but also from the aspect of application, very important ASs: captan, S-metolachlor, ethephon, clofentezine, abamectin, oxamyl, dithianon, dimoxystrobin, deltamethrin and others. All those involved in plant protection are eagerly awaiting the decisions of the Standing Committee on Plants, Animals, Food and Feed related to the further status of these ASs.

KEYWORDS: Pesticides active substances, mutagen, carcinogen, toxic for reproduction, unacceptable effects



CREVNA MIKROBIOTA KAO OSETLJIVA META DELOVANJA TOKSIČNIH SUPSTANCI

CHEMICALS ARE ALL
AROUNDS US

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Crevna mikrobiota ima važnu ulogu u održavanju homeostaze koju ostvaruje preko interakcija sa imunskim sistemom i gastrointestinalnim traktom. Toksične supstance ostvaruju direktan štetni efekat na sastav i funkcionalnost crevne mikrobiote. Kadmijum (Cd) je metal sa izraženom toksičnošću, koji se povezuje sa brojnim oboljenjima. U našoj studiji je predložen mehanizam zaštite koji bi se ostvario posredstvom bakterijskih egzopolisaharida (EPS) izolovanih iz soja *Lactiplantibacillus plantarum* BGAN8, a bazira se na visokom afinitetu adsorpcije kadmijumovih jona. Ciljevi rada su bili utvrđivanje efekata produženog delovanja kadmijuma na sastav crevne mikrobiote *Dark agouti* (DA) pacova, kao i izučavanje efekta EPS-AN8 na ublažavanje takvih štetnih efekata.

Pacovi su kroz vodu unosili nižu (5 ppm) i višu (50 ppm) dozu kadmijuma, dok je EPS-AN8 (100 µg/ml) dat kroz hranu za životinje. Totalna DNK je izolovana iz lumena duodenuma i 16S rDNK amplikon je sekvenciran strategijom uparenih krajeva na Illumina NovaSeq platformi. Dodatno, praćene su promene u inflamaciji i oksidativnom stresu homogenata duodenuma. Kadmijum je favorizovao rast oportunističkih patogenih bakterija poput pripadnika rodova *Blautia*, *Prevotella*, *Alloprevotella*, *Bacteroides*, dok je relativna zastupljenost laktobacila bila značajno smanjena. Obe doze kadmijuma su stimulisale produkciju proinflatornih citokina (IL-1β, TNF-α i IFN-γ) i povisile nivo oksidativnog stresa (MDA, CAT i GST). Oralni unos EPS-AN8 je značajno ublažio sve navedene promene. Oralna izloženost kadmijumu uzrokuje nedvosmislene promene u sastavu mikrobiote, koje mogu biti praćene i pojačanim nivoima inflamacije i oksidativnog stresa. Nasuprot tome, istovremeni unos EPS-AN8 redukuje pojavu takvih promena.

KLJUČNE REČI: kadmijum, crevna mikrobiota, egzopolisaharidi



GUT MICROBIOTA AS A TARGET OF TOXIC COMPOUNDS

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The gut microbiota has important role in maintaining homeostasis via its effect on the immune system and healthy gastrointestinal tract. Environmental toxicants directly influenced the changes in gut microbiota composition. Cadmium (Cd) is a hazard, world widely distributed metal. We have proposed the putative mechanism of protection against Cd harmful effect in the organism by using exopolysaccharides derived from *Lactiplantibacillus plantarum* BGAN8 (EPS-AN8) which has great ability to sequester Cd ions. Aims of our study were to examine Cd related changes in the gut microbiota of Dark agouti (DA) rats after prolonged oral exposure (30 days) to this metal and to determine impact of EPS-AN8 in preventing those changes.

Rats were given via water lower (5 ppm) and higher (50 ppm) Cd's dose. In paralel, EPS-AN8 was administrated through food (100 µg/ml). Total DNA from duodenum was isolated and PCR amplicon for 16SrRNA was sequenced on Illumina NovaSeq paired end platform. Moreover, we have followed changes in oxidative stress and inflammation in duodenum. Cd favored growth of opportunistic pathogen bacteria genera (*Blautia*, *Prevotella*, *Alloprevotella*, *Bacteroides*) while the relative abundance of lactobacilli was decreased. The both doses of Cd increased production of inflammatory cytokines (IL-1 β , TNF- α , and IFN- γ). Also, parameters of oxidative stress were significantly upregulated (MDA, CAT, and GST). All the mentioned changes were alleviated by EPS-AN8. Oral exposure to Cd lead to inevitable changes in the microbiota structure followed by inflammation and oxidative stress of surrounding tissue, while administration of EPS-AN8 reversed those changes.

KEYWORDS: cadmium, gut microbiota, exopolysaccharides



RAZLIČITI ASPEKTI EPIGENETIKE U TOKSIKOLOŠKIM ISTRAŽIVANJIMA

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Epigenetika opisuje mehanizme koji regulišu stabilni i tkivno-specifični obrazac genske ekspresije bez promene primarne sekvence DNK, uključujući i sledeće procese: DNK metilacija, različite forme post-translacionih modifikacija histona (histonskog koda) i regulatorna dejstva nekodirajućih RNK molekula. Reverzibilne kovalentne modifikacije DNK i histonskih proteina su definisane kao epigenetička obeležja koja su odgovorna za pakovanje hromatina u jezgro time omogućavajući transkripcionoj mašineriji pristup delovima genoma dok drugi regioni ostaju utišani. Epigenetička toksičnost je pojava u kojoj hemijska supstanca utiče na epigenom i ispoljava neželjeni efekat na žive organizme, što može objasniti dugoročne efekte hemijskih supstanci i predispoziciju ka bolestima usled faktora životne sredine.

Epigenetičke oznake su stabilne u diferenciranim ćelijama ali nakon izlaganja farmaceutskim proizvodima, nanomaterijalima, metalima, faktorima životne sredine i komponentama u ishrani, postaju podložne promenama koje vode ka razvoju različitih patoloških stanja. Izazvane promene obuhvataju izmene tkivno-specifičnog epigenetičkog profila koji je direktno povezan sa poremećajem u regulaciji genske ekspresije. Štaviše, pokazalo se da epigenetičke promene (epimutacije) u kombinaciji sa genetičkim mutacijama povećavaju proliferativni kapacitet ćelijama raka isključujući tumor supresorske gene i/ili aktivirajući protoonkogene. Dodatno, promene ćelijskog metabolizma izazvane izloženošću faktorima spoljašnje sredine mogu dodatno doprineti epigenetičkoj promeni proizvodnjom metabolita koji inhibiraju ključne enzime uključene u epigenetičku regulaciju. Iako ne postoje potpuno efikasne strategije za prevenciju ili lečenje zbog komplikovane etiologije različitih nezaraznih bolesti, sama plastična priroda epigenoma predstavlja potencijalnu metu u dizajnu budućih terapijskih strategija (editovanje epigenoma) i novih epigenetičkih lekova (epilekova).

KLJUČNE REČI: Epigenetika, genska ekspresija, toksikologija



THE VARIOUS ASPECTS OF EPIGENETICS IN TOXICOLOGY RESEARCH

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Epigenetics describes mechanisms that regulate stable and tissue-specific patterns of gene expression without changing the primary sequence of DNA, including the following processes: DNA methylation, various forms of post-translational modifications of histones (histone code) and regulatory actions of noncoding RNA molecules.

The reversible covalent modifications on DNA and histone proteins are defined as epigenetic marks that are responsible for chromatin packaging in the nucleus making the parts of the genome regions accessible for transcription machinery while silencing other regions. Epigenetic toxicity is a phenomenon in which a chemical substance affects epigenomes and exerts undesirable effects on living organisms, which may explain the long-term effects of chemical substances and the predisposition to diseases due to environmental factors. The epigenetic marks are stable in differentiated cells and become dysregulated following exposure to different stimuli such as pharmaceuticals, dietary components, environmental chemicals, nanomaterials or metals.

This can alter tissue-specific epigenetic profile, thereby leading to disturbed gene expression. Furthermore, it has been shown that epigenetic changes (epimutations) can collaborate with genetic mutations to provide cancer cells with growth advantage by shutting down tumor suppressor genes or switching on proto-oncogenes. In addition, exposure-induced alterations of cellular metabolism can further contribute to epigenetic perturbation by producing metabolites that inhibit crucial epigenetic regulators. Although there are no completely effective preventive or curing strategies due to the multifactorial etiology of different non-communicable diseases, the very plastic nature of the epigenome represents a potential target in the design of future therapeutic strategies (epigenome editing) and novel epigenetic drugs (epidrugs).

KEYWORDS: Epigenetics, gene expression, toxicology



URGENTNA OPŠTA MEDICINA / EMERGENCY/GENERAL MEDICINE

EKG PROMENE U TROVANJIMA LEKOVIMA I DRUGIM KSENOBIOTICIMA

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Elektrokardiogram (EKG) je jednostavno i lako dostupno dijagnostičko sredstvo koje se rutinski koristi u svakodnevnoj lekarskoj praksi. Naročito je važna njegova pravilna interpretacija u urgentnoj medicini, između ostalog, u slučaju akutnih intoksikacija. Ksenobiotik je hemijska supstanca koja je prisutna u organizmu, ali se u njemu ne proizvodi niti se očekuje da bude prisutna. To su takođe i supstance koje su prisutne u organizmu u mnogo većoj koncentraciji od uobičajene. I lekovi su ksenobiotici za ljude, jer ih ljudsko telo ne proizvodi, niti su deo njihove normalne ishrane.

Sa aspekta kliničke toksikologije važno je prepoznati klasične EKG promene koje sugerišu blokadu jonskih kanala na ćelijskoj membrani, promene adrenergičkog tonusa ili poremećaje metaboličke aktivnosti miokarda. Kardiotoksičnost lekova i ksenobiotika se može manifestovati dejstvom na miokardne ćelije i/ili autonomni nervni sistem. Njihov uticaj može biti direktno na srčanu frekvencu, krvni pritisak i srčanu kontraktilnost ili indirektno usled metaboličkih poremećaja: hipoksija, acidobazni ili elektrolitni poremećaji. Neke od EKG promena koje se vide u intoksikacijama su: poremećaji srčanog ritma i sprovođenja, širenje QRS kompleksa, produženje QT intervala, ishemijske promene ST segmenta i T talasa. Oni se viđaju i kod intoksiciranih pacijenata bez prethodnih kardioloških oboljenja. Ovo predavanje će prikazati strukturirani pristup EKG interpretaciji kod akutno intoksiciranih pacijenata sa fokusom na kliničke implikacije i preporuke za inicijalnim zbrinjavanjem.

KLJUČNE REČI: ksenobiotici, trovanja lekovima, kardiotoksičnost, EKG promene, zbrinjavanje



ECG CHANGES IN POISONING WITH DRUGS AND OTHER XENOBIOTICS

EMERGENCY/GENERAL
MEDICINE

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Electrocardiogram (ECG) is a simple and available diagnostic tool that is routinely used in everyday medical practice. Its accurate interpretation is especially important in emergency medicine, among other things, in the case of acute intoxications. A xenobiotic is a chemical substance that is present in an organism but is not produced or expected to be present inside. These are also substances that are present in the body in a much higher concentration than usual. And drugs are also xenobiotics for humans because they are not produced by the human body, nor are they part of their normal diet.

In clinical toxicology, it is important to recognize classic ECG changes that suggest ion channels blockage on the cell membrane, adrenergic tone changes or myocardial metabolic disorders. Cardiotoxicity of drugs and xenobiotics can be manifested by effects on myocardial cells and/or the autonomic nervous system. Their influence can be directly on heart rate, blood pressure and cardiac contractility or indirectly due to metabolic disorders: hypoxia, acid-base or electrolyte disorders. Some of the ECG changes seen in intoxications are: heart rhythm and conduction disorders, QRS complex widening, QT interval prolongation, ST segment and T waves ischemic changes. They are seen in intoxicated patients without previous cardiac diseases, as well. This lecture will present a structured approach to ECG interpretation in acutely intoxicated patients with a focus on clinical implications and recommendations for initial care.

KEYWORDS: xenobiotics, drug poisonings, cardiotoxicity, ECG changes, initial care



OPŠTI PRINCIPI URGENTNOG ZBRINJAVANJA TROVANJA U AMBULANTI LEKARA OPŠTE MEDICINE - IZAZOVI I PREPREKE

EMERGENCY/GENERAL
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Blagovremeno prepoznavanje akutnog i hroničnog trovanja kao i farmakodinamskih mehanizama i patofiziologije trovanja od izuzetnog je značaja u ambulanti opšte medicine. Na primarnom nivou zdravstvene zaštite u poslednjih 5 godina registrovan je porast stope trovanja lekovima, preparatima i biološkim supstancama sa 0.14 na 0.21, dok se trovanje supstancama nemedicinskog porekla nalazi u značajnom padu sa 0.30 na 0.19 na 1000 stanovnika. Iskustvo lekara zaposlenih u opštoj službi vezano za slučajeve akutnog i hroničnog trovanja smo procenili na osnovu upitnika koji je elektronskom poštom prosleđen lekarima.

Od anketiranih 70 se odazvao 51. Pokazalo se da se 74,5 % lekara susretalo u praksi sa ovim stanjem. Lekari su se češće sretali 47,0%, sa zadesnim slučajevima trovanja, dok se 23,6% više puta susrelo sa namernim trovanjem. Veći procenat ovih slučajeva 47,0% je zbrinjavano na licu mesta. O postojanju Nacionalnog centra za kontrolu trovanja informisano je 56,9%, a mere detoksikacije potpuno su poznate za 60,8% lekara. Dodatna edukacija iz oblasti trovanja je poželjna po mišljenju 76,5% lekara. Unošenje otrova različitim putem u organizam može biti uzrok smrtnosti pacijenata ukoliko se pravovremeno ne prepozna pri prvom kontaktu sa lekarom u ambulanti opšte medicine. Kontinuirana edukacija predstavlja meru neophodnu za unapređenje kvaliteta pružanja zdravstvene pomoći u slučajevima akutnih i hroničnih trovanja.

KLJUČNE REČI: trovanje, opšta medicina, edukacija



GENERAL PRINCIPLES OF EMERGENCY TREATMENT OF POISONING IN A GENERAL MEDICINE DOCTOR'S PRACTICE - CHALLENGES AND IMPEDIMENTS

EMERGENCY/GENERAL
MEDICINE

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Timely recognition of acute and chronic poisoning, as well as pharmacodynamic mechanisms and pathophysiology of poisoning, is exceptionally important in the outpatient clinic of general medicine. At the primary level of health care, in the last 5 years, an increase in the rate of poisoning by drugs, preparations and biological substances has been registered from 0.14 to 0.21, while poisoning by substances of non-medical origin has decreased significantly from 0.30 to 0.19 per 1000 inhabitants. We evaluated the general practitioner's experience on acute and chronic poisoning, based on a questionnaire that was sent to doctors by e-mail. Of 70 surveyed, 51 responded.

About 74.5% of doctors encountered this condition in their practice. More often they had experience with accidental poisoning (47.0%), while 23.6% of them had patients with intentional poisoning more than once. A higher percentage of these cases, 47.0%, were treated on the spot. 56.9% of doctors were informed about the existence of the National Center for Poison Control, and 60.8% of them were fully aware on detoxification measures. Additional education in the field of poisoning is desirable in the opinion of 76.5% of doctors. Poisons that enter the body in different ways can be the cause of serious illness and death, if they are not recognized in time, during the first contact with a doctor in a general medicine clinic. Continuous education is a necessary measure for improving the quality of providing health care in cases of acute and chronic poisoning.

KEYWORDS: poisoning, general medicine, education



OKRUGLI STO / ROUND TABLE - DISSEMINATION OF SCIENCE IN TOXICOLOGY

ASSESSMENT OF THE INTERNATIONALISATION AT HOME OF THE HIGHER EDUCATION IN TOXICOLOGY

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Internationalisation at home is being assessed in terms of its potential usefulness for the achievement of a knowledge-based society and reducing regional inequalities. Similarly, problem-based learning is considered to be an effective tool to promote active learning. Teaching innovation using tools evaluated in the field of higher education science improves the teaching of toxicology content. The objective was to assess the impact of the teaching project "Problem-based learning in Toxicology in a virtual framework of internationalisation of higher education" for internationalisation at home Program (PRODIC-UMH).

Students enrolled in Toxicology courses at the Faculty of Experimental Sciences at Miguel Hernandez University of Elche (Spain), the Faculty of Food Technology and Biotechnology at University of Zagreb (Croatia), the Faculty of Pharmacy at University of Belgrade (Serbia) and the Faculty of Pharmacy at Gazi University (Ankara, Turkey) were included in the study. The retention in the program, the degree of completion of the problems, scores and success rate were recorded. The impact of the internationalisation at home was assessed in terms of retention in the course and success rate.



OKRUGLI STO / ROUND TABLE - DISSEMINATION OF SCIENCE IN TOXICOLOGY

Ninety-five students participated in 4 theory sessions and 4 problems of Toxicology over 9 weeks. Preliminary, evaluation of the acquired data revealed an achievement of 85%. Retention in the international program was considered to be acceptable, success rates were maintained at a reasonable level and student opinions about the internationalisation at home were considered to be positive. The evaluation of teaching practice based on educational science leads to better teaching design and, consequently, to better academic results. The formative assessment increases student engagement and consequently student achievement. Internationalisation at home promotes the enrichment of the contents of the toxicology courses and an international experience among the participants.

KEYWORDS: higher education; internationalisation; toxicology

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RAISING AWARENESS AND UNDERSTANDING OF TOXICOLOGY IN DAILY LIFE THROUGH EDUCATIONAL TOOLS

ROUND TABLE -
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There are universities, institutions, schools, non-profit organizations and governmental bodies with a mission to disseminate knowledge, in certain areas, through educational tools or through multimedia. Such tools are helpful in decision-making and increasing awareness on the safety of environmental and daily life chemical, biological and physical agents such as the safety of chemicals, food, pharmaceuticals, and cosmetics. Herewith public understanding and popularizing the toxicology in daily life through educational tools for different segments of the general public, health professionals, students, teachers etc. will be highlighted. In this manner, toxicology concepts-based multimedia activities and educational tools are overlooked especially focused on children. In the preparation and subsequent dissemination of the multimedia activities and educational tools; the target population-based social, psychological, intellectual, age-related properties are the main determinants to define the learning outcomes and to choose the appropriate language.

Those tools are expected to be scientifically reviewable, reliable and easily accessible. Multi-disciplinary approaches are the most comprehensive method to obtain positive and significant results in enhancing awareness. While toxicologists are the principal scientists orchestrating the content from visual professionals to educational scientists, engineers and specialists are the architects of the tools. Awareness on the basic safety concepts of environmental exposures is crucial to protect the health of the public, safe use of chemicals and raise conscious future generations. These tools have organic bonds with risk perception, risk communication and risk management.

KEYWORDS: Awareness, toxicology, risk perception, educational multi-media tools



VETERINARSKA EKSPERIMENTALNA TOKSIKOLOGIJA / VETERINARY EXPERIMENTAL TOXICOLOGY

ZA I PROTIV PRIMENE MARIHUANE (KANABISA) U VETERINARSKOJ MEDICINI

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Poznato je da se marihuana (i proizvodi od nje), danas sve više koristi, kako za medicinske, tako i rekreativne svrhe kod ljudi. U skladu sa ovim jeste i činjenica da je poslednjih godina značajno poraslo i naučno interesovanje za primenu marihuane. Najbolji dokaz za to je i broj citata u PubMedu, koji je u periodu od 2000 do 2002 iznosio svega 40, da bi u periodu od 2014. do 2016. porastao na 458, a danas je još veći. U Severnoj Americi i Evropi su mnoge zemlje donele zakone koji dozvoljavaju medicinsku upotrebu određenih proizvoda proizvedenih od kanabisa kod ljudi, a neke zemlje su donele zakone koji dozvoljavaju i rekreativnu upotrebu.

Međutim, sa porastom ove primene, rastao je i broj slučajeva toksikoza kod kućnih ljubimaca, nastalih usled trovanja marihuanom. Ovaj problem je postao još veći kada su vlasnici životinja u želji da pomognu svojim ljubimcima, kod raznih stanja i poremećaja pokušali iste da izleče, upravo primenom proizvoda od marihuane. Već dugo vremena vlasnici životinja širom sveta postavljaju pitanja:

„Da li su marihuana i njeni proizvodi legalni, sigurni i efikasni za lečenje raznih poremećaja kod životinja?“.



VETERINARSKA EKSPERIMENTALNA TOKSIKOLOGIJA / VETERINARY EXPERIMENTAL TOXICOLOGY

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Imajući ovo u vidu, cilj ovog rada je da se naše kolege (kroz prikaz različitih vrsta marihuane i njenih proizvoda, kao i primera primene istih kod životinja kroz istoriju, te postojeće zakonske regulative i propisa), bar malo upoznaju sa primenom istih (kao lekova i/ili suplemenata) u kliničkoj veterinarskoj praksi, ali i sa mogućim trovanjima i (usled toga) potrebnim upozorenjima vezanim za njihovu primenu.

**KLJUČNE REČI:** marihuana (kanabis), primena, veterinarska medicina, terapija, trovanja





## FOR AND AGAINST THE USE OF MARIJUANA (CANNABIS) IN VETERINARY MEDICINE

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It is known that marijuana (and its products) is used more and more today, both for medical and recreational purposes by people. In line with this is the fact that scientific interest in the use of marijuana has grown significantly in recent years. The best evidence for this is the number of citations in PubMed, which in the period from 2000 to 2002 was only 40, and in the period from 2014 to 2016, it increased to 458, and today it is even higher. In North America and Europe, many countries have passed laws allowing the medical use of certain cannabis products in humans, and some countries have passed laws allowing recreational use as well. However, with the increase in this application, the number of cases of toxicosis in pets, caused by marijuana poisoning, also increased.

This problem became even greater when animal owners, in their desire to help their pets, tried to cure them of various conditions and disorders, precisely by using marijuana products. For a long time, animal owners around the world have asked the question: "Are marijuana and its products legal, safe and effective for treating various disorders in animals?". Bearing this in mind, the aim of this paper is that our colleagues (through the presentation of different types of marijuana and its products, as well as examples of their use in animals throughout history, and the existing laws and regulations), become a bit familiar with the application of the same (as medicines and/or supplements) in clinical veterinary practice, but also with possible poisonings and (as a result) necessary warnings related to their use.

**KEY WORDS:** marijuana (cannabis), application, veterinary medicine, therapy, poisoning



## PROGRESS IN ECOTOXICITY TESTING WITH DUCKWEEDS

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The influx of municipal, agricultural and industrial waste into natural waters has toxic effects on all forms of aquatic life. Duckweeds, or Lemnaceae, have long been used as test organisms to assess water contaminant toxicity to aquatic higher plant life. Officially recognized test procedures (e.g., OECD 221) determine toxicity in terms of inhibition of the growth of the small leaf-like floating fronds of *Lemna minor* or *Lemna gibba* in the presence of water contaminants. Test endpoints of frond number, area or weight or chlorophyll content are determined for  $\geq 100$  ml culture batches after 7 days.

Recently, the protocols of the standardized toxicity tests with duckweeds have been modified and diversified to improve the practicability and the informative value of the tests.

The batch size of the test cultures and the test duration have been considerably reduced, and alternative end-points such as root regrowth, chlorophyll fluorescence and FTIR spectroscopy have been introduced as diagnostic criteria for evaluating toxicity. The use of duckweed species other than *L. minor* and *L. gibba* can be of advantage in specific experimental contexts. To understand the actual toxic effects of water contaminants on duckweeds, toxicity tests have long been accompanied by the determination of complementary biomarkers: physiological and metabolic effects associated with and possibly causally correlated with the toxicity. The continuous accumulation of biomarker information - now including gene expression data - corresponding to the toxicity of ever more types of water-contaminating substances may enable the identification of unspecified water-borne substances responsible for toxicity to duckweeds.

**KEYWORDS:** duckweeds, toxicity tests, biomarkers



## NEUROTOKSIČNO DEJSTVO VENOMA *VIPERA AMMODYTES* NA MODELU IZOLOVANE DIJAFRAGME PACOVA

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Poskok (*Vipera ammodytes*) predstavlja najzastupljeniju zmiju otrovnicu u Srbiji i na Balkanu, kako po brojnosti tako i po arealu rasprostranjenosti. U poređenju sa venomima ostalih otrovnica iz familije Viperidae na Balkanu, toksičnost venoma poskoka je najveća. Neurotoksičnost sirovog venoma poskoka smo ispitivali u in vitro uslovima na osnovu kontrakcija preparata dijafragme izazvanih poljnom električnom stimulacijom i aktivnosti enzima: acetilholinesteraze (AChE) i ukupnih ATP-aza u dijafragmi.

Formirano je 5 grupa pacova (n=5): kontrola (dijafragma bez venoma), dijafragma sa venomom, dijafragma sa smešom venom/antivenom („Viekvin“, Torklak, Srbija) u odnosima 1:2, 1:10 i 1:20 (m/m). Kontrakcije dijafragme su opale na 50% kontrolnih kontrakcija: pod uticajem venoma za 62,00±2,31 minuta, a sa smešom venom/antivenom 1:2 za 78,50±7,51 minuta, 1:10 za 150,00±17,32 minuta, 1:20 za 307,50±2,89 minuta. Statistička razlika (p<0,05) pod dejstvom venoma. Venom inhibira aktivnost ukupnih ATP-aza dijafragme za 51,81% u odnosu na kontrolu. Venom poskoka ne deluje na AChE i nikotinske receptore u neuro-mišićnoj sinapsi dijafragme, već narušava energetske metabolizam mitohondrija.

**KLJUČNE REČI:** *Vipera ammodytes*, neurotoksičnost, izolovana dijafragma, pacov



## NEUROTOXIC EFFECT OF VIPERA AMMODYTES VENOM ON THE RAT ISOLATED DIAPHRAGM MODEL

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Vipera ammodytes is the most common venomous snake in Serbia and the Balkans, both in terms of numbers and area of distribution. Compared to the venoms of other poisonous snakes from the family Viperidae in the Balkans, the toxicity of the V. ammodytes venom is the highest. We investigated the neurotoxicity of crude venom in vitro using contractions of the diaphragm preparation induced by electric field stimulation and enzyme activity: acetylcholinesterase (AChE) and total ATPases in the diaphragm. Five groups of rats were formed (n=5): control (diaphragm without venom), diaphragm with venom, diaphragm with mixture of venom/antivenom (“Viekvin”, Torlak, Serbia) in the ratio 1:2, 1:10 and 1:20 (m/m).

Diaphragmatic contractions decreased to 50% of control contractions: under the influence of venom for 62.00±2.31 minutes, and with venom/antivenom mixture 1:2 for 78.50±7.51 minutes, 1:10 for 150.00±17.32 minutes, 1:20 for 307.50±2.89 minutes. A statistical difference (p 0.05) under the influence of the venom. Venom inhibits the total ATPases activity of the diaphragm by 51.81% compared with control. The venom of V. ammodytes does not act on AChE and nicotinic receptors in the neuromuscular synapse of the diaphragm, but interferes with mitochondrial energy metabolism.

**KEYWORDS:** Vipera ammodytes, neurotoxicity, isolated diaphragm, rats



## BIOMARKERI OKSIDATIVNOG STRESA U JETRI PACOVA SUBAKUTNO TRETIRANIH DIAZINOM

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Diazinon (DZN) je organofosfatni pesticid koji se široko koristio u poljoprivredi. Svoje toksične efekte ostvaruje inhibicijom acetilholinesteraze (AChE) i indukovanjem oksidativnog stresa. U ovoj studiji, Wistar pacovi su oralno tretirani diazinonom (55 mg/kg) tokom četiri različita perioda: 7, 14, 21 i 28 dana. Posle svakog vremenskog perioda uzorkovano je tkivo jetre za ispitivanje enzimskih i neenzimskih biomarkera oksidativnog stresa. Analizirane su aktivnosti enzima AChE, superoksid-dizmutaze 1 (SOD1), katalaze (CAT), mijeloperoksidaze (MPO) i paraoksonaze 1 (PON1). Step en oštećenja ćelijske membrane i proteina praćen je određivanjem koncentracije malondialdehida (MDA) i karbonilnih grupa (CO).

Najveća inhibicija AChE zabeležena je između 7. ( $p < 0,01$ ) i 14. dana ( $p < 0,05$ ), a najveća inhibicija SOD1 između 14. ( $p < 0,01$ ) i 21. dana ( $p < 0,05$ ) tretmana u odnosu na kontrolu. Aktivnost CAT je bila značajno povećana ( $p < 0,001$ ) u sva četiri vremenska perioda. Aktivnost MPO raste 14., 21. ( $p < 0,01$ ) i 28. dana ( $p < 0,05$ ), dok su markeri oštećenja proteina povećani 21. i 28. dana tretmana ( $p < 0,001$ ). U sva četiri posmatrana vremenska perioda, postoji veoma jaka korelacija između aktivnosti AChE i MDA, kao i između AChE i PON1. Takođe, veoma jaka korelacija je zabeležena 14. dana između aktivnosti AChE i MPO, a 7. dana između AChE i CAT aktivnosti. Prolongirana primena DZN dovodi do izraženog oksidativnog stresa u jetri. Biomarkeri oksidativnog stresa se nalaze u jakoj korelaciji sa holinergičkim mehanizmom delovanja DZN, odnosno inhibicijom AChE i parametrima inflamacije.

**KLJUČNE REČI:** diazinon, oksidativni stres, jetra, pacov



## BIOMARKERS OF OXIDATIVE STRESS IN THE LIVER OF RATS TREATED SUBACUTELY WITH DIAZINON

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Diazinon (DZN) is an organophosphate pesticide widely used in agriculture. It achieves its toxic effects by inhibiting acetylcholinesterase (AChE) and inducing oxidative stress. In this study, Wistar rats were treated orally with diazinon (55 mg/kg) for four different periods: 7, 14, 21, and 28 days. After each period, liver tissue was examined for enzymatic and non-enzymatic biomarkers of oxidative stress. The activities of AChE, superoxide dismutase 1 (SOD1), catalase (CAT), myeloperoxidase (MPO), and paraoxonase 1 (PON1) were analyzed. The degree of cell membrane and protein damage was monitored by determining the concentration of malondialdehyde (MDA) and carbonyl groups (CO).

The greatest inhibition of AChE was observed between days 7 ( $p < 0.01$ ) and 14 ( $p < 0.05$ ), and the greatest inhibition of SOD1 between days 14 ( $p < 0.01$ ) and 21 ( $p < 0.05$ ) of treatment compared with control. CAT activity was significantly increased ( $p < 0.001$ ) in all four time periods. MPO activity increased on days 14, 21 ( $p < 0.01$ ) and 28 ( $p < 0.05$ ), whereas protein damage markers increased on days 21 and 28 of treatment ( $p < 0.001$ ). In all four observed time periods, there is a very strong correlation between AChE and MDA activity and between AChE and PON1. In addition, a very strong correlation was observed between AChE and MPO activity on day 14 and between AChE and CAT activity on day 7. Prolonged administration of DZN leads to pronounced oxidative stress in the liver. Oxidative stress biomarkers correlate strongly with the cholinergic mechanism of action of DZN, i.e., AChE inhibition and inflammatory parameters.

**KEYWORDS:** diazinon, oxidative stress, liver, rat



## VITAMIN B1 KAO POTENCIJALNI „ANTIDOT“ U LEČENJU JAPANSKIH PREPELICA TROVANIH HLORPIRIFOSOM

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Hlorpirifos (CPF) je hlorovani organofosfatni pesticid, odnosno insekticid, koji se već dugo sa uspehom koristio u poljoprivredi. Ovaj insekticid svoje delovanje kod sisara ostvaruje na najmanje tri načina: inhibicijom aktivnosti enzima acetilholinesteraze (AChE), izazivanjem oksidativnog stresa i izazivanjem funkcionalnog poremećaja endokrinih žlezda. Za vitamin B1 (tiamin) je poznato da spada u grupu hidrosolubilnih vitamina, kao i da poseduje antioksidativni efekat. Osim toga, tiamin preko acetil koenzima A (acetil-CoA) učestvuje u sintezi acetilholinesteraze pa njegov nedostatak potencira delovanje organofostata.

Cilj naših ispitivanja bio je da se na japanskim prepelicama, trovanih hlorpirifosom, ispita da li i u kojoj meri vitamin B1 utiče na parametre oksidativnog stresa, inflamacije (interleukina 1(IL-1) i interleukina 6 (IL-6)), apoptoze (inducibilne azot-oksidaže (iNOS) i ciklooksigenaze-2 (COX-2)), kao i aktivnost enzima acetil i butirilholinesteraze (AChE i BChE). Rezultati ovih ispitivanja pokazali su da tiamin efikasno dovodi do oporavka aktivnosti AChE i BuChE u plazmi, jetri i mozgu, koja je značajno bila inhibisana dejstvom CPF-a. Osim toga, tiamin pokazuje i antioksidativna svojstva, pošto povećava ukupnu koncentraciju redukovanog glutationa u mozgu, zatim smanjuje stepen lipidne peroksidacije, redukuje produkciju IL-1 i IL-6, snižava ekspresiju iNOS i COX-2. Dobijeni rezultati su pokazali da vitamin B1 ima povoljno dejstvo kod prepelica, trovanih hlorpirifosom.

**KLJUČNE REČI:** vitamin B1, japanske prepelice, organofosfatni pesticidi, hlorpirifos, parametri oksidativnog stresa



## VITAMIN B1 AS A POTENTIAL "ANTIDOTE" IN THE TREATMENT OF JAPANESE QUAIL POISONED WITH CHLORPYRIFOS

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Chlorpyrifos (CPF) is a chlorinated organophosphate pesticide, i.e. insecticide, which was successfully used in agriculture for a long time. This insecticide exerts its action in mammals in at least three ways: by inhibiting the activity of the enzyme acetylcholinesterase (AChE), by causing oxidative stress, and by causing functional disruption of the endocrine glands. Vitamin B1 (thiamine) is known to belong to the group of water-soluble vitamins, and has an antioxidant effect. In addition, thiamine through acetyl coenzyme A (acetyl-CoA) participates in the synthesis of acetylcholinesterase, so its deficiency potentiates the action of organophosphates.

The aim of our work was to investigate whether and to what extent vitamin B1 affects the parameters of oxidative stress, inflammation (interleukin 1 (IL-1) and interleukin 6 (IL-6)), apoptosis (inducible nitric oxide (iNOS) and cyclooxygenase-2 (COX-2)), as well as the activity of the enzymes acetyl and butyrylcholinesterase (AChE and BChE) and cyclooxygenase (COX), in Japanese quail poisoned with chlorpyrifos. The results of these tests showed that thiamine effectively led to the recovery of AChE and BuChE activity in plasma, liver and brain, which was significantly inhibited by the action of CPF. In addition, thiamine also shows antioxidant properties as it increases the total concentration of reduced glutathione in the brain, then decreases the degree of lipid peroxidation, reduces the production of IL-1 and IL-6, and lowers the expression of iNOS and COX-2. The obtained results showed that vitamin B1 has a beneficial effect on quail poisoned by chlorpyrifos.

**KEYWORDS:** vitamin B1, Japanese quail, organophosphate pesticides, chlorpyrifos, oxidative stress parameters





## DEBATA - ANALITIČKA TOKSIKOLOGIJA MOĆNO ORUŽJE I VEĆITI IZAZOV / DEBATE - ANALYTICAL TOXICOLOGY IS A POWERFUL WEAPON AND A GREAT CHALLENGE

### ZNAČAJ I PRIMJENA HROMATOGRAFIJE U TOKSIKOLOŠKOJ ANALIZI

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Testiranje bioloških tkiva i/ili tečnosti na droge, isparljive supstance i druge hemikalije je izazovan proces koji zahtijeva visoko obučene analitičare i sofisticiranu opremu. Glavni problem sa kojim se analitičari suočavaju su niske koncentracije u kojima su toksična jedinjenja i metaboliti prisutni u ovakvim uzorcima. Međutim, danas je moguće detektovati niske koncentracije zahvaljujući ogromnom razvoju hromatografskih tehnika koje su sposobne za kvalitativnu i kvantitativnu analizu. Hromatografija je analitička procedura koja se koristi za odvajanje različitih organskih jedinjenja/hemikalija i to primjenom tačne hromatografije visokih performansi (HPLC) ili gasne hromatografije (GC), obje spojene sa masenom spektrometrijom (MS). U toksikologiji ovo je najpouzdanija tehnika koja se koristi za dokazivanje strukture nepoznatih supstanci. Tokom posljednjih 20 godina primijećeno je dramatično povećanje sposobnosti MS-a, a njegovo povezivanje sa tehnikama tačne hromatografije bilo je izuzetno važan događaj.

Zaista, nove i moćne tehnologije omogućile su naučnicima u svim analitičkim oblastima da detektuju ono što nisu mogli samo nekoliko godina ranije. Razvoj komercijalne MS tehnologije po pristupačnoj cijeni pretvorio je GC-MS i HPLC-MS (obično poznat kao LC-MS) u sve popularnije alate u savremenim toksikološkim laboratorijama. U stvari, uspješno povezivanje tačne hromatografije sa masenom spektrometrijom (LC-MS) donijelo je novo svjetlo u bioanalitičke i forenzičke nauke jer omogućava otkrivanje ne samo lijekova i metabolita u koncentracijama koje je teško analizirati korišćenjem najčešće prihvaćenih GC-MS ili HPLC tehnikama već i velikog broja pesticida i drugih hemikalija u hrani i životnoj sredini.

**KLJUČNE RIJEČI:** tačna hromatografija, masena spektrometrija, toksikologija, gasna hromatografija



## SIGNIFICANCE AND APPLICATION OF CHROMATOGRAPHY IN TOXICOLOGICAL ANALYSIS

DEBATE -  
ANALYTICAL TOXICOLOGY  
IS A POWERFUL WEAPON  
AND A GREAT CHALLENGE

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Testing biological tissues and/or fluids for drugs, volatile substances and other chemicals is a challenging process that requires highly trained analysts and sophisticated equipment. The main problem that analysts face is the low concentrations at which toxic compounds and metabolites are present in such samples. However, today it is possible to detect these low concentrations thanks to the enormous development of chromatographic techniques capable of qualitative and quantitative determination. Chromatography is an analytical procedure used to separate various organic compounds/chemicals using High Performance Liquid Chromatography (HPLC) or Gas Chromatography (GC), both coupled with Mass Spectrometry (MS). In toxicology, this is the most reliable technique used to prove the structure of unknown substances.

The last 20 years have seen a dramatic increase in the capabilities of MS, and its coupling with liquid chromatography techniques has been an extremely important development. Indeed, new and powerful technologies have enabled scientists in all analytical fields to detect what they could not just a few years before. The development of affordable commercial MS technology has made GC-MS and HPLC-MS (commonly known as LC-MS) increasingly popular tools in modern toxicology laboratories. In fact, the successful coupling of liquid chromatography with mass spectrometry (LC-MS) has brought new light to the bioanalytical and forensic sciences because it allows the detection not only of drugs and metabolites at concentrations that are difficult to analyze using the most commonly accepted GC-MS or HPLC techniques, but also of a large number of pesticides and other chemicals in food and the environment.

**KEYWORDS:** liquid chromatography, mass spectrometry, toxicology, gas chromatography



## TEČNA HROMATOGRAFIJA - MOĆNO ORUŽJE U SKRININGU LEKOVA I PSIHOAKTIVNIH SUPSTANCI

DEBATE -  
ANALYTICAL TOXICOLOGY  
IS A POWERFUL WEAPON  
AND A GREAT CHALLENGE

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Toksikološki skrining predstavlja set standardnih metoda za detekciju toksičnih supstanci, koji može potvrditi ili negirati trovanje. Tečna hromatografija u sprezi sa UV skenirajućim (PDA) i/ili maseno-spektrometrijskim (MS) detektorom može se koristiti u identifikaciji lekova, psihoaktivnih supstanci i njihovih metabolita. Identifikacija supstanci se vrši na osnovu poređenja dobijenih UV ili MS spektara sa kompjuterskom bibliotekom. Maseno spektrometrijska identifikacija je veoma pouzdana. U zavisnosti od tipa masenog detektora, u sprezi sa tečnim hromatografom može se izvršiti kompletna identifikacija nepoznatih molekula koji se ne nalaze u biblioteci pomoću egzaktno mase u koncentraciji reda veličine pg/mL. Cilj ovog rada je da predstavi prednosti i značaj primene tečne hromatografije u toksikološkom skriningu lekova i psihoaktivnih supstanci. Tečna hromatografija sa PDA i/ili MS detektorima primenjene su u identifikaciji nepoznatog uzročnika trovanja. Biološki uzorci pripremani su čvrsto-faznom ekstrakcijom, a čvrsti rastvaranjem u metanolu. U identifikaciji su korišćeni softveri za toksikološki skrining sa bibliotekama UV i MS spektara.

Toksikološkim skriningom uzoraka krvi i urina primenom HPLC-PDA i LC-MS/MS metoda izvršena je identifikacija nepoznatih uzročnika trovanja kod pacijenata u komi ili sa nepoznatom etiologijom trovanja, što je olakšalo njihovo lečenje. Takođe analizom čvrstih uzoraka identifikovano je prisustvo lekova i psihoaktivnih supstanci, potencijalnih uzročnika trovanja. Akutna trovanja su često životno ugrožavajuća i zahtevaju brzu dijagnostiku. Primena tečne hromatografije sa PDA i/ili MS detektorom predstavlja metodu izbora u identifikaciji nepoznatog uzročnika trovanja jer je brza, pouzdana i osetljiva metoda koja ne zahteva posebnu pripremu uzoraka.

**KLJUČNE REČI:** tečna hromatografija, toksikološki skrining, akutno trovanje



## LIQUID CHROMATOGRAPHY - A POWERFUL WEAPON IN THE SCREENING OF DRUGS AND PSYCHOACTIVE SUBSTANCES

DEBATE -  
ANALYTICAL TOXICOLOGY  
IS A POWERFUL WEAPON  
AND A GREAT CHALLENGE

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Toxicological screening is a set of standard methods for the detection of toxic substances. It can confirm or deny poisoning. Liquid chromatography coupled with a UV scanning (PDA) and/or mass spectrometric (MS) detector can be used in the identification of drugs, psychoactive substances and their metabolites. Identification of substances is performed on the basis of comparison of obtained UV or MS spectra with a computer library. Mass spectrometric identification is very reliable. Depending on the type of mass detector, coupled with a liquid chromatograph, a complete identification of unknown molecules that are not in the library can be performed using an exact mass in a concentration of the order of pg/mL.

The aim of this paper is to present the advantages and importance of the application of liquid chromatography in the toxicological screening of drugs and psychoactive substances.

Liquid chromatography with PDA and/or MS detectors were used to identify the unknown cause of poisoning. Biological samples were prepared by solid-phase extraction, and powders by dissolving in methanol. Software for toxicological screening with libraries of UV and MS spectra was used in the identification. Toxicological screening of blood and urine samples using HPLC-PDA and LC-MS/MS methods identified unknown causes of poisoning in patients in coma or with unknown etiology of poisoning, which facilitated their treatment. The analysis of powders also identified the presence of drugs with a potentially harmful effect on health. Acute poisonings are often life-threatening and require rapid diagnosis. The application of liquid chromatography with a PDA and/or MS detector is the method of choice in identification of the unknown cause of poisoning because it is a fast, reliable and sensitive method that does not require special sample preparation.

**KEY WORDS:** Liquid chromatography, toxicological screening, acute poisoning



## GASNA HROMATOGRAFIJA - PRIMENA U SUDSKO-TOKSIKOLOŠKOJ PRAKSI

DEBATE -  
ANALYTICAL TOXICOLOGY  
IS A POWERFUL WEAPON  
AND A GREAT CHALLENGE

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Gasna hromatografija (GC), pored tečne hromatografije danas predstavlja jednu od najzastupljenijih analitičkih tehnika za analizu postmortem uzoraka u sudskoj toksikologiji. U kombinaciji sa odgovarajućim detektorima, GC omogućuje identifikaciju i kvantifikaciju širokog spektra toksičnih agenasa od gasova, organskih rastvarača, pesticida do najrazličitijih prirodnih i sintetičkih droga i lekova. Osnovni preduslovi za izbor gasne hromatografije kao analitičke tehnike u toksikološkim analizama su laka isparljivost, termostabilnost i male molekulske mase analiziranih jedinjenja. Proširivanje obima analize GC na analite veće mase i manje isparljivosti, postiže se uvođenjem hidrolize i/ili derivatizacije u postupke pripreme uzoraka. Priprema i analiza postmortem uzoraka kao što su krv, urin, kosa i tkiva organa predstavljaju veliki izazov za analitičare, naročito ukoliko su uzorci već u poodmakloj fazi raspadanja. Dobro pripremljeni uzorci i adekvatan izbor detektora u GC, predstavljaju ključnu kombinaciju za uspešno određivanje veoma niskih koncentracija otrova u postmortem uzorcima.

Određivanje komponenata smeše u kompleksnom lešnom materijalu u GC može se vršiti pomoću detektora različite osetljivosti i specifičnosti. Jedan od najosetljivijih detektora je maseni spektrometar koji snima masene spektre veoma značajne za sudsko-medicinska veštačenja i u nekim slučajevima presudne za utvrđivanje uzroka smrti. Uz svoje prednosti i mane, GC u kombinaciji sa plamenojonizujućim detektorom danas predstavlja „zlatni standard“ za analizu lakoisparljivih otrova, prvenstveno alkohola, dok sa masenim detektorom omogućuje snimanje masenih spektara tzv. „otisak prsta“ jedinjenja, koji za sud predstavljaju neoborivi dokaz prisustva otrova u organizmu. Cilj ove prezentacije je predstavljanje značaja i primene gasne hromatografije sa masenim i plamenojonizujućim detektorima kroz primere sudsko-medicinskih veštačenja akreditovanog Odeljenja za toksikološku dijagnostiku Zavoda za sudsku medicinu u Nišu. Bilo na terenu ili u laboratoriji, gasna hromatografija u kombinaciji sa različitim detektorima nudi jednostavnost i visoku efikasnost u radu toksikoloških laboratorija, pri čemu se dobijaju pouzdani i tačni rezultati korisni za sudsko-medicinska veštačenja.

**KLJUČNE REČI:** gasna hromatografija, postmortem uzorci, sudska toksikologija



## GAS CHROMATOGRAPHY - THE APPLICATION IN FORENSIC TOXICOLOGY

DEBATE -  
ANALYTICAL TOXICOLOGY  
IS A POWERFUL WEAPON  
AND A GREAT CHALLENGE

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Gas chromatography (GC), in addition to liquid chromatography, is today one of the most common analytical techniques for analyzing postmortem samples in forensic toxicology. In combination with different detectors, GC enables the identification and quantification of a wide range of toxic agents from gases, organic solvents, and pesticides to a wide variety of natural and synthetic medicines and illicit drugs. The essential preconditions for selecting gas chromatography as an analytical technique in toxicological analyses are high volatility, thermostability, and low molecular weight of the analyzed compounds.

Expanding the scope of GC analysis to analytes of higher molecular weight and lower volatility is achieved by introducing hydrolysis and/or derivatization into sample preparation procedures. Preparation and analysis of postmortem samples such as blood, urine, hair, and organ tissues present a great challenge for analysts, especially if the samples are already in an advanced stage of decomposition. Well-prepared samples and an appropriate selection of detectors in GC are a key combination for the successful determination of trace concentrations of poisons in postmortem material. In GC, the component determination in a complex mixture of postmortem material may be carried out by detectors with different sensitivity and specificity. One of the most sensitive detectors is a mass spectrometer that records spectra significant for forensic-medical expertise. In some cases, mass spectra can be crucial evidence for determining the cause of death. With its advantages and disadvantages, GC in combination with a flame ionization detector today represents the "gold standard" for the analysis of highly volatile poisons, primarily alcohol, while with a mass detector, it enables the recording of mass spectra of the so-called "fingerprint" of the compound, which for the court is unbeatable evidence of the poison present in the body. The goal of this presentation is to indicate the importance and application of gas chromatography coupled with mass and flame ionization detectors through cases of forensic medical expertise, using the results of the accredited Department of Toxicological Diagnostics of the Institute of Forensic Medicine in Niš. Whether in the field or the laboratory, gas chromatography combined with various detectors offers simplicity and high efficiency in the work of toxicology laboratories, providing reliable and accurate results relevant to forensic examinations.

**KEYWORDS:** gas chromatography, postmortem samples, forensic toxicology



# **PARTICIPANTS**

## abstracts

**13<sup>th</sup> INTERNATIONAL  
CONGRESS**  
OF THE SERBIAN SOCIETY  
OF TOXICOLOGY



**1<sup>st</sup> TOXSEE  
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CONFERENCE**

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Present and Future of toxicology: Challenges and opportunities

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## ANALITIČKA TOKSIKOLOGIJA / ANALYTICAL TOXICOLOGY

### ZADESNO TROVANJE KOLHICINOM (COLCHICUM AUTUMNALE) SA LETALNIM ISHODOM - PRIKAZ SLUČAJA

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Poslednjih pet godina u Nacionalnom centru za kontrolu trovanja prijavljeno je oko trideset pacijenata trovanih biljkama sa toksičnim alkaloidima. Trovanja mrazovcem (*Colchicum autumnale*) su retka i slučajna. Nastaju usled zamene medvedeg luka (sremuša) mrazovcem. Kolhicin je alkaloid koji odgovoran za toksičnost mrazovca. Cilj ovog rada bio je analiza alkaloida kolhicina u postmortem materijalu primenom tečne hromatografije sa UV skenirajućim detektorom (HPLC/PDA). Pacijent starosne dobi 50 godina primljen je na Kliniku za urgentnu i kliničku toksikologiju Vojnomedicinske akademije sa podatkom da je konzumirao sremuš (medvedi luk). Od simptoma imao je mučninu, grčeve, usporeno disanje, probleme sa jetrom i dehidratacijom.

Zbog sumnje da je došlo do zamene sremuša mrazovcem, urađena je HPLC-PDA analiza na prisustvo kolhicina, koji je detektovan u uzorku urina. Letalni ishod je nastao 24 sata nakon prijema. Postmortem uzorci su ekstrahovani hloroformom uz dodatak amonijaka. Analiza uzoraka je rađena HPLC/PDA metodom koja je bila linearna u rasponu od 0,02-1,0 mg/L, sa limitom detekcije od 0,01 mg/L. U analiziranim uzorcima dokazan je kolhicin u sledećim koncentracijama: urin 0,051 mg/L, krv 0,011 mg/L, bubreg 0,008 mg/L i jetra 0,007 mg/L. Potvrda uzroka smrti najčešće nije moguće bez toksikološko-hemijskih analiza, a u proceni distribucije toksičnog agensa potrebno je analize raditi i u parenhimskim tkivima. HPLC/PDA metoda je dovoljno osetljiva i pouzdana za detekciju kolhicina i potvrdu trovanja ovim jedinjenjem.

**KLJUČNE REČI:** *Colchicum autumnale*, trovanje, kolhicin, HPLC/PDA





# ACCIDENTAL POISONING WITH COLCHICINE (COLCHICUM AUTUMNALE) WITH FATAL OUTCOME - CASE REPORT

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In the last five years, about thirty patients poisoned by plants with toxic alkaloids have been reported to the National Poison Control Center. Colchicum autumnale poisonings are rare and accidental. They are created due to the replacement of bear's garlic (ramsons) with autumn crocus. Colchicine is an alkaloid responsible for the toxicity of frostbite. The aim of this work was analysis of colchicine alkaloids in postmortem material using liquid chromatography with UV scanning detector (HPLC/PDA). A 50-year-old patient was admitted to the Clinic for Clinical Toxicology of Military Medical Academy with information that he had consumed bear's garlic (ramsons). His symptoms included nausea, cramps, slow breathing, liver problems and dehydration. Due to the suspicion that there was a replacement of ramsons by autumn crocus, an HPLC-PDA analysis was performed for the presence of colchicine, which was detected in the urine sample.

The fatal outcome occurred 24 hours after admission. Postmortem samples were extracted with chloroform with the addition of ammonia. Analysis of samples was done by HPLC/PDA method, which was linear in the range of 0.02-1.0 mg/L, with a detection limit of 0.01 mg/L. In the analyzed samples, colchicine was detected in the following concentrations: urine 0.051 mg/L, blood 0.011 mg/L, kidney 0.008 mg/L and liver 0.007 mg/L. Confirmation of the cause of death is usually not possible without toxicological-chemical analyses, and in assessing the distribution of toxic agent, it is necessary to perform analyses in parenchymal tissues. HPLC/PDA method is sensitive and reliable enough to detect colchicine and confirm poisoning with this compound.

**KEYWORDS:** Colchicum autumnale poisoning, colchicin, HPLC/PDA



## ODREĐIVANJE POLIHLOROVANIH BIFENILA U PAPIRU I KARTONU METODOM GASNE HROMATOGRAFIJE GC/MS

ANALYTICAL  
TOXICOLOGY

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Polihlorovani bifenili (PCB) su grupa toksičnih hemikalija koje su se obično koristile u različitim industrijskim i komercijalnim industrijama, uključujući proizvodnju proizvoda od papira i kartona. Za određivanje prisustva PCB-a u papiru i kartonu, gasna hromatografija-masena spektrometrija (GC-MS) je uobičajena analitička metoda. Koraci uključeni u ovu metodu su priprema uzorka, GC i MS analiza. Uzorak papira ili kartona se prvo ekstrahuje odgovarajućim rastvaračem, kao što je heksan ili smeša heksana i acetona, da bi se ekstrahovali PCB-ovi.

Ekstrakt se zatim prečišćava da bi se uklonile sve supstance koje mogu ometati analizu. Prečišćeni ekstrakt se zatim ubrizgava u GC sistem opremljen kapilarnom kolonom i detektorom masenog spektrometra. GC odvaja pojedinačne PCB kongenere na osnovu njihovih hemijskih i fizičkih svojstava, kao što su njihove tačke ključanja i pritisci pare. Pojedinačni PCB kongeneri odvojeni GC-om se zatim detektuju i identifikuju pomoću MS detektora na osnovu njihovih karakterističnih masenih spektara. Zabeleži se zastupljenost svakog kongenera i izračunava se ukupna količina PCB-a u uzorku. Važno je napomenuti da GC-MS metoda za određivanje PCB-a u papiru i kartonu može zahtevati optimizaciju različitih parametara, kao što su izbor rastvarača za ekstrakciju, GC kolona i temperaturni program, i radni uslovi MS, da bi se postigli optimalno odvajanje i kvantifikacija kongenera PCB-a.

**KLJUČNE REČI:** Polihlorovani bifenili, metoda, gasna hromatografija



## DETERMINATION OF POLYCHLORINATED BIPHENYLS (PCBS) IN PAPER AND BOARD BY GAS CHROMATOGRAPHY METHOD (GC/MS)

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Polychlorinated biphenyls (PCBs) are a group of toxic chemicals that were commonly used in various industrial and commercial applications, including the manufacture of paper and cardboard products. To determine the presence of PCBs in paper and cardboard, gas chromatography-mass spectrometry (GC-MS) is a commonly used analytical method. The steps involved in this method are sample preparation, GC and MS analysis. The paper or cardboard sample is first extracted with a suitable solvent, such as hexane or a mixture of hexane and acetone, to extract the PCBs. The extract is then purified to remove any interfering substances that may interfere with the analysis. The purified extract is then injected into a GC system equipped with a capillary column and a mass spectrometer detector.

The GC separates the individual PCB congeners based on their chemical and physical properties, such as their boiling points and vapor pressures. The individual PCB congeners separated by the GC are then detected and identified by the MS detector based on their characteristic mass spectra. The abundance of each congener is recorded, and the total amount of PCBs in the sample is calculated. It is important to note that the GC-MS method for the determination of PCBs in paper and cardboard may require optimization of various parameters, such as the choice of extraction solvent, the GC column and temperature program, and the MS operating conditions, to achieve optimal separation and quantification of the PCB congeners.

**KEYWORDS:** Polychlorinated biphenyls, method, gas chromatography



## AKUTNO TROVANJE ETANOLOM I ANALGOANTIPIRETICIMA – PRIKAZ SLUČAJA

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Prema podacima Nacionalnog centra za kontrolu trovanja Srbije, najčešći uzročnik akutnih intoksikacija pacijenata je etanol iz alkoholnih pića, dok su vodeći uzročnici trovanja kod hospitalizovanih osoba lekovi. Često su to polimedikamentozna teška trovanja u kombinaciji sa etanolom. Određivanje etanola u krvi metodom gasne hromatografije sa plameno-jonizacionim detektorom (GC/FID) i analgoantipiretika u plazmi metodom tečne hromatografije sa UV skenirajućim detektorom (HPLC/PDA).

Pacijentkinja starosti 55 godina, primljena je na Kliniku za urgentnu i kliničku toksikologiju Vojnomedicinske akademije (VMA) zbog akutne intoksikacije alkoholom i lekovima. Pri prijemu registrovano je stanje svesti nivoa kome, afebrilna, eupnoična, cijanotična, bez vidljivih spoljnih znakova svežeg povređivanja. Nakon viščasovne opservacije, pacijentkinja je intubirana, bez oporavka stanja svesti i hipotenzivna.

Zbog sumnje da se radi o polimedikamentoznom trovanju, urađen je toksikološki skrining uzorka plazme pacijentkinje metodom HPLC/PDA. Inicijalno dokazana koncentracija etanola u krvi iznosila je 6,46 mg/ml, dok je koncentracija paracetamola iznosila 32,57 mg/l i ibuprofena 203,65 mg/l. Kontrolnom analizom nakon šest sati dobijene su sledeće koncentracije: etanol 8,23mg/ml, paracetamol 80,52 mg/l i ibuprofen 189,68 mg/l. Naredna dva dana analizom krvi dobijene su sledeće koncentracije: etanol (6,15 mg/ml, 5,97 mg/ml i 2,06 mg/ml), paracetamol (57,67 mg/l, 35,86 mg/l i 9,44 mg/l) i ibuprofen (14,25 mg/l, 6,7 mg/l i 13,1 mg/l). Etanol se u potpunosti eliminisao iz krvi nakon tri dana. Nakon stabilizacije stanja svesti i vitalnih parametara pacijentkinja se otpušta sa klinike. Toksikološko-hemijske analize su značajne u skriningu, dijagnozi i sprovođenju adekvatne terapije. HPLC/PDA metoda je korisna u skriningu i brzom detekciji potencijalnih uzročnika trovanja, dok je GC/FID zlatni standard za određivanje koncentracije etanola u krvi.

**KLJUČNE REČI:** etanol, GC/FID, paracetamol, ibuprofen, HPLC/PDA



## CASE REPORT: ACUTE INTOXICATION WITH ETHANOL AND ANALGESIC ANTIPYRETICS

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According to data from National Poison Control Center of Serbia, most common cause of acute intoxication at patients is ethanol from alcoholic beverages, while leading causes of poisoning in hospitalized persons are drugs. These are often severe poly-drug poisonings in combination with ethanol. Determination of ethanol in the blood is done by gas chromatography with a flame ionization detector (GC/FID) and analgoantipyretics in plasma by liquid chromatography with UV scanning detector (HPLC/PDA). A 55-year-old female patient was admitted to Clinic for Emergency and Clinical Toxicology of Military Medical Academy (VMA) due to acute intoxication with alcohol and drugs. At admission time, a coma-level state of consciousness was registered, afebrile, eupnoic, cyanotic, without visible external signs of fresh injuries. After several hours of observation, patient was intubated, without recovery of consciousness and hypotensive.

Due to suspicion of polydrug poisoning, toxicological screening was performed using the HPLC/PDA. Initially proven concentration of ethanol in blood was 6.46 mg/ml, concentration of paracetamol 32.57 mg/l and ibuprofen 203.65 mg/l. After six hours concentrations were: ethanol 8.23 mg/ml, paracetamol 80.52 mg/l and ibuprofen 189.68 mg/l. The following two days results were: ethanol (6.15mg/ml, 5.97mg/ml, 2.06mg/ml), paracetamol (57.67 mg/l, 35.86 mg/l, 9.44 mg/l) and ibuprofen (14.25 mg/l, 6.7 mg/l, 13.1 mg/l). Ethanol was completely eliminated from blood after three days. After stabilization of the patient's condition, patient was discharged from the clinic. Toxicological-chemical analyzes are important in screening, diagnosis and implementation of adequate therapy. HPLC/PDA is useful in screening and rapid detection of potential poisoning agents, while GC/FID is gold standard for determining ethanol concentration.

**KEYWORDS:** ethanol, GC/FID, paracetamol, ibuprofen, HPLC/PDA



## BRZA KVANTITATIVNA METODA ZA ANALIZU GHB TEČNOM HROMATOGRAFIJOM SA TANDEM MASENOM SPEKTROMETRIJOM

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$\gamma$ -Hidroksibuterna kiselina (GHB) je endogena supstanca sa depresivnim delovanjem. Katabolit je neurotransmitera  $\gamma$ -aminobuterne kiseline. Endogene koncentracije u urinu su do 10 mg/L. GHB se koristi u medicinske svrhe, ali i kao sredstvo zloupotrebe. Poznat je i kao droga za silovanje. Zbog endogenog prisustva GHB-a analiza rezultata kod zloupotebe ove supstance je veliki analitički izazov. Razvijanje UPLC-MS/MS metode za određivanje GHB-a u urinu. Za određivanje GHB-a uvedena je metoda tačne hromatografije sa tandem masenim detektorom (UPLC-MS/MS). Uzorci urina su nakon zakišeljavanja pripremani čvrsto-faznom ekstrakcijom na Oasis<sup>®</sup> MCX kertridžima.

Razdvajanje GHB-a od komponenti matriksa vršeno je na C18 koloni uz korišćenje 5 mmol/L amonijum acetata (pH 3,5) i 0,1% rastvora sirćetne kiseline u acetonitrilu (80:20), kao mobilne faze, protoka 0,3 mL/min. Retenciono vreme GHB-a bilo je 1,06 min. Identifikacija GHB-a vršena je u negativnom jonskom režimu (ESI<sup>-</sup>), MRM modu, prema tranzicijama: m/z 103→85, 103→57, 103→103 i kolizionim enegijama od 10-15 V. UPLC-MS/MS metoda bila je linearna u opsegu od 2,0-50,0 mg/L sa koeficijentom korelacije  $r^2 > 0,99$ . Granica kvantifikacije (LOQ) bila je 2,0 mg/L, a granica detekcije (LOD) 1,0 mg/L. Analizom uzoraka urina pacijenata sa sumnjom na zloupotrebu GHB-a potvrđeno je prisustvo GHB-a u koncentracijama većim od 30 mg/L. Razvijena metoda tačne hromatografije sa tandem masenom spektrometrijom je jednostavna, brza i efikasna i može se koristiti za pouzdano određivanje nivoa GHB u urinu.

**KLJUČNE REČI:** GHB, UPLC-MS/MS, urin



## A RAPID QUANTITATIVE METHOD FOR THE ANALYSIS OF GHB BY LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY

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$\gamma$ -Hydroxybutyric acid (GHB) is an endogenous substance with depressant effects. It's a catabolite of neurotransmitter  $\gamma$ -aminobutyric acid. Endogenous GHB concentrations in urine are up to 10 mg/L. GHB is used for medical purpose, but also as a drug of abuse. It's known as a rape drug. Due to endogenous presence, the analysis of the results in abusing of GHB is a big analytical challenge. Development of a UPLC-MS/MS method for the determination of the GHB in urine. The method of a UPLC-MS/MS for determination of GHB in urine was developed. After acidification, urine samples were prepared by solid-phase extraction on Oasis<sup>®</sup> MCX cartridges.

Separation of GHB was performed on a C18 column using 5 mmol/L ammonium acetate (pH 3.5) buffer and 0.1 % acetic acid solution in acetonitrile (80:20) as the mobile phase, with a flow rate of 0.3 mL/min. The retention time of GHB was 1.06 min. The identification of GHB was performed in negative ion mode (ESI<sup>-</sup>), MRM mode, according to transitions: m/z 103→85, 103→57, 103→103 and collision energies of 10-15 V. UPLC-MS/MS method was linear in the range of 2.0-50.0 mg/L with the correlation coefficient  $r^2 > 0.99$ . LOQ was 2.0 mg/L. LOD was 1.0 mg/L. Analysis of urine samples taken from the patients suspected of GHB abuse, was confirmed presence of GHB in concentrations higher than 30 mg/L. The developed UPLC-MS/MS method is simple, fast and efficient and can be used for reliable determination of GHB level in urine.

**KEYWORDS:** GHB, UPLC-MS/MS, urine



## EFEKTI RAZLIČITIH HEMIJSKIH KOZMETIČKIH TRETMANA NA SADRŽAJ NIKOTINA U KOSI

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Analiza kose pruža pouzdane informacije o dugotrajnoj izloženosti nikotinu, u zavisnosti od dužine kose. Sposobnost kose da očuva supstance kao što je nikotin može biti ugrožena hemijskim tretmanom kose koji može oštetiti strukturu kose i uticati na tačnost detekcije nikotina u kosi. Cilj ove studije bio je da se ispita da li različiti hemijski tretmani za kosu (izbeljivanje, farbanje i preliv) smanjuju nivo nikotina u kosi. Sadržaj nikotina u kosi određen je u netretiranim i hemijski tretiranim segmentima kose u 20 uzoraka primenom metode mikroekstrakcije u čvrstoj fazi (HS-SPME) i gasnom hromatografijom kaplovanom sa spektrometrijom masa (GC-MS). Maseni udeo nikotina su bili niži u hemijski tretiranoj kosi u poređenju sa kosom bez tretmana.

Studija je takođe pokazala da različite vrste hemijskog tretmana imaju različite efekte na maseni udeo nikotina u kosi. Dok je izbeljivanje najviše uticalo na nivo nikotina (smanjenje 23 – 88%), neke druge vrste tretmana kose (prelivi koje se ispiru) nisu imale značajan efekat (manje od 8%). Farbanje je smanjilo nivo nikotina u kosi do 40%, u zavisnosti od formule za bojenje. S obzirom na to da postoji ograničen broj sličnih studija, ova bi mogla dati pravac za druge studije o uticaju različitih hemijskih tretmana na nivo sličnih supstanci u kosi. Ovi podaci takođe sugerišu da se efekat hemijskog kozmetičkog tretmana na kosu mora uzeti u obzir kada se tumače rezultati ilegalnih sredstava zavisnosti koji se standardno analiziraju u kosi, a to su uglavnom bazična jedinjenja kao što je nikotin.

**KLJUČNE REČI:** nikotin, kosa, kozmetički tretman, HS-SPME, GC-MS





## EFFECTS OF DIFFERENT CHEMICAL COSMETIC TREATMENTS ON HAIR NICOTINE LEVELS

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Hair analysis provides reliable information on long-term nicotine exposure, depending on hair length. The ability of hair to preserve substances such as nicotine incorporated into its shaft may be compromised by the chemical treatment of hair that can damage the structure of the hair and affect the accurate detection of nicotine in it. The aim of this study was to investigate whether different hair chemical treatments (bleaching, dyeing, and wash-out coloring) reduce hair nicotine levels.

Hair nicotine content was determined in non-treated and chemically treated hair segments in 20 samples using the headspace solid phase microextraction (HS-SPME) method followed by gas chromatography-mass spectrometry (GC-MS). Nicotine levels were lower in chemically treated hair in comparison to hair without treatment. The study also showed that different types of chemical treatment had different effect on hair nicotine levels. While bleaching influenced nicotine levels the most (23–88% decrease), certain other types of hair treatment (wash-out coloration) did not have a significant effect (less than an 8% decrease). Dyeing reduced hair nicotine levels up to 40%, depending on the coloring formula. Given that there are a limited number of similar studies, this one could provide a direction for other studies concerning the influence of diverse chemical treatments on drug levels in hair. These data also suggest that the effect of chemical cosmetic treatment on hair has to be taken into account when interpreting results of drug abuse standardly analysed in hair, which are mainly basic compounds such as nicotine.

**KEYWORDS:** nicotine, hair, cosmetic treatment, HS-SPME, GC-MS



## ODREĐIVANJE ETILEN OKSIDA I NJEGOVOG METABOLITA 2-HLOROETANOLA GC-MS/MS TEHNIKOM U HRANI BILJNOG POREKLA

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Zaštita hrane biljnog porekla tokom skladištenja važan je parametar kvaliteta u lancu proizvodnje i distribucije. Jedna od metoda zaštite je fumigacija sa etilen-oksdom. Fumigacija etilen oksidom dokazana je u zaštiti hrane od gljivica, virusa i bakterija. Međutim, korišćenje etilen-oksida u Evropskoj Uniji je zabranjeno prema Uredbi 396/2005 jer je klasifikovan kao kancerogen za ljude. Zbog visoke aktivnosti kao fumigant, ipak se koristi za fumigaciju silosa u nekim zemljama trećeg sveta. Otkriće nezakonite fumigacije semena susama etilen oksidom u avgustu 2020. učinilo je ovo pitanje gorućim sa stajališta analize ostataka i osiguranja kvaliteta.

Usled svega navedenog akreditovana je metoda Određivanja etilen oksida i njegovog metabolita 2-hloretanola (EURL-SRM Analysis of Ethylene Oxide and its Metabolite 2-Chloroethanol by the QuOil or the QuEChERS Method and GC/MS/MS). Proces verifikacije metode je urađen na uzorku cerealija i proizvoda od cerealija, na gasnom hromatografu Agilent Technologies 7890A sa tandem masenim spektrometrom Agilent Technologies 7000C sa autoinjektorom i 7693 autosamplerom, i na koloni za GC Agilent J&W HP-VOC UI 30 m × 0.20 mm × 1.12 μm. Metoda je linearna za opseg odabrane koncentracije od 0,020 mg/kg do 2 mg/kg uzorka. Na osnovu statističkih parametara, zahtevani kriterijumi za tačnost i preciznost na nivou limita kvantifikacije su u potpunosti zadovoljeni i metoda se može koristiti u rutinskom radu, za definisano područje primene. Zadovoljen je i postavljeni kriterijum za specifičnost određivanja etilen oksida i 2-hloroetanola datom metodom u navedenom matriksu, odnosno ne postoje značajnije smetnje koje bi uticale na rezultate.

**KLJUČNE REČI:** etilen-oksid, 2-hloroetanol, fumigacija, GC-MS/MS, hrana



## DETERMINATION OF ETHYLENE OXIDE AND ITS METABOLITE 2-CHLOROETHANOL BY GC-MS/MS TECHNIQUE IN FOOD OF PLANT ORIGIN

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Protection of plant-based food during storage is an important quality parameter. One of the protection methods is fumigation with ethylene oxide. Ethylene oxide fumigation has been proven to protect food from fungi, viruses and bacteria. The use of ethylene oxide in the European Union is prohibited under Regulation 396/2005 because it is classified as a human carcinogen. Still, it is used for silo fumigation in some Third countries.

The discovery of illegal fumigation of sesame seeds with ethylene oxide in August 2020 brings this issue into focus from the standpoint of residue analysis and quality assurance. The method for the determination of ethylene oxide and its metabolite 2-chloroethanol (EURL-SRM Analysis of Ethylene Oxide and its Metabolite 2-Chloroethanol by the QuOil or the QuEChERS Method and GC/MS/MS) was accredited. The method verification process was performed on a sample of cereal products, on an Agilent Technologies 7890A gas chromatograph with an Agilent Technologies 7000C tandem MS with an autoinjector and a 7693 autosampler, and on a GC column Agilent J&W HP-VOC UI 30 m × 0.20 mm × 1.12 μm. The method is linear for the selected concentration range: 0.020mg/kg to 2mg/kg of sample.

The required criteria for accuracy and precision at the level of the quantification limit are fully satisfied and the method can be used in routine work. The set criterion for the specificity of the determination of ethylene oxide and 2-chloroethanol in the specified matrix is satisfied, there are no significant interferences that would affect the results.

**KEYWORDS:** ethylene oxide, 2-chloroethanol, fumigation, GC-MS/MS, food



## VALIDACIJA METODE TEČNE HROMATOGRAFIJE SA FLUORESCENTIM DETEKTOROM (HPLC-FLD) ZA ODREĐIVANJA BISFENOLA A U MATERIJALIMA I PREDMETIMA KOJI DOLAZE U KONTAKT SA HRANOM

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Materijali i predmeti u kontaktu sa hranom mogu da sadrže bisfenol A koji se dodaje kao plastifikator ili antioksidans pri izradi plastike ali je i sastavni deo epoksi smola koje se koriste kao unutrašnji premazi za konzerve u kojima se čuvaju namirnice. U kontaktu sa hranom, bisfenol A se može osloboditi u sadržaj, preko hrane dospeti u organizam čoveka i ispoljiti štetne efekte. Bisfenol A može da dovode do pojave ranog puberteta, steriliteta kod muškaraca i žena, poremećaja u razvoju nervnog sistema, kardiotoksičnosti i gojaznosti, a prema nalazima nekih studija može se dovesti u vezu sa pojavom karcinoma. Za uspešnu kontrolu i prevenciju izloženosti ljudi, obavezna je, između ostalog, kontrola migracije bisfenola A iz predmeta i materijala koji dolaze u kontakt sa hranom. Cilj rada je bio da se razvije i validira metoda tečne hromatografije sa fluorescentim detektorom (HPLC-FLD) za određivanja bisfenola A u materijalima i predmetima koji dolaze u kontakt sa hranom i da se primeni u navedene svrhe. Predložena HPLC-FLD metoda zadovoljava sve preporučene kriterijume validacije. Metoda je osetljiva, precizna i tačna za određivanje bisfenola A u model rastvorima 95% etanol, 3% sirćetna kiselina i dejonizovana voda i može se koristiti za rutinsku analizu bisfenola A u uzorcima predmeta koji dolaze u kontakt sa hranom. Od 12 ispitivanih predmeta, zdravstveno neispravnim smatraju se plastični tanjir i aluminijumska limenka sa premazom jer u testu migracije otpuštaju bisfenol A u koncentracijama većim od granične vrednosti postavljene evropskom regulativom. Koncentracije bisfenola A izmerene ovom metodom mogu se koristiti za procenu unosa bisfenola A preko hrane u koju dospevaju migracijom iz materijala ili predmeta.

**KLJUČNE REČI:** bisfenol A, HPLC-FLD, validacija, predmeti u kontaktu sa hranom



## VALIDATION OF THE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION METHOD (HPLC-FLD) FOR THE DETERMINATION OF BISPHENOL A IN FOOD CONTACT MATERIALS

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Food contact materials can contain bisphenol A added as a plasticizer or antioxidant as well as a component of epoxy resins that are a coating material for food cans. Bisphenol A could be released in food and then enter the human body and exerts its adverse effect. After exposure, bisphenol A could cause early puberty, infertility in males and females, neurodevelopmental disorders, heart diseases, and obesity, while some evidence indirectly links exposure to bisphenol A and carcinogenesis. For the purpose of controlling and preventing human exposure to bisphenol A, it is obligatory to test levels of bisphenol A in food contact materials.

The aim of this study was to develop and validate the high-performance liquid chromatography with fluorescence detection method (HPLC-FLD) for the determination of bisphenol A in food contact materials. The developed HPLC-FLD method meets all the recommended validation criteria. The method is a reliable, precise, and accurate assay for quantitative analysis of bisphenol A in model solutions, 95% ethanol, 3% acetic acid, and deionized water. From a total of 12 tested samples, plastic plate, and varnished aluminum can were not safe according to the migration limit set by EU Legislation. It can be used for the routine analysis of bisphenol A in samples of food contact materials and for assessment of bisphenol A intake by food consumption after migration from plastics and food contact materials.

**KEYWORDS:** bisphenol A, HPLC-FLD, validation, plastics, food contact materials



## ANALITIČKE METODE U DIJAGNOSTICI I BIOMONITORINGU AKUTNIH TROVANJA ORGANOFOSFATIMA - PRIKAZ DVA SLUČAJA TROVANJA DIAZINONOM

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Organofosforni insekticidi su neurotoksična jedinjenja koja se široko koriste u agrikulturni. Opisani su brojni slučajevi namernih ili slučajnih trovanja širom sveta sa smrtnim ishodom. Diazinon je organofosforni insekticid koji daje simptome trovanja slične drugim organofosforinim insekticidima. Cilj ovog rada je pregled bioanalitičkih metoda za detekciju diazinona kako bi se potvrdila dijagnoza trovanja. Posebna pažnja je posvećena holinesteraznim bioesejima gde se na osnovu praćenja stepena inhibicije enzima utvrđuje izbor i doza antidota. Prikazana su dva slučaja namernog trovanja kod osoba različitog pola ingestijom rastvora diazinona. Kao materijal za dokazivanje diazinona korišćen je lavat želuca, krv i urin. Identifikacija diazinona i njegovih metabolita izvršena je metodom gasne hromatografije kombinovane mase- nom spektrometrijom (GC-MS).

Klasičnim spektrofotometrijskim metodama određivana je aktivnost holinesteraza u serumu (ChE) i eritrocitima (AChE). Dokazano je prisustvo diazinona u krvi, lavatu i urinu kod obe osobe. Metabolički produkt diazinona, diazinon-okson nađen je u urinu takođe kod obe osobe. Acetilholinesteraza (AChE) u eritrocitima i pseudoholinesteraza (ChE) u serumu pokazale su visok stepen inhibicije nakon incidenta u odnosu na referentne vrednosti. Kod osobe ženskog pola Ache se oporavila nakon 7 dana i ušla u opseg referentnih vrednosti. Pseudoholinesteraza se oporavila samo 20% nakon 15 dana. Acetilholinesteraza kod osobe muškog pola se oporavila 30% nakon 18 dana, dok je pseudoholines- teraza i dalje pokazivala visok stepen inhibicije, oporavljeno je samo 10% nakon 25 dana. Obe osobe su preživele. Specifičnim metodama identifikovan je diazinon i njegov metabolički produkt diazinon-okson, značajni za dijagnozu trovanja. Klasičnim metodama, kao što je određivanje aktivnosti holinesteraza, praćen je monitoring zdravstvenog statusa trovanih.

**KLJUČNE REČI:** pesticidi, organofosfati, prikaz slučaja, diazinon



# ANALYTICAL METHODS IN DIAGNOSIS AND BIOMONITORING OF ORGANOPHOSPHATE POISONING - CASE STUDY OF TWO CASES OF DIAZINON POISONING

ANALYTICAL  
TOXICOLOGY

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Organophosphorus insecticides are neurotoxic compounds widely used in agriculture. Numerous cases of intentional or accidental poisoning with fatal outcome have been described worldwide. Diazinon is an organophosphorus insecticide that produces poisoning symptoms similar to other organophosphorus insecticides. The aim of this paper is to review bioanalytical methods for the detection of diazinon in order to confirm the diagnosis of poisoning. Special attention is paid to cholinesterase bioassays, where the choice and dose of the antidote is determined based on monitoring the degree of enzyme inhibition. Two cases of intentional poisoning in persons of different sexes by ingestion diazinon solution are presented. Gastric lavage, blood and urine were used as material for proving diazinon. The identification of diazinon and its metabolites was performed using the method of gas chromatography combined with mass spectrometry (GC-MS).

Classical spectrophotometric methods were used to determine the activity of cholinesterase in serum (ChE) and erythrocytes (AChE). The presence of diazinon in the blood, lavage and urine of both persons was proven. The metabolic product of diazinon, diazinon-oxon, was also found in the urine of both individuals. Acetylcholinesterase (AChE) in erythrocytes and pseudocholinesterase (ChE) in serum showed a high degree of inhibition after the incident compared to reference values. In a female subject, AChE recovered after 7 days and entered the range of reference values. Pseudocholinesterase recovered only 20% after 15 days. Acetylcholinesterase in the male subject recovered 30% after 18 days, while pseudocholinesterase still showed a high degree of inhibition, only 10% recovered after 25 days. Both people survived. Specific methods were used to identify diazinon and its metabolic product diazinon-oxon, important for the diagnosis of poisoning. Classical methods, such as determining the activity of cholinesterase, were used for the monitoring of the health status of the poisoned.

**KEYWORDS:** pesticides, organophosphates, case report, diazinon



## ODREĐIVANJE 11-NOR- $\Delta$ 9-TETRAHIDROKANABINOL-9- KARBOKSILNE KISELINE U URINU TEČNOM HROMATOGRAFIJOM ULTRAVISOKIH PERFORMANSI SA TANDEM MASENOM SPEKTROMETRIJOM

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Kanabis je u svetu najčešće korišćena droga. Farmakološki aktivna jedinjenja biljke su kanabinoidi. Jedan od izomera tetrahidrokanabinola (THC),  $\Delta$ 9THC odgovoran je za većinu psihoaktivnih efekata kanabisa. Potvrda zloupotrebe je prisustvo njegovog metabolita, 11-nor- $\Delta$ 9tetrahidrokanabinol-9-karboksilne kiseline (THC-COOH) u urinu. Može se detektovati u urinu do 7 dana nakon jednokratnog konzumiranja, a kod zavisnika i do mesec dana. Cilj ovog rada bio je uvođenje UPLC/MS/MS metode za određivanje THC-COOH u urinu. Za izolovanje slobodne THC-COOH iz uzorka urina, posle hidrolize amonijum hidroksidom na 60°C, korišćena je metoda čvrsto-tečne ekstrakcije pomoću Oasis HLB kertridža.

Hromatografsko razdvajanje vršeno je na C18 koloni u linearnom gradijentu mobilne faze: 5 mmol/L amonijum acetat pH 3,5 (A) i acetonitril sa 0,1 % sirćetne kiseline (B). Protok je bio 0,4 mL/min. Za jonizaciju je korišćen elektrosprej jonski izvor u pozitivnom modu (ESI+). Energija fragmentacije je bila u intervalu od 20-70 V, sa energijom kolizione ćelije od 3-20 V. Praćene su dve MS/MS tranzicije: m/z 345,10→299,10 i 345,10→193,15. Metoda je bila linearna u opsegu koncentracija od 40,2-402,0 ng/mL, sa koeficijentom korelacije  $r^2 > 0,99$ , granicom detekcije (LOD) 0,94 ng/mL i granicom kvantifikacije (LOQ) 3,14 ng/mL. Retenciono vreme THC-COOH bilo je 6,2 min. Razvijena UPLC-MS/MS metoda je jednostavna i brza za određivanje niskih koncentracija THC-COOH u urinu, i pouzdana za potvrdu zloupotrebe kanabisa.

**KLJUČNE REČI:** THC-COOH, UPLC-MS/MS, urin





## DETERMINATION OF 11-NOR- $\Delta$ 9-TETRAHYDROCANNABINOL-9-CARBOXYLIC ACID IN URINE BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY

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Cannabis is the most widely used drug in the world. The pharmacologically active compounds of the plant are cannabinoids. One of the isomers of tetrahydrocannabinol (THC),  $\Delta$ 9THC is responsible for most of the psychoactive effects of cannabis. Confirmation of abuse is the presence of its metabolite, 11-nor- $\Delta$ 9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in the urine. It can be detected in the urine for up to 7 days after a single consumption, and in addicts for up to a month. This research aimed to introduce a UPLC-MS/MS method for the determination of THC-COOH in urine. To isolate free THC-COOH from a urine, after hydrolysis with ammonium hydroxide at 60°C, a solid-liquid extraction method using an Oasis HLB cartridge was used. Chromatographic separation was performed on a C18 column in a linear mobile phase gradient: 5 mmol/L ammonium acetate pH 3.5 (A) and acetonitrile with 0.1% acetic acid (B); flow rate was 0.4 mL/min.

For ionization, an electrospray ion source in positive mode (ESI+) was used. The fragmentation energy was in the range of 20-70 V, with a collision cell energy of 3-20 V. Two MS/MS transitions were monitored:  $m/z$  345.10 $\rightarrow$ 299.10 and 345.10 $\rightarrow$ 193.15. The method was linear in the range of 40.2-402.0 ng/mL, with correlation coefficient  $r^2 > 0.99$ . LOD was 0.94 ng/mL. LOQ was 3.14 ng/mL. The retention time of THC-COOH was 6.2 min. The developed UPLC-MS/MS method is simple and fast for determining low concentrations of THC-COOH in urine, and reliable for confirming cannabis abuse.

**KEYWORDS:** THC-COOH, UPLC-MS/MS, urine



## ODREĐIVANJE KONCENTRACIJE KVETIAPINA U SERUMU TEHNIKOM TEČNE HROMATOGRAFIJE SA PDA DETEKCIJOM

ANALYTICAL  
TOXICOLOGY

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Kvetiapin pripada grupi lekova koji se zovu antipsihotici. Primenjuje se oralno, a indikovano je u terapiji shizofrenije, bipolarnog poremećaja i u kombinaciji sa drugim lekovima kod velikih depresivnih poremećaja. Depresija je udružena sa povećanim rizikom od pojave suicidalnih misli i samopovređivanja. Zbog toga je potrebno posebno praćenje ovih pacijenata, što podrazumeva određivanje koncentracije leka tokom terapije, a posebno ukoliko postoji sumnja na predoziranje ili trovanje ovim lekom.

Cilj ovog rada je prikaz metode za određivanje kvetiapina u serumu tehnikom tečne hromatografije sa UV skenirajućim detektorom (HPLC/PDA). Izolovanje kvetiapina iz seruma rađeno je na Oasis HLB kertridžima, metodom čvrsto-fazne ekstrakcije. Razdvajanje kvetiapina od ostalih komponenti matriksa vršeno je na C8 koloni koja je grejana na 30°C. Kao mobilna faza korišćena je mešavina acetonitrila i 50 mM natrijum dihidrogenfosfata (pH 3,6) u gradijent modu. Detekcija kvetiapina je vršena na talasnoj dužini od 230 nm. HPLC-PDA metoda za određivanje kvetiapina u serumu bila je linearna u opsegu koncentracija od 0,25-2,50 mg/L, sa koeficijentom korelacije  $r^2 > 0,99$ , granicom detekcije (LOD) 0,01 mg/L i granicom kvantifikacije (LOQ) 0,05 mg/L. Retenciono vreme kvetiapina bilo je 13,7 min. Uvedena HPLC / PDA metoda je jednostavna, brza i specifična i može se primeniti za određivanje koncentracija kvetiapina u serumu u rutinskoj analizi, kod predoziranja i kod akutnih trovanja.

**KLJUČNE REČI:** Kvetiapin, HPLC/PDA, serum



## DETERMINATION OF QUETIAPINE CONCENTRATION IN SERUM USING THE LIQUID CHROMATOGRAPHY TECHNIQUE WITH PDA DETECTION

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Quetiapine belongs to a group of drugs called antipsychotics. It is administered orally, and is indicated in the treatment of schizophrenia, bipolar disorder and in combination with other drugs in major depressive disorders. Depression is associated with an increased risk of suicidal thoughts and self-harm. This is why special monitoring of these patients is necessary, which means determining the concentration of the drug during therapy, especially if there is a suspicion of overdose or poisoning with this drug. Introduction of a HPLC/PDA method for the determination of quetiapine in serum. Isolation of quetiapine from serum was performed on Oasis HLB cartridges, using the solid-phase extraction method.

Separation of quetiapine from other matrix components was performed on a C8 column heated to 30°C. A mixture of acetonitrile and 50 mM sodium dihydrogen phosphate (pH 3.6) was used as the mobile phase in gradient mode. Detection of quetiapine was performed at a wavelength of 230 nm.

The HPLC-PDA method for the determination of quetiapine in serum was linear in the concentration range of 0.25-2.50 mg/L, with a correlation coefficient of  $r^2 > 0.99$ . LOD was 0.01 mg/L. LOQ was 0.05 mg/L. The retention time of quetiapine was 13.7 min. The developed HPLC/PDA method is simple, fast and specific and can be applied for the determination of quetiapine concentrations in serum in routine analysis, in overdose and in acute poisoning.

**KEYWORDS:** Quetiapine, HPLC/PDA, serum



## PRIKAZ SLUČAJA AKUTNOG TROVANJA TEOFILINOM I MOGUĆNOSTI ANALITIČKE POTVRDE

ANALYTICAL  
TOXICOLOGY

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Akutno predoziranje teofilinom može izazvati ozbiljne znake i simptome trovanja. U toksikokinetici teofilina, postoje interindividualne varijacije i ne postoji jasna korelacija između koncentracije teofilina u serumu i toksičnih efekata. Koncentracije teofilina u serumu kod akutnih trovanja su znatno veće nego kod hroničnih predoziranja, ali su toksični efekti slabiji. Metode izbora za analitiku teofilina u serumu su enzimski imunoesej (EMIT) i tačna hromatografija sa masenom detekcijom (HPLC/MS). Cilj rada je bio detekcija i određivanje koncentracije teofilina u serumu akutno otrovane pacijentkinje kao pomoć u dijagnostici, lečenju i praćenju toksičnih efekata. U radu je opisano akutno trovanje teofilinom, pacijentkinje starosti 48 godina, koja je u cilju suicida popila 20 tableta Aminofilin R<sup>®</sup> (aminofilin) 350 mg, nakon čega je hospitalizovana u Centru za kontrolu trovanja VMA.

Određivanje koncentracije teofilina izvršeno je metodom tačne hromatografije sa UV skenirajućim detektorom (HPLC/PDA). Pacijentkinja je na prijemu imala mučninu, povraćanje, sinusnu tahikardiju što se komplikovalo rabdomiolizom lakšeg stepena. Toksikološko-hemijske analize krvi potvrdile su prisustvo teofilina u koncentraciji od 55,89 mg/L na prijemu, 18 h posle prve analize 62,65 mg/L i 1,67 mg/L 24 h nakon prijema. Nakon primene suportivne terapije i stabilizacije opšteg stanja, pacijentkinja je otpuštena kući. Metoda HPLC/PDA je specifična, osetljiva i relativno brza metoda za određivanje koncentracije teofilina u serumu. Pogodna je za rutinsku analizu teofilina u uzorcima seruma akutno trovanih osoba.

**KLJUČNE REČI:** HPLC/PDA, teofilin, akutno trovanje



## CASE REPORT: ACUTE THEOPHYLLINE OVERDOSE AND POSSIBILITY OF ANALYTIC CONFIRMATION

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Acute theophylline overdose can cause serious signs and symptoms of poisoning. In the toxicokinetics of theophylline, there are individual variations and there is no clear correlation between the concentration of theophylline in the serum and toxic effects. Concentrations of theophylline in serum in acute poisoning are significantly higher than in chronic overdose, but toxic effects are weaker. Methods of choice for analysis of theophylline are enzyme-immunoassay (EMIT) and liquid chromatography with mass detection (HPLC/MS). The aim of this work was detection and determination of the concentration of theophylline in the serum of acutely poisoned patients as a help in diagnosis, treatment, and monitoring of toxic effects.

The study describes acute poisoning with theophylline in a 48-year-old patient who, in an attempt to commit suicide, took 20 tablets of Aminofilin R® (aminophylline) 350 mg, after which she was hospitalized in Poison Control Center at Military Medical Academy. Determination of theophylline concentration was carried out by liquid chromatography with UV scanning detector (HPLC/PDA). On admission, the patient had nausea, vomiting, sinus tachycardia, which was complicated by mild rhabdomyolysis.

Toxicological-chemical blood analysis confirmed the presence of theophylline in the concentration of 55.89 mg/L on admission, 18 hours after first analysis 62.65 mg/L and 1.67 mg/L 24 hours after admission. After the application of supportive therapy and stabilization of the general condition, the patient was discharged home. HPLC/PDA method is a specific, sensitive and relatively fast method for determining the concentration of theophylline. It is suitable for routine analysis of theophylline in serum samples of acutely poisoned persons.

**KEY WORDS:** HPLC/PDA, theophylline, acute poisoning



## UTICAJ PRIMENE OTC PREPARATA SA KODEINOM NA REZULTAT IMUNOHROMATOGRFSKE ANALIZE URINA NA OPIOIDE: PRIKAZ SLUČAJA

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U radu je ispitivan uticaj primene OTC preparata sa kodeinom na pojavu lažno pozitivnih rezultata preliminarne analize opioida u urinu osobe koja OTC preparat sa kodeinom koristi u terapiji glavobolje. U istraživanju je učestvovao jedan ispitanik, muška osoba starosti 47 godina sa poznatim periodima glavobolja koje se javljaju jednom nedeljno ili češće, i koji, prema preporuci lekara, uzima jednu ili dve tablete leka Caffetin® (250 mg paracetamola + 50 mg kofeina + 10 mg kodeina + 210 mg propifenazona). Ispitanik je nakon pojave glavobolje koristio Caffetin® u terapiji olakšanja bola tenzionog tipa, pri čemu je prvog dana primenio dve tablete Caffetin®, a trećeg dana jednu tabletu Caffetin® usled ponovnog javljanja glavobolje. Sakupljeno je 15 uzoraka urina tokom 5 uzastopnih dana i izvršena je njihova analiza imunohromatografskim test trakama na opioide AbuGnost MOP300®. Pozitivni rezultati su dobijeni u periodu od 5 sati do 27 sati nakon primene dve tablete Caffetin®, odnosno, periodu od 7 sati do 30,5 sati nakon jedne tablete Caffetin®.

Uzorci urina su čuvani u frižideru zbog konfirmativne analize. Nakon 6 meseci čuvanja uzoraka, potvrđen je njihov nepromenjen integritet i dobijeni su identični rezultati ponovljene imunohromatografske analize, kao i pri analizi izvedenoj neposredno nakon sakupljanja uzoraka. S obzirom na to da se slična ispitivanja mogu preduzeti prilikom rutinskih testiranja, npr. zaposlenih, sportista, kao i u slučaju saobraćajnih nesreća i drugih događaja u kojima dolazi do povređivanja, ističe se značaj uzimanja anamnestičkih podataka, kako u smislu korišćenja određenih lekova, tako i vremenskog perioda koji je prošao od trenutka njihove primene.

**KLJUČNE REČI:** OTC preparat, kodein, opioidi, test trake, lažno pozitivni rezultati



## THE EFFECT OF THE USE OF OTC PREPARATIONS WITH CODEINE ON THE RESULT OF IMMUNOCHROMATOGRAPHIC ANALYSIS OF URINE FOR OPIOIDS: A CASE REPORT

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This study examines the effect of codeine-based OTC drugs on the occurrence of false positive results of preliminary urine analysis of opioids. The study included one 47-year old men with known periods of headache that occur once a week, or more often, and who takes one or two tablets of Caffetin® (250 mg paracetamol + 50 mg caffeine + 10 mg codeine + 210 mg propifenazone), following recommendations by a physician. After headache symptoms onset, the subject used 2 tablets of Caffetin® on the first day and 1 tablet on the third day because of the relapse. 15 urine samples were collected for 5 consecutive days and analysed by AbuGnost MOP300® immunochromatographic test panels on opioids.

Positive results were obtained in the period from 5 hours to 27 hours after administration of 2 tablets of Caffetin®, and from 7 hours to 30.5 hours after administration of 1 tablet of Caffetin®. Urine samples were stored for confirmatory analysis. After 6 months, their unchanged integrity was confirmed and identical results were obtained by repeated immunochromatographic analysis like those obtained immediately after the collection of samples. Considering that similar tests can be used in routine testing of employees, athletes, in case of traffic accidents or in events of injuries, the importance of taking anamnestic data is emphasized, not only in terms of using certain drugs, but also in terms of the time period that has passed since their application.

**KEYWORDS:** OTC drugs, codeine, opioids, test strips, urine analysis, false positive result



## BIOMARKERI TOKSIČNOSTI / BIOMARKERS OF TOXICITY

### DA LI SU CINK I BAKAR UKLJUČENI U TOKSIČNE EFEKTE U MOZGU WISTAR PACOVA INDUKOVANE PRIMENOM BDE-209?

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Dekabromovani difenil etar (BDE-209) je široko rasprostranjena toksična supstanca u životnoj sredini, posebno na mestima odlaganja električnog i elektronskog otpada. Sa deponija se BDE-209 može osloboditi u životnu sredinu i dospeti do organizma čoveka, i eventualno uzrokovati štetne efekte.

Predmet istraživanja ove studije bio je kako BDE-209 remeti homeostazu Zn i Cu u mozgu Wistar pacova. Pacovi su tokom 28 dana oralnom gavažom primali BDE-209 u doznim grupama: 1000, 2000 i 4000 mg/kg/dan; a grupa koje je primala vodu je bila kontrola. Nivoi Zn i Cu su mereni atomskom apsorpcionom spektrofotometrijom nakon mineralizacije uzorka tkiva mozga.

Promene u nivou Zn i Cu indukovane primenom BDE-209 su testirane procenom donje granice pouzdanosti benchmark doze od 10% (BMDL10) primenom softvera PROAST (RIVM; Holandija) u cilju modelovanja odnosa doze i odgovora. BDE-209 nije izazvao dozno zavisne promene koncentracije Cu u mozgu pacova, dok su promene koncentracije Zn bile dozno zavisne. Procenjena BMDL10 iznosila je 2890 mg/kg/dan. Imajući u vidu da je mozak identifikovan kao ciljni organ za BDE-209 i efekat na koncentraciju Zn, u sledećim eksperimentima bi suplementacija Zn mogla da da odgovore koji ključni događaji su uključeni u navedeni put štetnog ishoda.

**KLJUČNE REČI:** BDE-209, bioelementi, mozak





## ARE THE ZINC AND COPPER INVOLVED IN TOXIC EFFECTS INDUCED BY BDE-209 IN THE BRAIN OF WISTAR RATS?

BIOMARKERS  
OF TOXICITY

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Deca brominated diphenyl ether (BDE-209) is a widely present toxic substance in the environment, particularly in electrical and electronic waste sites. From landfill sites, BDE-209 can be released into the environment, reach humans and possibly induce adverse effects.

The objective of this study was to assess how BDE-209 impairs Zn and Cu homeostasis in the brain of Wistar rats. Rats were treated by oral gavage for 28 days with three doses of BDE-209 of 1000, 2000, and 4000 mg/bw/day, and water as a control group. Cu and Zn levels were measured by flame atomic absorption spectrophotometer after brain tissue samples mineralization. Changes in the level of Zn and Cu induced by BDE-209 were tested by deriving a lower confidence limit of a Benchmark dose of 10% (BMDL10) using PROAST software (RIVM, Netherlands) for the dose-response modeling. BDE-209 did not cause dose-dependent changes in Cu level, however Zn level in brain tissue is dependent on the dose of BDE-209. Derived BMDL10 in the experiment was 2890 mg/kg/day. Since the brain is identified as the target tissue for the BDE-209, for the effect on Zn concentration, in further experiments supplementation with Zn could elucidate what type of key events are involved in described adverse outcome pathway.

**KEYWORDS:** BDE-209, bioelements, brain



## OKSIDATIVNI STRES KAO POSLEDICA IZLOŽENOSTI DUVANSKOM DIMU

BIOMARKERS  
OF TOXICITY

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Prevelika produkcija slobodnih radikala ili smanjenje antioksidantnih protektivnih mehanizama organizma, dovodi do oksidativnog stresa i oštećenja ćelija i tkiva. Oksidativni stres koji se javlja kao posledica dejstva otrova, smatra se jednim od najznačajnijih mehanizama koji doprinose toksičnosti mnogih jedinjenja. Otrovi mogu da izazovu oksidativni stres direktno izazivajući pojačani nastanak slobodnih radikala, ali i indirektno, utičući na antioksidativni sistem zaštite. Oksidativni stres koji je izazvan otrovima može da izazove direktno oštećenje ćelije usled produkcije jakih oksidanasa, ali i da iskaže svoj uticaj na signalizaciju i regulaciju genske ekspresije preko redoks senzitivnih mehanizama. Duvanski dim sadrži slobodne radikale koji doprinose potenciranju oksidativnog stresa i stvaranju pro-oksidativne sredine.

Ovakva sredina pogoduje nastanku i razvoju lipidne peroksidacije i porastu oksidativne modifikacije brojnih biomolekula. U jednom gramu duvanskog dima nalazi se  $10^{15}$ - $10^{17}$  čestica slobodnih radikala, a njihova stabilnost se kreće od nekoliko sekundi, do onih čija se stabilnost meri čak i mesecima. Superoksidni radikal, koji se nalazi u duvanskom dimu, dismutira do peroksida, zatim reaguje sa fero ( $Fe^{2+}$ ) jonom stvarajući hidroksil radikale. Superoksidni radikal se uglavnom generiše u metaboličkim reakcijama u mitohondrijama, reakcijama u kojima učestvuju ksantin i aldehid oksidaze, metaboličkim reakcijama arahidonske kiseline. Vodonik peroksid i hlorid, u prisustvu mijeloperoksidaze neutrofila, formiraju hipohlornu kiselinu koja predstavlja veoma snažan oksidans.

**KLJUČNE REČI:** slobodni radikali, oksidativni stres, dejstvo otrova, toksične supstance



## OXIDATIVE STRESS AS A CONSEQUENCE OF EXPOSURE TO TOBACCO SMOKE

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Excessive production of free radicals or reduction of the antioxidant protective mechanisms leads to oxidative stress and damage to cells and tissues. Oxidative stress, which occurs as a consequence of the effects of poisons, is considered one of the most important mechanisms of toxicity. Toxic substances can cause oxidative stress directly by causing increased production of free radicals, but also indirectly by affecting the antioxidant protection system. Oxidative stress caused by poisons can cause direct cell damage due to the production of strong oxidants, but also affect its influence on signaling and regulation of gene expression through redox-sensitive mechanisms. Tobacco smoke contains free radicals that contribute to the potentiation of oxidative stress and the creation of a pro-oxidative environment.

This environment favors the development of lipid peroxidation and the increase of oxidative modification of numerous biomolecules. One gram of tobacco smoke contains  $10^{15}$ - $10^{17}$  particles of free radicals, and their stability ranges from a few seconds to months. The superoxide radical, found in tobacco smoke, dismutates to peroxide, then reacts with the ferrous ( $\text{Fe}^{2+}$ ) ion to form hydroxyl radicals. The superoxide radical is mainly generated in metabolic reactions in the mitochondria, reactions involving xanthine and aldehyde oxidase, and metabolic reactions of arachidonic acid. Hydrogen peroxide and chloride, in the presence of neutrophil myeloperoxidase, form hypochlorous acid, which is a very strong oxidant.

**KEYWORDS:** free radicals, oxidative stress, effect of toxins, toxic substances



## IN VITRO EVALUACIJA ELEMENATA SINAPTIČKE TRANSMISIJE KAO POTENCIJALNIH BIOMARKERA EFEKTA NEUROAKTIVNIH SUPSTANCI

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Intenzivna i globalna upotreba neuroaktivnih supstanci (NS) dovodi do njihovog konstantnog oslobađanja u životnu sredinu, a naročito u površinske vode. Zabrinutost zbog mogućih štetnih efekata NS na ne-ciljne organizme, sa posledicama po ljudsku populaciju, probudila je i potrebu za razvojem strategije zasnovane na biomarkerima, za procenu uticaja NS u vodenoj sredini. Cilj ovog istraživanja je da se ispita osetljivost ključnih elemenata uključenih u sinaptičku transmisiju na NS od ekotoksikološkog značaja iz grupe lekova, pesticida i stimulanasa, korišćenjem humanih neuroblastoma ćelija SH-SY5Y kao in vitro modela.

Ekspresija odabranih gena izmerena je RQ-PCR analizom, dok je aktivnost enzima katabolizma neurotransmitera izmerena odgovarajućim enzimskim esejima. Nekoliko elemenata signalnih puteva serotonina i dopamina (serotoninski receptor 3A, dopaminski receptor D2, monoamin oksidaza A i B) i sinaptotagmin 10 uključen u egzocitozu neurotransmitera istakli su se kao najosetljiviji parametri za testirane NS. Primećene su i razlike u efektima NS koje pripadaju različitim grupama, što može predstavljati osnovu za karakterizaciju smeša supstanci nepoznatog primarnog mehanizma dejstva. Sveobuhvatno, rezultati ukazuju na parametre koji su potencijalno dobri kandidati za biomarkere toksičnih efekata NS različitog primarnog mehanizma dejstva.

**KLJUČNE REČI:** biomarkeri, neuroaktivne supstance, SH-SY5Y, signalni putevi neurotransmitera

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# IN VITRO EVALUATION OF ELEMENTS OF SYNAPTIC TRANSMISSION AS POTENTIAL BIOMARKERS OF EFFECT OF NEUROACTIVE COMPOUNDS

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Intensive and global use of neuroactive compounds (NCs) results in their constant release in the environment, with surface waters being strongly affected. The concern related to their possible adverse effects in non-target organisms, with implications for human population, raised the necessity for development of biomarker-based strategy for impact assessment of NCs in the aquatic environment. The aim of this study was to evaluate responsiveness of key elements involved in synaptic transmission to relevant NCs from the group of pharmaceuticals, pesticides and stimulants, using human neuroblastoma SH-SY5Y cells as in vitro model.

The expression of selected genes was determined using RQ-PCR analyses, while the activity of enzymes involved in catabolism of neurotransmitters was measured by corresponding enzyme activity assays. Several elements of serotonin and dopamine pathways (serotonin receptor 3A, dopamine receptor D2, monoamine oxidase A and B) and synaptotagmin 10 involved in exocytosis of neurotransmitters distinguished as the most sensitive parameters to tested NCs. Differential effects of various groups of NCs were noticed, which might have relevance for characterization of mixtures of compounds with unknown primary mode of action. Overall findings imply to the promising candidates for biomarkers of toxic effects of NCs with various primary modes of action.

**Keywords:** biomarkers, neuroactive compounds, SH-SY5Y, neurotransmitter pathways

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## REFERENTNE VREDNOSTI I STANDARDNA METODA ZA MERENJE AKTIVNOSTI ACETILHOLINESTERAZE U HUMANIM ERITROCITIMA

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Stvarna fiziološka uloga enzima acetilholinesteraze (AChE) u humanim eritrocitima još uvek nije u potpunosti poznata. Postoje eksperimentalni rezultati ali i hipoteze o ulozi AChE u stabilizaciji membrane eritrocita, ali i njene uključenosti u pojedina oboljenja eritrocitne loze. Humani eritrociti poseduju 91% neuron specifičnu AChE aktivnost u odnosu na nespecifičnu holinesteraznu (ChE) aktivnost. Lokalizacija AChE je dominantno (74%) u solubilnoj frakciji eritrocita. Zbog toga, eritrocitna AChE može poslužiti kao marker u dijagnostici i praćenju primenjene terapije kod pacijenata otrovanih organofosfatnim jedinjenjima.

Takođe, može biti dobar periferni biomarker za praćenje delovanja lekova koji su inhibitori AChE u mozgu kod tretmana pacijenata sa Alzheimer-ovom bolešću. Rezultati ove studije nude jednu poboljšanu i pojednostavljenu standardnu metodu za rutinsko merenje AChE aktivnosti u hemolizatu humanih eritrocita, kao i referentne vrednosti u populaciji zdravih ispitanika. Dobijene referentne vrednosti kod zdravih odraslih ispitanika sa teritorije grada Beograda iznosile su 16.4 - 28.4 IU/g Hb, i 328 - 1321 IU/109 bez statistički značajne razlike između muškaraca i žena.

**KLJUČNE REČI:** eritrocitna AChE, standardna metoda, referentne vrednosti



## REFERENT VALUE AND STANDARD METHOD FOR ACETYLCHOLINESTERASE ACTIVITY IN HUMAN ERYTHROCYTE

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A physiological role of the erythrocyte acetylcholinesterase (AChE) is still obscure. However, there are reports and hypotheses of its involvement in erythrocyte membrane stability and in some form of hemolytic disorders. Human erythrocytes possess a 91% of neuronal specific AChE versus non-specific ChE, and mostly (74%) in soluble fraction. Because of that, erythrocyte AChE could be a good marker in diagnose and efficiency of therapy of organophosphate poisoning patients. However, could be a good peripheral biomarker for acting of AChE inhibitors in the treatment of Alzheimer's disease. The results from this study offer an improved and simplified standard method for routine measurement of AChE activity and a new referent value. The referent value for adults in Belgrade population obtained by the method presented in the study was 16.4 - 28.4 IU/g Hb, and 328 - 1321 IU/109 erythrocytes without difference between males and females.

**KEYWORDS:** human erythrocyte AChE, standard method, referent value



## COVID-19 VS TOKSIKOLOGIJA / COVID-19 VS TOXICOLOGY

### UPOTREBA ALKOHOLA U DOBA KOVID-19 PANDEMIJE - ŠTA NAM GOVORE REZULTATI POSTMORTEM ANALIZE?

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Period kovid-19 pandemije doneo je epidemiološke mere i socijalnu izolaciju a prema mnogobrojnim studijama u svetu i povećanu upotrebu alkohola. Psihološki pritisak, neizvesnost i uznemirenost poznati su faktori rizika za povećeni unos alkohola. Cilj ovog rada je prikaz rezultata alkoholemija kod smrtnih slučajeva obdukovanih u Institutu za sudsku medicinu u Beogradu u godini pre pandemije (2019) i u jeku pandemije 2020. godine kada su bile na snazi i mere vanrednog stanja. Analize etanola su urađene u toksikološkoj laboratoriji metodom gasne hromatografije sa plameno jonizacionim detektorom. U 2020. godini je bilo za 6,9% više smrtnih slučajeva sa pozitivnom alkoholemijom u odnosu na 2019. godinu.

Od proglašenja pandemije korona virusa i uvođenja vanrednog stanja, marta 2020. godine beleži se porast pozitivnih alkoholemija kod preminulih osoba u odnosu na isti period 2019. godine, i to sa 6,6% na 16,4% u martu, 7,5% na 24% u maju i sa 6,8% na 27,9% u junu mesecu. U aprilu postoji slična zastupljenost pozitivnih slučajeva u obe godine. U pogledu stepena alkoholisanosti nije bilo značajnih razlika za alkoholemije do 0,5 mg/ml, kao ni onih od 1,0 do 2,0 mg/ml. Uočava se viši procenat smrtnih slučajeva (17%) u 2020. godini sa alkoholemijama od 2,0 do 3,0 mg/ml u odnosu na 2019. godinu (13,3%). U 2020. godini bilo je više smrtnih slučajeva sa alkoholemijom višom od 4 mg/ml (6%) u odnosu na 2019. godinu (3,9%). Rezultati postmortem analiza ukazuju na veći broj kao i viši stepen alkoholisanih slučajeva preminulih u godini izolacije u odnosu na godinu pre korona virusa.

**KLJUČNE REČI:** postmortem alkoholemija, pandemija, kovid-19





## ALCOHOL USE DURING THE COVID-19 PANDEMIC - WHAT DO THE RESULTS OF THE POSTMORTEM ANALYSIS TELL US?

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The period of the covid-19 pandemic brought epidemiological measures and social isolation, and according to numerous studies in the world, increased alcohol use. Psychological and emotional reaction are known risk factors for increased alcohol intake. The aim of analysis is to present the results of alcoholemia in death cases autopsied at the Institute of Forensic Medicine in Belgrade in the year before the pandemic (2019) and in the height of the pandemic in 2020. Ethanol analyzes were performed in the toxicology laboratory using the gas chromatography method with a flame ionization detector. In 2020, there were 6.9% positive alcohol cases more than in 2019.

Since the first days of pandemic, there has been an increase in positive alcohol levels in deceased persons compared to the same period in 2019 from 6.6% to 16.4% in March, from 7.5% to 24% in May and from 6.8% to 27.9% in June. In April, we had no significant differences results. Also, there were no significant differences for alcoholemias up to 0.5 mg/ml, as well as those from 1.0 to 2.0 mg/ml. There was a higher percentage of cases (17%) in 2020 with alcohol levels of 2.0 to 3.0 mg/ml compared to 2019 (13.3%). In 2020, there were more deaths with alcoholemia higher than 4 mg/ml (6%) compared to 2019 (3.9%). The results of the postmortem analyzes indicate a higher number and a higher degree of alcohol-related deaths in the year of isolation compared to the year before the corona virus.

**KEYWORDS:** postmortem alcoholemia, pandemia, covid-19



## SUICIDNOST U DOBA PANDEMIJE KORONA VIRUSOM

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Samoubistvo je svesno i namerno uništavanje sopstvenog života. Zbog porasta stope suicida među osetljivim kategorijama stanovništva, ono predstavlja značajan zdravstveni i društveni problem. Pandemija korona virusom je izložila celokupno stanovništvo brojnim neizvesnostima koje su uslovile pojavu novih i pogoršanje već postojećih psihijatrijskih fenomena među pojedincima, u koje spadaju i pokušaji samoubistva. Pretpostavlja se da će izloženost populacije višestrukim stresnim okolnostima i problemima uticati na porast stope samoubistava u doba pandemije korona virusom. Cilj istraživanja je utvrđivanje učestalosti suicidalnog ponašanja među bolesnicima lečenim na Klinici za psihijatriju Kliničkog centra Vojvodine (KCV) za vreme pandemije korona virusom i utvrđivanje psihopatoloških i sociodemografskih karakteristika ove kohortne grupe.

Istraživanje je obuhvatilo 112 bolesnika čiji anamnestički podaci, dobijeni upotrebom kliničkog informacionog sistema KCV, sadrže podatak o pokušaju suicida u 2020. godini. Ispitivana obeležja su statistički obrađena u statističkom programu JASP 0.14.1 i Microsoft Excel 2016. Rezultati su predstavljeni tabelarno i grafički. Utvrđeno je da je pokušaj suicida bio češći među osobama ženskog pola, starosti 11-24 i 35-44 godina. Ne postoji statistički značajna povezanost između suicidalnosti i COVID-19 okolnosti u ispitivanom uzorku. Najčešći metod pokušaja samoubistva je bilo namerno samotrovanje lekovima. Učestalost pokušaja suicida je bila veća u drugoj polovini godine. Pojedini bolesnici su više puta u godini pokušali da izvrše samoubistvo. Kod većine bolesnika su prisutni psihijatrijski komorbiditeti. U doba pandemije korona virusom značajna je rana dijagnoza mentalnih poremećaja, kao i održavanje kontakata u cilju prevencije pokušaja suicida među osetljivim populacionim grupama.

**KLJUČNE REČI:** suicid, pokušaj suicida, pandemija, samotrovanje, COVID-19



## SUICIDALITY IN THE PERIOD OF CORONAVIRUS PANDEMIC

COVID-19 VS  
TOXICOLOGY

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Suicide is conscious and deliberate attempt at taking one's own life. Due to the increase in suicide rates, suicide is considered a significant health and social problem. The uncertainties about coronavirus pandemic led to new and/or exacerbation of existing psychiatric problems among which are also suicide attempts. Such stress will assumably lead to an increase of suicide rates during the coronavirus pandemic. The aim of the study was to determine a frequency of suicide behavior among patients that were treated at Psychiatry Clinic at the Clinical Center of Vojvodina and to determine psychopathological and sociodemographic characteristics of this cohort. A total of 112 patients' anamnestic data that contained information about a suicide attempt in 2020 were analysed. The data were statistically analysed in JASP 0.14.1 and Microsoft Excel 2016.

The results were presented in tables and graphs. The analysis of collected data showed that suicide attempts were more frequent among female patients, 11-24 and 35-44 years old. We found no statistically significant correlation between suicidality and COVID-19 motivation for suicide attempt. The most frequent method used for attempting suicide was drug intoxication. The frequency of suicide attempts was higher in the latter half of the year. Some patients attempted suicide more than once in 2020. Most of the patients have psychiatric comorbidities. During the coronavirus pandemic, an early diagnosis of psychiatric illness is of great importance. It is also significant for vulnerable groups to stay socially engaged in order to prevent as many as possible suicide attempts.

**KEYWORDS:** suicide, suicide attempt, pandemic, self-poisoning, COVID-19



## IN SILICO ANALIZA TOKSIKOGENOMSKIH PODATAKA O UTICAJU TOKSIČNIH METALA NA KOMPLIKACIJE BOLESTI COVID-19

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Izloženost ljudi na radnom mestu ili iz životne sredine toksičnim metalima može da izazove štetne efekte na plućima, bubrezima, jetri, itd. Sa pojavom infektivnog oboljenja, COVID-19, koje izaziva novi korona virus 2 (SARS-CoV-2), nametnulo se pitanje da li izloženost toksičnim metalima može da doprinese razvoju komplikacija ove bolesti, budući da virus dominantno deluje na pluća. Međutim, ostavlja posledice i na druge organe. Cilj ovog rada je bio da in silico analizom toksikogenomskih podataka ispita uticaj toksičnih metala (arsena, kadmijuma, olova, žive, nikla i hroma) na komplikacije bolesti COVID-19. Korišćenjem Komparativne toksikogenomske baze podataka (engl. Comparative Toxicogenomic Database, CTD) i My Venn alata, pronađeno je 5 zajedničkih gena od interesa (IL1B, CXCL8, IL6, IL10 i TNF) za svih 6 toksičnih metala i utvrđeno da toksični metali pretežno povećavaju ekspresiju tih gena.

Daljom analizom je pronađeno 20 gena srodnih zajedničkim i istraženi su tipovi interakcija među njima pomoću alata GeneMania (<https://genemania.org/>). Fizičke interakcije su bile dominantne između gena od interesa, dok je kod gena pojedinačnih metala dominirala ko-ekspresija. Biološki procesi, molekularne funkcije i molekularni putevi u kojima učestvuje ispitivanih 25 gena (5 zajedničkih i 20 srodnih), dobijeni Gene Ontology analizom, prvenstveno su odgovorni za regulaciju aktivnosti citokina. Prekomeran imunski odgovor i produkcija proinflamatornih citokina koji dovode do citokinske oluje su odgovorni za razvoj komplikacija COVID-19. Dobijeni rezultati ukazali su na to da geni na koje deluju ispitivani toksični metali utiču na citokine i njihovu aktivnost. Stoga, izloženost ovim supstancama bi mogao biti jedan od faktora koji doprinosi razvoju komplikacija COVID-19.

**KLJUČNE REČI:** toksični metali, komplikacije COVID-19, citokini, in silico, CTD baza



## IN SILICO ANALYSIS OF TOXICOGENOMIC DATA ON THE INFLUENCE OF TOXIC METALS ON THE COMPLICATIONS OF COVID-19 DISEASE

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Exposure of people to toxic metals in workplace or environment can have harmful effects on the lungs, kidneys, liver, etc. With the emergence of an infectious disease, COVID-19, caused by the new coronavirus 2 (SARS-CoV-2), the question arose whether exposure to toxic metals contributes to the development of complications of this disease, since this virus mainly affects lungs. However, it also leaves consequences on other organs. This work aimed to investigate the influence of toxic metals (arsenic, cadmium, lead, mercury, nickel and chromium) on the complications of COVID-19 disease by in silico analysis of toxicogenomic data.

Using the Comparative Toxicogenomic Database (CTD) and the My-Venn tool, 5 common genes of interest (IL1B, CKSCL8, IL6, IL10 and TNF) were found to be of predominantly elevated expression after the exposure to all 6 metals. In further analysis, 20 related genes were found, and the nature of interactions between them was investigated by GeneMania (<https://genemania.org/>). Physical interactions between genes of interest were the most prominent, while co-expression dominated in the case of individual metal genes. The biological processes, molecular functions and pathways involving these 25 genes (5 common and 20 related), identified by Gene Ontology analysis were primarily related to cytokines. Excessive immune response and production of pro-inflammatory cytokines leading to cytokine storm are responsible for development of COVID-19 complications. The obtained results suggest that the genes affected by the studied toxic metals influence cytokines and, therefore, their activity could be one of the factors contributing to the development of COVID-19 complications.

**KEYWORDS:** toxic metals, COVID-19 complications, cytokines, in silico, CTD base



## TOKSIČNOST I ZLOUPOTREBA IVERMEKTINA TOKOM PANDEMIJE COVID-19

COVID-19 VS  
TOXICOLOGY

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Pandemija COVID-19 (Corona virus disease) već više od tri godine predstavlja ozbiljnu pretnju po javno zdravlje širom sveta. Tretman teških COVID 19 infekcija podrazumeva kombinaciju antimikrobnih, imunosupresivnih, imunomodulatornih lekova, i simptomatsko suportivnu terapiju. Međutim, širenje dezinformacija i naučno neutemeljenih informacija je doprinelo povećanju samolečenja i zloupotrebi lekova, pre svega antiparazitika ivermektina. Ivermektin efikasno deluje na larvene i odrasle oblike gastrointestinalnih i plućnih nematoda; artopode/ektoparazite poput: insekta, krpelja, buva, šugarca, vaši kod goveda, ovaca, svinja, konja, pasa i mačaka, kao i protiv infektivnih larvi srčanih crva kod pasa. Kao humani lek koristi se protiv infestacije crvima, prvenstveno u lečenju onhocercijaze, strongiloidijaze, askarijaze ili filarijaze, epidermalnih parazitskih kožnih bolesti, uključujući šugu i vaške. Za sada nema kliničkih potvrda o efikasnosti u lečenju virusnih infekcija.

Međutim, podaci in vitro studija koji sugerišu da ivermektin inhibira  $\alpha/\beta$  -1 transportne proteine koje virusi koriste u suzbijanju antivirusnog odgovora domaćina su bile osnov za pojedine naučne studije u kojima su dokazane ozbiljne greške u metodologiji rada i predstavljanju rezultata. Naime, senzacionalističko objavljeno, nepouzdana i lažni naučni podaci, naneli su ogromnu štetu i doprineli zloupotrebi ivermektina u terapiji COVID-19, usled čega su širom sveta zabeležena višestruka trovanja i predoziranje ivermektinom zbog neadekvatne primene. Upravo, nepravilna primena ivermektina uzrokuje brojna neželjena dejstva, od blažih gastrointestinalnih u vidu dijareje i povraćanja, do izuzetno ozbiljnih toksičnih efekata na nervni i mišićni sistem, poput konfuzija, halucinacija, somnolencije, ataksije, pareza, mišićnih spazama, koji mogu završiti letalnim ishodom.

**KLJUČNE REČI:** ivermektin, COVID-19, zloupotreba, toksičnost



## IVERMECTIN TOXICITY AND MISUSE DURING THE COVID-19 PANDEMIC

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The pandemic COVID-19 (Corona virus disease) has been a serious threat to public health for more than three years. Treatment of severe COVID 19 infections includes a combination of antimicrobial, immunosuppressive, immunomodulatory drugs, and symptomatic supportive therapy. However, the spread of false and scientifically unsupported information has contributed to the increase in self-medication and abuse of drugs, including ivermectin. Ivermectin is effective against larval and adult forms of gastrointestinal and pulmonary nematodes, ectoparasites in farm and companion animals, as well as against heartworm larvae in dogs.

In human medicine, it is used against worm infestation, primarily in the treatment of onchocerciasis, strongyloidiasis, ascariasis or filariasis, epidermal parasitic skin diseases, including scabies and head lice. So far, there are no clinical confirmations of effectiveness in the treatment of viral infections. However, data from in vitro studies suggesting that ivermectin inhibits the  $\alpha/\beta$  -1 transport proteins that viruses use to inhibit the host's antiviral response were the basis for certain scientific studies with serious errors in the methodology and results. Sensational, unreliable and false scientific data caused enormous damage and contributed to the abuse of ivermectin in the treatment of COVID-19, as a result of which poisonings and overdoses of ivermectin were recorded worldwide. Precisely, improper use of ivermectin causes numerous side effects, from mild gastrointestinal (diarrhea and vomiting), to serious toxic effects on the nervous and muscular system, such as confusion, hallucinations, somnolence, ataxia, paresis, muscle spasms, which can end in a fatal outcome.

**KEYWORDS:** ivermectin, COVID-19, misuse, toxicity



## THE CHARACTERISTICS OF INTENTIONAL POISONING DURING COVID-19 OUTBREAK IN SOUTH KOREA

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To evaluate the impact of coronavirus disease (COVID-19) on intentional poisoning trends, we analyzed patients visited emergency department (ED) following intentional poisoning. We retrospectively collected the data of patients who visited ED following intentional poisoning before (March to December 2019) and after (March to December 2020) the COVID-19 outbreak in one university hospital in South Korea. 927 and 996 patients visited ED following intentional poisoning and after the COVID-19 outbreak, respectively. The number of female was 566 vs. 641 ( $p=0.008$ ), and the number of those in their twenties (20s) was 223 vs. 285 ( $p<0.001$ ), respectively. The proportion of patients with intentional poisoning increased after the outbreak (44% vs. 6.4%,  $p=0.009$ ). The outcomes of patients following hospital admission were significantly different in terms of increased safe discharge numbers, and less number of deaths during admission. During the COVID-19 outbreak, we found notable characteristic changes in the rate of intentional poisoning in young adults with decreased severity of intentional poisoning among patients who visited ED.

**KEYWORDS:** poisoning, COVID-19





## EKOTOKSIKOLOGIJA / ECOTOXICOLOGY

### REZIDUE KOFEINA I NESTEROIDNIH ANTIINFLAMATORNIH LIJEKOVA U POVRŠINSKIM VODAMA U BIH

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Lijekovi i njihovi metaboliti sve se češće detektiraju u otpadnim vodama, površinskim vodama, podzemnim vodama, morima i tlu. Nepostojanje striktne zakonske regulative o otpuštanju lijekova u okoliš i njihova sve veća upotreba svakako je razlog njihovog globalnog prisustva u okolišu, tipično u koncentracijama između ng/l i µg/l. Međutim, ove koncentracije su dovoljne da ispolje značajne neželjene efekte na vodene i kopnene organizme u okolišu. Cilj ovog rada jeste odrediti rezidue nesteroidnih antiinflamatornih lijekova (NSAIL) i kofeina u površinskim vodama u Bosni i Hercegovini. Kofein je korišten kao marker kontaminacije rijeka otpadnim vodama. Sakupljeni su uzorci vode iz rijeka Miljacke, Željeznice, Bosne i Neretve (N=9). Nakon filtracije, ekstrakcija na čvrstim fazama (SPE) provedena je na Select HLB kertridžima.

Za derivatizaciju korišten je N-metil-N-(trimetilsilil)-trifluoracetamid (MSTFA), a uzorci su analizirani GC-MS metodom. Ibuprofen, naproksen, salicilna kiselina i kofein detektovani su u većini uzoraka, dok nijedan uzorak nije sadržavao ketoprofen i diklofenak. Koncentracije analgetika bile su jednake ili manje u odnosu na koncentracije određene u rijekama drugih evropskih zemalja. Dobar status imala je rijeka Neretva, gdje je pronađena samo salicilna kiselina. Generalno, veće koncentracije analita odgovarale su uzorcima područja sa većim brojem stanovnika i većom gustinom naseljenosti.

Kako bi se dobila cjelokupna slika ekološkog statusa rijeka u Bosni i Hercegovini, odnosno njihovog zagađenja lijekovima i otpadnim vodama, korisno bi bilo provesti studiju sa analizom sedimenta.

**KLJUČNE REČI:** gasna hromatografija, masena spektrometrija, NSAIL, kofein, površinske vode



## RESIDUES OF CAFFEINE AND NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN SURFACE WATERS IN BIH

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Pharmaceuticals and their metabolites are increasingly reported in wastewater, surface water, groundwater, seas, and soil. The lack of strict legal regulations regarding the release of pharmaceuticals into the environment and their increasing use is certainly the reason for their global presence in the environment, typically in ng/l to µg/l range. However, these concentrations are sufficient to show significant adverse effects on aquatic and terrestrial organisms in the environment. The aim of this study is residue determination of non-steroidal anti-inflammatory drugs (NSAIDs) and caffeine in surface waters of Bosnia and Herzegovina. Caffeine is used as a marker for the presence of contamination by wastewater in surface waters.

Samples of water from rivers Miljacka, Željeznica, Bosna, and Neretva were collected (N=9). After filtration, solid phase extraction (SPE) was performed on Select HLB cartridges. N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was used for derivatization, and the samples were analyzed by GC-MS. Ibuprofen, naproxen, salicylic acid and caffeine were detected in most samples, while none of the samples contained ketoprofen or diclofenac. The concentrations of analgetics were similar to or lower than the concentrations found in rivers of other European countries. The Neretva river had a good status, with samples only containing salicylic acid. Generally, higher concentrations of analytes corresponded to sampling areas that had a larger population and higher population density. In order to obtain a complete picture of the ecological status and pollution by pharmaceuticals and waste water of rivers in Bosnia and Herzegovina, it would be useful to conduct a sediment analysis study.

**KEYWORDS:** gas chromatography, mass spectrometry, NSAID, caffeine, surface water



# STANJE ZAGAĐENOSTI I EKOLOŠKI RIZIK ODABRANIH NEORGANSKIH I ORGANSKIH POLUTANATA U SEDIMENTU JEZERA MODRAC

ECOTOXICOLOGY

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Voda iz najvećeg vještačkog jezera Modrac, u opštini Lukavac, Bosna i Hercegovina, koristi se za snabdijevanje industrijskih postrojenja, ali i za javnu potrošnju u Kantonu Tuzla. Prethodne analize vode i uzoraka riba, pokazale su prisustvo visokih koncentracija metal(oid)a (npr. As, Pb, Cd) i nekih organskih polutanata (npr. polihlorovanih bifenila, PCB). Pošto sediment ima ulogu rezervoara za teške metale i organske polutante, može imati značajnu ulogu u transportu i sudbini polutanata u vodenoj sredini. Iz tog razloga, procjena statusa zagađenosti sedimenta, vrlo je važna u upravljanju kvalitetom površinskih voda. Kako bi se procijenilo stanje zagađenosti i izračunao ekološki rizik povezan sa prisustvom metala/metaloida (Pb, Cd, Ni, As, Se, Cr, Zn, Mn, Cu) i organskih polutanata npr. 7 indikatorskih PCB i organohlornih pesticida (ciklodieni, lindan), 5 površinskih uzoraka sedimenta sakupljeno je sa različitih lokacija na jezeru Modrac.

Stepen kontaminacije metalima/metaloidima procijenjen je korištenjem nekoliko indeksa (indeks geokumulacije, indeks opterećenja polutantima, faktor kontaminacije, stepen kontaminacije, indeks potencijalnog ekološkog rizika). Prosječne koncentracije metala/metaloida (analizirani pomoću GF-AAS i živinog analizatora) u sedimentu imale su slijedeći opadajući raspored Ni>Mn>Se>Cr>Pb>As>Cu>Hg>Zn>Cd. Indeksi zagađenja ukazali su na niske ili umjerene nivoe kontaminacije za Cr, Zn, Mn, Cu i Cd. Indeks opterećenja polutantima (raspon: 2,42-4,31) i indeks potencijalnog ekološkog rizika (raspon 688,55-1879,1) na svih pet lokacija uzorkovanja pokazao je značajno opterećenje polutantima sa vrlo visokim ekološkim rizikom. Sadržaj organskih polutanata, određen pomoću GC-ECD metode bio je u rasponu od n.d do 11,4 µg/kg suhe mase, n.d.-0,223 µg/kg suhe mase i n.d.-0,065 µg/kg suhe mase za sumu 7PCB, aldrin i lindan, respektivno.

**KLJUČNE REČI:** stanje zagađenosti, ekološki rizik, organski polutanti, teški metali



# POLLUTION STATUS AND ECOLOGICAL RISK OF SELECTED INORGANIC AND ORGANIC POLLUTANTS IN SEDIMENT OF MODRAC LAKE

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Water from the largest artificial lake Modrac located in the municipality of Lukavac, Bosnia, and Herzegovina, is used for the industrial, but also public water supply in Tuzla Canton. Previous analysis of water and fish samples showed high concentrations of metal(oid)s (i.e. As, Pb, Cd) and some organic pollutants (i.e. polychlorinated biphenyls, PCBs). Since sediment is a sink for heavy metals and organic pollutants it plays a significant role in the transport and fate of pollutants in aquatic environments. Therefore, the assessment of sediment pollution status is very important in water quality management. In order to evaluate pollution status and calculate the ecological risk of metals/metalloids (Pb, Cd, Ni, As, Se, Cr, Zn, Mn, Cu) and organic pollutants i.e. 7 indicators PCBs and organochlorine pesticides (cyclodienes, lindane), 5 grab-samples of sediments were collected from different locations at the Modrac Lake.

The degree of contamination with metals/metalloids was assessed using the contamination indices (geoaccumulation index; pollution load index; contamination factor, contamination degree, potential ecological risk index). The average concentration of metals/metalloids (analyzed by GF-AAS and mercury analyzer) in sediment followed a decreasing order of Ni>Mn>Se>Cr>Pb>As>Cu>Hg>Zn>Cd. Pollution indices showed low or moderate levels of contamination for Cr, Zn, Mn, Cu, and Cd. The pollution load index (range: 2.42-4.31) and Potential ecological risk index (range 688.55-1879.1) at all five sampling sites showed a strong indication of pollution with very high ecological risk. The content of organic pollutants, determined by means of GC-ECD ranged from n.d to 11.4 µg/kg d.w., n.d.-0.223 µg/kg d.w. and n.d.-0.065 µg/kg d.w, for the sum of 7PCBs, aldrin, and lindane, respectively.

**KEYWORDS:** pollution status, ecological risk, organic pollutants, heavy metals



## UTICAJ INDIVIDUALNIH FAKTORA I STILA ŽIVOTA NA NIVOE OLOVA I KADMIJUMA U KRVI STANOVNIKA BEOGRADA

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Olovo (Pb) i kadmijum (Cd) su toksični metali kojima je stanovništvo primarno izloženo iz životne i/ili radne sredine. Dugotrajna izloženost čak i niskim koncentracijama Pb i Cd može doprineti razvoju različitih bolesti. Cilj istraživanja je određivanje nivoa Pb i Cd u punoj krvi stanovnika Beograda dobrovoljnih davaoca krvi i identifikovanje faktora koji utiču na njihove koncentracije. Analiziranje je izvršeno metodom ICP-MS. Faktori čiji su uticaji ispitivani su pol, starost, indeks telesne mase, poreklo, pušenje, bavljenje sportom, blizina saobraćajnica, obrazovanje i ekonomski status. Dobijene koncentracije Pb i Cd u krvi stanovnika Beograda su u opsegu vrednosti dobijenih u zemljama u okruženju (sr.vr.±std: 21,7±15,6 µg/L za Pb, 0,79±0,87 µg/L za Cd).

Faktori koji značajno utiču na nivoe Pb i Cd su pušenje, godine života i pol, a na Cd i blizina saobraćajnica. Aktivni i bivši pušači imaju 1,3 puta više nivoe Pb u odnosu na nepušače, a aktivni 2,5 puta više vrednosti Cd u odnosu na nepušače i bivše pušače. Mlađi učesnici (18–37 godina) imaju 1,3 puta niže vrednosti Pb i Cd. Muškarci u odnosu na žene imaju za skoro 50% više Pb i 30% niže nivoe Cd. Osobe čije je prebivalište udaljeno manje od 100 m od prometnih saobraćajnica imaju više vrednosti Cd u krvi. Dobijeni podaci potvrđuju uticaj pušenja, starosti i pola na povišene nivoe Pb i Cd, kao i da stanovanje blizu prometnih saobraćajnica nosi rizik od veće ekspozicije Cd.

**KLJUČNE REČI:** olovo, kadmijum, pušenje, starost, pol, blizina saobraćajnica



## THE INFLUENCE OF INDIVIDUAL FACTORS AND LIFESTYLE ON THE LEVEL OF LEAD AND CADMIUM IN THE BLOOD OF BELGRADE RESIDENTS

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Lead (Pb) and cadmium (Cd) are toxic metals whose primary exposure is through the living and/or working environment. Even long-term exposure to low concentrations can affect the development of some disorders. The aim of this study was to determine the levels of Pb and Cd in the whole blood of the residents of Belgrade, voluntary blood donors, and to identify factors that most influence their concentrations. The analysis was performed using the ICP-MS method. Factors whose influences were examined were gender, age, body mass index, origin, smoking, sports, proximity of the place of residence to heavy traffic, education and economic status.

The obtained Pb and Cd concentrations were in the range of values obtained in neighboring countries (mean ± std: 21.7±15.6 µg/L for Pb, 0.79±0.87 µg/L for Cd). Factors that significantly affected Pb and Cd were smoking, age and sex, and proximity to traffic roads for Cd. Active and ex-smokers had similar Pb levels, and 1.3 times higher than non-smokers. Active smokers had 2.5 times higher Cd than non-smokers and ex-smokers. Younger participants (18–37 years old) had 1.3 times lower Pb and Cd values. Men had 50% more Pb and 30% less Cd. People living less than 100 m from heavy traffic had higher Cd values. The obtained data confirms the existing knowledge about the influence of smoking, age and gender on blood Pb and Cd levels, as well as that living near heavy traffics carries the risk of higher exposure to Cd.

**KEYWORDS:** lead, cadmium, smoking, age, gender, heavy traffic vicinity



## PROMENE PROTEOMA MODEL BAKTERIJE PSEUDOMONAS AERUGINOSA SAN AI EKSPONIRANE NA CITOTOKSIČNU NANOCERIJU

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Nanočestice su u fokusu brojnih istraživanja zbog svojih jedinstvenih osobina i raznovrsne primene. Povećana komercijalna upotreba nanocerije (NC) zahteva i proučavanje potencijalnih rizika povezanih sa njenim efektima na žive organizme. Kao model organizam za proučavanje i dublje razumevanje uticaja nanomaterijala korišćen je ekološki izolat Gram-negativne bakterije *Pseudomonas aeruginosa* san ai. Praćene su promene u ukupnom proteomu, kako bi se identifikovao uticaj NC na procese anabolizma i katabolizma glavnih biomolekula, kao i očuvanje redoks homeostaze.

Podaci proteomskih studija su kuplovani sa promenama profila određenih sekundarnih metabolita. Kvantitativna analiza ukupnog proteoma *P. aeruginosa* san ai izloženog delovanju NC verus kontrola je urađena pomoću sveobuhvatne, visokopropusne bioanalitičke nLC-MS/MS platforme kuplovane sa bioinformatikom. Promene u sekundarnom metabolizmu su rađene klasičnim metodama analize ciljanog metaboloma. Povećana je produkcija proteina povezanih sa redoks homeostazom, biosintezom aminokiselina i katabolizmom lipida pri izlaganju NC. Kao dokaz promena u redoks homeostazi, povećana je produkcija piocijanina, ključnog redoks molekula, i pioverdina, siderofore odgovorne za homeostazu gvožđa. Iako NC nije pokazala letalan citotoksičan efekat, izazvala je brojne promene kod *P. aeruginosa* san ai, što jasno pokazuje da treba biti oprezan sa upotrebom nanomaterijala. Osobito zbog povećane proizvodnje faktora virulencije, *P. aeruginosa* može postati potentan patogen, iako je po prirodi oportunistički.

**KLJUČNE REČI:** nanoceria, *Pseudomonas aeruginosa*, proteome, metaboliti



## CHANGES IN THE PROTEOME OF THE MODEL BACTERIUM PSEUDOMONAS AERUGINOSA SAN AI EXPOSED TO CYTOTOXIC NANOCERIA

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Nanoparticles have gained the attention of the scientific community due to their unique properties and diverse applications. The increased commercial use and spread of nanoceria (NC) raises concerns about the risks associated with its effects on living organisms. An environmental isolate of the Gram-negative bacterium *Pseudomonas aeruginosa* san ai was used as a model organism for the study and deeper understanding of the impact of nanomaterials. In order to identify the influence of NC on the processes of metabolism of the main biomolecules, and preservation of redox homeostasis, changes in the total proteome were monitored, coupled with changes in the profile of targeted secondary metabolites. Comparative proteomic analysis was performed using a comprehensive, high-throughput bioanalytical nLC-MS/MS platform coupled with bioinformatics.

Changes in secondary metabolism were performed using classical methods of analysis of the targeted metabolome. Increased production of proteins related to redox homeostasis, amino acid biosynthesis and lipid catabolism upon NC exposure was observed. As evidence of changes in redox homeostasis, increased production of pyocyanin, a key redox molecule, and pyoverdine, a siderophore responsible for iron homeostasis was detected. Although NC did not show a lethal cytotoxic effect, it induced numerous changes in *P. aeruginosa* san ai, which clearly indicates that caution should be exercised with the use of nanomaterials. Especially due to the increased production of virulence factors, *P. aeruginosa* can become a potent pathogen, although it is opportunistic in nature.

**KEYWORDS:** nanoceria, *Pseudomonas aeruginosa*, proteome, metabolites





## PSEUDOMONAS U BIOREMEDIJACIJI TOKSIČNIH ORGANSKIH JEDINJENJA

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Zagađenje životne sredine fenolnim jedinjenjima, naftnim ugljovodonicima, pesticidima, plastikom i drugim organskim zagađivačima predstavlja opasan rizik, kako za ekosistem u celini, tako i za zdravlje ljudi. Ekološki izolat poliektremofilne, hidrokarbonoklastične bakterije *Pseudomonas aeruginosa* san ai poseduje značajan potencijal za razgradnju različitih ksenobiotika, zahvaljujući postojanju razgranatih puteva sekundarnog metabolizma. Plastičnost metabolizma ugljenika je jedna od adaptivnih strategija vrsta roda *Pseudomonas* na izloženost toksičnim organskim jedinjenjima. Ključ za razumevanje mehanizama prilagođavanja *Pseudomonas* na kontaminirano okruženje leži u promenama proteoma i metaboloma, koji se mogu efikasno pratiti novim, sveobuhvatnim, visokopropusnim bioanalitičkim omiks platformama.

Cilj rada je ispitivanje potencijala *Pseudomonas aeruginosa* san ai za biodegradaciju različitih organskih jedinjenja, proučavanjem mehanizama adaptacije mikroorganizma pomoću kvantitativnog proteomskog pristupa i puteva transformacije zagađujućih supstanci pomoću targetiranog/ciljanog metabolomskog pristupa. Ukupni proteom i ciljani metabolom *P. aeruginosa* san ai gajenog na nekoliko različitih izvora ugljenika: natrijum-benzoat (3 mM), 2,6-di-terc-butilfenol (100 mg/L) i fluoren (10 mg/L), versus glukoza (5 mM) i suncokretovo ulje (100 µL/L), su analizirani pomoću platformi nLC-MS/MS kuplovane sa bioinformatikom (proteom) i GCxGC-MS (metabolom). Proteomska analiza *P. aeruginosa* san ai eksponiranog na organske zagađivače, uporedo sa analizom genoma i ciljanog metaboloma, je potvrdila postojanje ključnih enzima i metabolita β-ketoadipatne putanje za degradaciju aromatičnih jedinjenja, uključujući disupstituisani alkilfenol. Zahvaljujući potencijalu za razgradnju organskih zagađivača aromatične strukture, *P. aeruginosa* san ai bi mogao poslužiti u bioremedijaciji životne sredine, ali bi i pažljivo selektovani proteini/enzimi i metaboliti, mogli biti biomarkeri kontaminacije aromatičnim, zagađujućim supstancama.

**KLJUČNE REČI:** biodegradacija, *Pseudomonas*, organska jedinjenja, proteom, metabolom, ekotoksikoproteomiks



# PSEUDOMONAS IN BIOREMEDIATION OF TOXIC ORGANIC COMPOUNDS

ECOTOXICOLOGY

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Environmental pollution with phenolic compounds, petroleum hydrocarbons, pesticides, plastic and other organic pollutants is a dangerous risk to the ecosystem and human health. The ecological isolate, polyextremophilic, hydrocarbonoclastic *Pseudomonas aeruginosa* strain has a significant potential for the degradation of various xenobiotics due to its plasticity of carbon metabolism and branched pathways of secondary metabolism. The key to understanding the mechanisms of adaptation of *Pseudomonas* to a contaminated environment lies in the changes in the proteome and metabolome, which can be efficiently monitored by new, comprehensive, high-throughput bioanalytical omics platforms.

The aim of the work is to examine the potential of *Pseudomonas aeruginosa* strain for the biodegradation of various organic compounds, by studying the mechanisms of microorganism adaptation using a quantitative proteomic approach and the pathways of transformation of polluting substances using a targeted metabolomic approach. Total proteome and targeted metabolome of *P. aeruginosa* strain grown on several different C-sources: sodium-benzoate, 2,6-di-tert-butylphenol, fluorene, versus glucose, sunflower oil, were analyzed using nLC-MS/MS coupled with bioinformatics (proteome) and GCxGC-MS (metabolome) platforms. Proteomic analysis of *P. aeruginosa* strain exposed to organic pollutants, along with genome and targeted metabolome analysis, confirmed the existence of key enzymes and metabolites of the  $\beta$ -ketoacid pathway for the degradation of aromatic compounds, including disubstituted alkylphenol. Thanks to the potential for the decomposition of organic pollutants with an aromatic structure, *P. aeruginosa* strain could serve in environment bioremediation, but carefully selected proteins/enzymes and metabolites could also be biomarkers of contamination.

**KEYWORDS:** biodegradation, *Pseudomonas*, organic compounds, proteome, metabolome, ecotoxicoproteomics



## WATER AND SOIL CONTAMINANTS - ARE CHALLENGES TO ECOSYSTEM AND HUMAN HEALTH ARSENIC, NITRATES AND NITRITES?

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The quality of the water we drink directly impacts our health. For primary prevention, especially for kids, it is essential to assess and prevent water-borne illnesses. One of the greatest environmental disasters in one area is the high concentrations of the carcinogenic metalloid arsenic (As) in groundwater system. The main purpose of this study was to investigate the quality of drinking water and soil in various areas. The level of nitrates, nitrites and arsenic was determined, as well as the way in which they affect human health and the surrounding ecosystem.

Nitrites and nitrates are largely spread in the environment and we can naturally find them in vegetables and fruit as part of the azote cycle. Due to their beneficial properties against the microbial contamination and chemical changes, they are frequently used in terms of additives in meat products, milk products or cereals. Some vegetables, as for example the unprocessed spinach, the green salad, celery, zucchini and beatroot may contain large concentrations of nitrites and nitrates, and the large consumption of these substances determine a high level of nitrates and nitrites in organisms. Along with the methemobinizing action of these compounds, they take into account the reduction of the risk of the occurrence of nitrosamines with cancer effect, nitrates and nitrites are also responsible for the formation of these compounds. Water samples were analyzed for various physicochemical parameter pH, turbidity, color, odor, taste, conductivity (EC), total hardness (Calcium + Magnesium), chloride, arsenic, phosphate, lead, ammonium ion, nitrate and nitrite. We consider it necessary to develop the research in this direction to monitorize both the contamination with arsenic and nitrates, nitrites in the drinkable water and soil.

**KEYWORDS:** arsenic, nitrates, nitrites, drinkable water, soil



## SILICIJUM U FLAŠIRANIM VODAMA

ECOTOXICOLOGY

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Cilj rada je utvrditi tačnu koncentraciju silicijuma u flaširanim prirodnim vodama na našem tržištu. Silicijum u vodama se analizira prema Pravilniku o higijenskoj ispravnosti vode za piće (Sl. list SRJ, 42/98 i 44/99) i Pravilniku o kvalitetu i drugim zahtevima za prirodnu mineralnu vodu, prirodnu izvorsku i stonu vodu (Sl. List SCG 53/2005 i Službeni glasnik RS 43/2013). Ispitano je 23 različitih voda na našem tržištu od slabomineralnih do jako gaziranih. Metoda koja se koristi prilikom određivanja je ICP OES, na aparatu Shimadzu 9820 ICPE. Pravilnicima nisu predviđene MDK vrednosti za Si u vodama.

Koncentracija Si u analiziranim flaširanim prirodnim vodama sa našeg tržišta kretala se od 2,24 mg/l do 54 mg/l. Većina prirodnih voda koje se flaširaju imaju izbalansiran odnos anjona i katjona, odličan balans minerala, čijim unosom zadovoljavamo naše dnevne potrebe za hidratacijom i esencijalnim mineralima. Silicijum dioksid (SiO<sub>2</sub>) je mineral koji usporava starenje i podmlađuje telo i u svetu je poznat i kao mineral lepote, jer učestvuje u formiranju proteina kolagena i elastina koji koži daju elastičnost, punoću i čvrstinu. Sa godinama količina ovog minerala u organizmu opada i važno je obezbediti njegov dodatni unos, koji je najlakši i najbrži putem vode.

**KLJUČNE REČI:** flaširana voda, silicijum, silicijumdioksid



## SILICATE IN BOTTLED WATERS

### ECOTOXICOLOGY

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The aim of the work is to determine the exact concentration of silicate in bottled natural waters on our market. Silicate in water is analyzed according to the Rulebook on the hygienic suitability of drinking water (Official Gazette of the FRY, 42/98 and 44/99) and the Rulebook on the quality and other requirements for natural mineral water, natural spring and table water (Official Gazette of SCG 53 /2005 and Official Gazette of RS 43/2013). We tested 23 different waters on our market, ranging from low-mineral to highly carbonated. The method used for the determination is ICP OES, on a Shimadzu 9820 ICPE apparatus.

The regulations do not provide MDK values for Si in water. The concentration of Si in the analyzed bottled natural waters from our market ranged from 2.24 mg/l to 54 mg/l. Most of the natural bottled waters have a balanced ratio of anions and cations, an excellent balance of minerals, with the intake of which we meet our daily needs for hydration and essential minerals. Silica dioxide (SiO<sub>2</sub>) is a mineral that slows down aging and rejuvenates the body, and is also known in the world as a mineral of beauty. Because it participates in the formation of collagen and elastine proteins that give the skin elasticity, fullness and firmness. With age, the amount of this mineral in the body decreases and it is important to ensure its additional intake, which is easiest and fastest through water.

**KEYWORDS:** bottled water, silicate, silica dioxide



## KRETANJE KONCENTRACIJE AMONIJAKA U AMBIJENTALNOM VAZDUHU POSLE AKCIDENTA

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Prema Uredbi o uslovima za monitoring i zahtevima kvaliteta vazduha (Sl. glasnik RS br.11/2010,75/2010 i 63/13) nivo zagađenosti vazduha prati se merenjem nekih drugih zagađujućih materija u vazduhu, a amonijak je prepoznat samo u delu koji se odnosi na namenska merenja. Cilja rada je da se prati kretanje koncentracije amonijaka u ambijentalnom vazduhu posle akcidenta, prilikom koga je veća količina gasa isticurela iz jedne od cisterni. Amonijak u uzorcima vazduha analiziran je validovanom spektrofotometrijskom metodom, metodom sa kalijumtetrajodomerkuratom, odnosno Neslerovim reagensom.

Uzorkovanje vazduha vršeno je u vremenskim intervalima od 24 h. Prvo uzorkovanje vazduha izvršeno je 24 h od akcidenta, i nastavljeno je sa uzorkovanjem jednom dnevno u narednih sedam dana. Merna mesta uzorkovanja u odnosu na mesto akcidenta su bila na 100 m, na 10 km i 60 km. Kretanje koncentracija amonijaka variralo je od dana do dana, u zavisnosti od atmosferskih faktora, vazdušnog pritiska, temperature i strujanja vazduha. Rezultati merenja koncentracije amonijaka na lokaciji 100 m od mesta akcidenta kretali su se od 230 µg/m<sup>3</sup> do 2041,7 µg/m<sup>3</sup>, a na lokaciji 10 km i 60 km od mesta akcidenta sva merenja su bila ispod MDK (100 µg/m<sup>3</sup>). Povišene vrednosti koncentracije amonijaka u ambijentalnom vazduhu posle akcidenta izmerene su samo na 100 m od mesta akcidenta. Kretanje vrednosti amonijaka tokom perioda uzorkovanja pokazalo je varijacije u odnosu na vreme uzorkovanja i uticaja udaljenosti mesta uzorkovanja.

**KLJUČNE REČI:** aerozagađenje, amonijak, akcident



## TREND OF AMMONIA CONCENTRATION IN THE AMBIENT AIR AFTER THE ACCIDENT

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According to the Regulation on conditions for monitoring and air quality requirements (Official Gazette of the RS No. 11/2010, 75/2010 and 63/13), the level of air pollution is monitored by measuring some other pollutants in the air, and ammonia is recognized only in the part that refers to dedicated measurements. The aim of the work is to monitor the movement of ammonia concentration in the ambient air after the accident, during which a large amount of gas leaked from one of the tanks. Ammonia in air samples was analyzed by a validated spectrophotometric method, the method with potassium tetraiodomercurate, i.e. Nessler's reagent. Sampling of the air was carried out in time intervals of 24 hours.

The first air sampling was performed 24 hours after the accident, and continued with sampling once a day for the next seven days. The sampling measurement points in relation to the accident site were at 100 m, 10 km and 60 km. The movement of ammonia concentrations varied from day to day, depending on atmospheric factors, air pressure, temperature and air flow. The results of ammonia concentration measurements at a location 100 m from the accident site ranged from 230 µg/m<sup>3</sup> to 2041.7 µg/m<sup>3</sup>, and at a location 10 km and 60 km from the accident site, all measurements were below MDK (100 µg/m<sup>3</sup>). Elevated values of ammonia concentration in the ambient air after the accident were measured only 100 m from the accident site. The movement of ammonia values during the sampling period showed variations in relation to the sampling time and the influence of the distance of the sampling site.

**KEYWORDS:** air pollution, ammonia, accident



## PRAŠINA OD RASTAVLJANJA E-OTPADA KAO IZVOR HEMIKALIJA KOJE OMETAJU RAD ENDOKRINOG SISTEMA: IN SILICO TESTIRANJE TOKSIČNOSTI SMEŠE NA ŠTITNU ŽLEZDU I MUŠKI REPRODUKTIVNI SISTEM

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Aktivnosti prilikom rastavljanja e-otpada predstavljaju značajan izvor mnogih štetnih komponenti, što dovodi do svakodnevnog izlaganja radnika toksičnim isparenjima koja se sastoje od različitih metala i organskih jedinjenja. Svaka od ovih hemikalija iz životne sredine ponaosob ispoljava toksične efekte ometajući homeostazu hormona, a njihov potencijal da deluju kao endokrini ometači je već potvrđen. Prema tome, primenom in silico metoda ispitivanja, imali smo za cilj da: (i) procenimo uticaj ispitivane toksične smeše (dekabromodifenil etar, dekabromodifenil etan, tetrabromobisfenol A, olovo (Pb) i kadmijum (Cd) na indukciju poremećaja štitne žlezde i muškog reproduktivnog sistema, (ii) otkrijemo potencijalne molekularne mehanizme uključene u toksičnost smeše.

U našem istraživanju, komparativna toksikogenomička baza podataka (engl. Comparative Toxicogenomics Database, CTD), ToppGene Suite portal i GeneMANIA su korišćeni kao glavni in silico alati. Naši rezultati su pokazali 4 zajednička gena za sve hemikalije prisutne u smeši i poremećaje koji su povezani sa recikliranjem e-otpada. Najzapaženije interakcije između gena bile su fizičke interakcije (77,64%). Vezivanje NADP-a i antioksidativna aktivnost su bile najznačajnije molekularne funkcije, dok je odgovor na oksidativni stres bio ključni put pogođen izlaganjem odabranoj smeši, a u vezi sa poremećajima muškog reproduktivnog sistema i štitne žlezde. Naši rezultati su ukazali na ulogu oksidativnog stresa kao zajedničkog mehanizma uključenog u patogenezu navedenih bolesti. Dobijeni podaci su takođe istakli potencijalne rizike usled ekspozicije smeši različitih hemikalija tokom rastavljanja e-otpada. Međutim, dalje studije su potrebne da bi se potvrdili tačni mehanizmi toksičnosti smeše.

**KLJUČNE REČI:** bromovani usporivači gorenja, teški metali, oksidativni stres, interakcije, toksikogenomika





## DUST FROM E - WASTE DISMANTLING AS A SOURCE OF ENDOCRINE DISRUPTING CHEMICALS: IN SILICO TESTING OF MIXTURE TOXICITY ON THYROID GLAND AND MALE REPRODUCTIVE SYSTEM

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E-waste dismantling activities constitute a significant source of many harmful components, resulting in everyday exposure of workers to toxic fumes consisting of different metals and organic compounds. Each of these environmental chemicals individually exerts toxic effects by interfering with hormone homeostasis and their potential to act as endocrine disruptors (EDs) has already been confirmed. Thus, by using in silico testing methods, we aimed to (i) assess the impact of investigated toxic mixture (decabromodiphenyl ether (DBDE), decabromodiphenyl ethane (DBDPE), tetrabromobisphenol A (TBBPA), lead (Pb) and cadmium (Cd)) on the development of thyroid gland and male reproductive system disorders, (ii) reveal the potential molecular mechanisms involved in the mixture toxicity. In our research, Comparative Toxicogenomics Database (CTD), ToppGene Suite portal and GeneMANIA were used as the main in silico tools.

Our results have shown 4 genes common to all chemicals present in the mixture and e-waste recycling related disorders. The most notable interactions among genes were physical interactions (77.64%). NADP binding and antioxidant activity were the most significant molecular functions, while oxidative stress response was the key pathway affected by exposure to the selected mixture and related to the disorders of the male reproductive system and thyroid. Our results indicated the role of oxidative stress as a common mechanism involved in the pathogenesis of the aforementioned diseases. Obtained data also emphasized the potential exposure risks to a mixture of various chemicals during e-waste disassembling. However, further studies are needed to confirm the exact mixture toxicity mechanisms.

**KEYWORDS:** brominated flame retardants, heavy metals, oxidative stress, interactions, toxicogenomics



## UTICAJ ZAGAĐENJA VAZDUHA NA POJAVU I RAZVOJ KARCINOMA: DA LI SU UGROŽENA SAMO PLUĆA?

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Zagađenje vazduha predstavlja ekološki faktor rizika koji je povezan sa 1% svih slučajeva razvoja karcinoma i 2% svih smrtnih slučajeva usled razvoja karcinoma u Evropi. Postoji veliki broj podataka koji ukazuju na vezu između zagađenja vazduha, posebno suspendovanih čestica (PM), i pojave karcinoma pluća. Suspendovane čestice predstavljaju najvažniju komponentu zagađenog vazduha a sastoje se od metala i organskih molekula koji mogu dovesti do promena u ćeliji i promovisati karcinogenezu. Pored karcinoma pluća, epidemiološki podaci dovode u vezu ambijentalno zagađenje vazduha sa povećanim rizikom od razvoja karcinoma mokraćne bešike i dojke.

Na razvoj karcinoma jajnika takođe može uticati dugotrajna izloženost zagađenom vazduhu, usled genotoksičnosti i estrogene aktivnosti metala kao što su Cd i Pb koji se nalaze u PM česticama. Studije su dovele u vezu incidencu papilarnog karcinoma štitaste žlezde sa povećanjem koncentracije PM<sub>2,5</sub>. Dodatno, studije ukazuju na vezu između povećanih koncentracija PM<sub>2,5</sub> i gastrointestinalnih maligniteta, a posebno karcinoma jetre i kolorektalnog karcinoma. Poznato je da izlaganje PM<sub>2,5</sub> povećava incidencu karcinoma u detinjstvu, uglavnom limfoidne leukemije, kao i promociju metastaza kroz povećanje faktora rasta. Iako su potrebna dalja istraživanja, zagađenje vazduha može potencijalno biti povezano sa manjom verovatnoćom preživljavanja od karcinoma. Prevencija nastanka karcinoma povezanih sa zagađenjem vazduha zahteva višedimenzionalni pristup uključujući sprovođenje mera javnog zdravlja i zdravstvene politike. Potrebno je sprovesti dalje epidemiološke studije, posebno u zemljama u razvoju, sa ciljem rasvetljavanja uticaja zagađenja vazduha na razvoj karcinoma.

**KLJUČNE REČI:** zagađenje vazduha, karcinom dojke, karcinom mokraćne bešike, karcinom jajnika



## EXPOSURE TO OUTDOOR AIR POLLUTION AND CANCER DEVELOPMENT: IS AIR POLLUTION RESPONSIBLE FOR MORE THAN LUNG CANCER?

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Air pollution represents environmental risk factor linked to 1% of all cancer incidences and 2% of all cancer deaths in Europe. There is a strong body of data linking outdoor (ambient) air pollution, particularly particulate matter (PM), with lung cancer incidence. Among air pollutants, the most important PM is made up of a variety of toxic metals and organic molecules which promote cell changes and, consequently, carcinogenesis. Besides lung cancer, epidemiological data linking outdoor air pollution to an increased risk of developing urinary bladder and breast cancer.

The development of ovarian tumors may also be influenced by long-term exposure to air pollution by conceivable pathways, estrogen-like effects of metals such as Cd and Pb, and genetic alterations. Furthermore, some studies correlated the incidence of papillary thyroid cancer with an increase in fine PM 2.5  $\mu\text{m}$  (PM<sub>2.5</sub>) in air pollution. There is some evidence of links between PM<sub>2.5</sub> and gastrointestinal malignancies, with the strongest connections being seen in cases of liver and colorectal cancer. Furthermore, Exposure to PM<sub>2.5</sub> increased the incidence of childhood cancer, mostly lymphoid leukemia and can promotes cancer metastasis through increased growth factor. Although further research is required, outdoor air pollution may potentially be linked to a lower likelihood of surviving cancers. Cancer prevention associated with air pollution, requires multidimensional public health and policy measures, with more study needed especially in low- and middle-income countries. Further epidemiological studies on cancer incidence as well as the impact of air pollution on cancer development are needed.

**KEYWORDS:** air pollution, breast cancer, urinary bladder cancer, ovarian cancer



## LABORATORIJSKI POKAZATELJI DOBRI, A OSOBA SE OSEĆA LOŠE - DA LI LEČITI PACIJENTA ILI LABORATORIJSKE PARAMETRE?

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U lekarskoj praksi se često dešava da su laboratorijski parametri pacijenta uredni, a da se on oseća loše i žali se na brojne polimorfne tegobe. Postavlja se pitanje da li i šta lečiti, obzirom da su parametri uredni i da nema dijagnoze? Cilj apstrakta je da ukaže da fokus zdravstvenog radnika bi trebalo da bude na pacijentu i njegovim zdravstvenim tegobama, a ne na laboratorijskim parametrima koji često mogu biti uredni i pri postojećem zdravstvenom poremećaju. Brojne tegobe mogu, ali i ne moraju nužno da budu praćene poremećenim laboratorijskim analizama. Ne moraju da se uoče ni promene na EKG-u kao ni promene prilikom ultrazvučnog pregleda stomaka i male karlice. U skladu sa doktrinom dijagnostike u klasičnoj medicini, ovoj osobi se ne može postaviti dijagnoza jer sve što je urađeno, rezultiralo je urednim nalazom. Iz tog razloga, ovoj osobi se ne može prepisati ni terapija lekovima (nema dijagnoze – nema terapije!). Međutim, tegobe koje osoba oseća su se javile usled narušene homeostaze na diskretnom, ćelijskom nivou, što je posledica poremećaja biohemijskih i biofizičkih mehanizama (delom mogu biti uzrokovani i zagađivačima životne sredine).

U ovakvim slučajevima se redovno predlaže „praćenje“ za neki vremenski period. „Praćenjem“ zdravstvenog stanja osobe u određenom vremenskom periodu, zapravo se čeka da se kod osobe razvije neka patologija koja će se manifestovati i u laboratorijskim analizama, na ultrazvučnom pregledu i sl. kako bi se postavila dijagnoza i onda započelo sa „lečenjem“, ili da te tegobe (nekim čudom) same od sebe nestanu. Ne samo da se osoba ne motiviše da promeni životne navike koje bi poboljšale njeno zdravstveno stanje, već se osoba motiviše da nastavi da živi „normalno“, da živi “kao do sada“...; praktično, sugeriše se da sve što je radila i što ju je dovelo u stanje u kojem se nalazi, treba da nastavi da radi i dalje, ali da očekuje da će joj biti bolje. Iskustvo je pokazalo da to nije najbolji savet koji lekari daju pacijentima. Lečenje pacijenata ne bi trebalo samo da se svode na prepisivanje lekova, već bi zdravstveni radnici trebalo da s pacijentima intenzivno razgovaraju kako bi stekli uvid u životni stil i prisutne faktore rizika kod svojih pacijenata; usmeravanjem pacijenata ka dobrim životnim navikama, može značajno uticati na poboljšanje njihovog zdravstvenog stanja i eliminaciju tegoba.

**KLJUČNE REČI:** životni stilovi, ekotoksikologija, hronične nezarazne bolesti, personalizovana terapija



## WHEN THE LABORATORY PARAMETERS ARE NORMAL, BUT THE PERSON FEELS BAD, SHOULD WE TREAT THE PATIENT OR THE LABORATORY PARAMETERS?

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It often happens in medical practice that the patient's laboratory parameters are normal, but person feels bad and complains of numerous polymorphic diseases. In that situation, the question is what we need to treat since the parameters are normal and there is no disease diagnosis? The aim of the summary is to indicate that the focus of the healthcare professional should be on the patient and his health problems, and not on the laboratory parameters that can often be normal even with an existing health disorder. Numerous complaints may be accompanied, but not necessarily, by impaired laboratory analyses. During the ultrasound examination of the abdomen and pelvis, or during monitoring ECG, no changes have to be revealed necessarily. In accordance with the doctrine of diagnostics in classical medicine, this person cannot be diagnosed because everything that was done resulted in normal findings. For this reason, no drug therapy can be prescribed for this person (no diagnosis – no therapy!). However, the complaints that a person feels are caused by disturbed homeostasis at a discrete, cellular level, which is a consequence of the disruption of biochemical and biophysical mechanisms (they can also be partly caused by environmental pollutants).

In such cases, regular "monitoring" over a period of time is suggested. By "monitoring" i.e. follow-up a person's health over a certain period of time, we are actually waiting for the person to develop some pathology that will manifest itself in laboratory analyses, ultrasound examinations, etc. in order to make a diagnosis and then to start "treatment", or to wait that all of these problems (by some miracle) disappear by themselves. The most often situation is that health care professionals do not motivate patients to change lifestyle habits that would improve their health condition, but they suggest them to continue living "normally", to live "as before"...; practically, patient is suggested to do the same things that brought him to current health condition, but to expect that he will be better in coming time. Experience has shown that this is not the best advice that doctors give to patients. Treatment of patients should not be done only by drugs prescription, but health professionals should talk intensively with their patients in order to gain insight into the lifestyle and risk factors that are present; by guiding patients towards good lifestyle habits, improvement of their health condition and elimination of ailments will be better and faster achieved.

**KEYWORDS:** lifestyles, ecotoxicology, chronic non-communicable diseases, personalized therapy



## DA LI ZDRAVSTVENI RADNICI OBRAĆAJU DOVOLJNO PAŽNJE NA MOGUĆE ŠTETNE ZDRAVSTVENE EFEKTE ZAGAĐIVAČA IZ ŽIVOTNE SREDINE?

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Prema Svetskoj zdravstvenoj organizaciji (SZO) zagađenje vazduha je jedan od najvećih ekoloških rizika po zdravlje. Kombinovani efekti zagađenja ambijentalnog vazduha i zagađenja vazduha u domaćinstvima povezani su sa 6,7 miliona prevremenih smrti godišnje, dok se procenjuje da je zagađenje ambijentalnog (spoljnog) vazduha izazvalo 4,2 miliona prevremenih smrti širom sveta u 2019.

Cilj rada je da ukaže na to da zdravstveni radnici treba da razmotre zagađivače kao mogući uzrok simptoma pacijenata dok ih leče protiv hroničnih nezazravnih bolesti (HNB). Teški metali mogu ući u telo na različite načine (npr. kontaminiranom vodom iz slavine, propisanim lekovima, živinim amalgamima u zubima, pušenjem i/ili udisanjem pasivnog dima, konzumiranjem hrane koja sadrži visoke nivoe teških metala, životom u blizini deponije, primanjem vakcinacije koje sadrže timerosal, kozmetičke promene kao što su pirsing i tetovaže itd.).

Najčešći teški metali u našem okruženju su živa, olovo, kadmijum, arsen. Uobičajeni simptomi kod nekoliko tipova trovanja teškim metalima su: dijareja, mučnina, nadimanje, bol u stomaku, povraćanje, kratak dah, peckanje u rukama i stopalima, jeza, slabost, nedostatak energije i hronični umor, višak kilograma, glavobolja, mentalna konfuzija, alergije na hranu... Osim teških metala, svi smo izloženi mikroplastici, hemijskim jedinjenjima koje ometaju rad endokrinog sistema, pesticidima, hormonima iz hrane (posebno živine) itd. Kod osoba koje imaju neke gore opisane simptome, često se ne sumnja na intoksikaciju malim dozama toksikanata, te se stoga i ne predlaže proces detoksikacije organizma. Zdravstvenim radnicima se savetuje da razmotre moguću intoksikaciju svojih pacijenata zagađivačima i da predlože programe detoksikacije. Adekvatnim unosom vode i promenom ishrane, a uporedo sa primenom dodatka ishrani i lekovitim biljem u cilju ishrane jetre i bubrega, proces detoksikacije se može uspešno obaviti. Nakon detoksikacije, dalji tretman HNB će biti mnogo efikasniji.

**KLJUČNE REČI:** teški metali, zagađenje, detoksikacija, sindrom hroničnog umora



## DO HEALTH CARE PROFESSIONALS PAY ENOUGH ATTENTION TO POSSIBLE HARMFUL HEALTH EFFECTS OF POLLUTANTS COMING FROM AN ENVIRONMENT?

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According to World Health Organization (WHO) air pollution is one of the greatest environmental risk to health. The combined effects of ambient air pollution and household air pollution are associated with 6.7 million premature deaths annually, while ambient (outdoor) air pollution is estimated to have caused 4.2 million premature deaths worldwide in 2019. Aim of the paper is to point out that health care professionals should consider pollutants as possible cause of patients' symptoms while treating them against chronic non-communicable diseases (CNCDs). Heavy metals may enter the body in various ways (e.g. by contaminated tap water, prescribed drugs, mercury amalgams in teeth, smoking and/or inhaling second-hand smoke, eating foods that contain high levels of heavy metals, living near a landfill, receiving vaccinations that contain thimerosal, cosmetic changes such as piercing and tattoos etc.).

The most common heavy metals in our environment are mercury, lead, cadmium, arsenic. Common symptoms across several types of heavy metal poisoning include: diarrhea, nausea, bloating, abdominal pain, vomiting, shortness of breath, tingling in hands and feet, chills, weakness, lack of energy and chronic fatigue, excess weight, headaches, mental confusion, allergies to food... Aside heavy metals, we all are exposed to micro-plastic, endocrine-disrupting chemicals, pesticides, hormones from food (especially poultry) etc. One facing symptoms described above may very often be misdiagnosed and therefore not treated properly. Health care professionals are advised to consider possible intoxication of their patients with pollutants and to suggest detoxification programs. By adequate volume of water intake and diet change concomitantly with nutritionals and herbs in order to nourish the liver and kidneys, detoxification process can be done successfully. Following detoxification, further treatment of primary CNCDs will be much more effective.

**KEYWORDS:** heavy metals, pollution, detoxification, chronic fatigue syndrome



## UPRAVLJANJE FARMACEUTSKIM OTPADOM NA PRIMERU GRADA BEOGRADA TOKOM 2022. GODINE

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Nadzor nad upravljanjem farmaceutskim otpadom (FO) je važan aspekt u očuvanju životne sredine (deponije, kanalizacija, otpadne vode). Pravilnik o načinu i postupku upravljanja FO definiše upravljanje FO kao skup mera koje obuhvataju sakupljanje, razvrstavanje, pakovanje, obeležavanje, skladištenje, transport i tretman otpada u cilju njegovog konačnog zbrinjavanja na bezbedan način po zdravlje ljudi, životinja i životnu sredinu. Prema Pravilniku o načinu i postupku upravljanja FO razlikuje: neopasan FO – nije opasnost za životnu sredinu i zdravlje ljudi i opasan FO – od lekova i dezinficijensa koji sadrže teške metale, lekova koji zahtevaju posebne postupke tretiranja, uključujući citotoksični i citostatički otpad i sav pribor za pripremu, zbog mogućeg kancerogenog, mutagenog i teratogenog efekta. Razvrstani farmaceutski otpad se pakuje: 1) farmaceutski otpad – u kese i kontejnere crvene boje; 2) citotoksični i citostatski otpad – u kese i kontejnere ljubičaste boje. U okviru pisanja ovog rada, od 9 apoteka koje su anketirane u Beogradu (Zvezdara, Palilula, Vračar), u 5 apoteka su na pitanje da li mogu preuzeti lekove sa isteklim rokom trajanja, odgovori bili odrični, dok je u 4 apoteke odgovor bio potvrđan, ali bez jasnog obaveštenja o mogućnosti preuzimanja neispravnih lekova, niti jasno opredeljenog prostora za tu namenu. Poznato je da farmaceutski aktivna jedinjenja u prirodi predstavljaju jedan od glavnih problema usled razvoja rezistencije na antibiotike i antivirusne, bioakumulacije i efekata endokrinih ometača. Potrebno je povećati odgovornost svih generatora medicinskog i farmaceutskog otpada, uz jasne smernice kako izvesti procenu toksikološkog rizika, odnosno smernica Specifičnih mera zaštite zdravlja živih organizama i životne sredine.

**KLJUČNE REČI:** farmaceutski otpad, upravljanje farmaceutskim otpadom, zaštita životne sredine, citostatski i citotoksični otpad





## MANAGEMENT OF PHARMACEUTICAL WASTE ON THE EXAMPLE OF THE CITY OF BELGRADE DURING 2022

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Management of pharmaceutical waste (PhW) is an important aspect in the protection of the environment (landfills, sewage, waste water). The Rulebook of waste management defines the management of waste as a set of measures that include collection, sorting, packaging, marking, storage, transport and treatment of waste with the aim of its final disposal in a safe manner for the health of people, animals and the environment.

By the Rulebook PhW: non-hazardous PhW – not a threat to the environment and human health, and hazardous PhW – from drugs and disinfectants containing heavy metals, drugs that require special treatment procedures, including cytotoxic and cytostatic waste and all accessories for preparation, due to a possible carcinogenic, mutagenic and teratogenic effect. Sorted PhW is packed: 1) PhW – in red bags and containers; 2) cytotoxic and cytostatic waste – in purple bags and containers. Our research included 9 pharmacies in Belgrade (Zvezdara, Palilula, Vračar), in 5 pharmacies the answer to the question of whether they can take expired medicines was negative, while in 4 pharmacies the answer was yes, but without a sign and space to accept defective medicines. It is known that pharmaceutical active compounds in nature represent one of the main problems due to the development of resistance to antibiotics and antivirals, bioaccumulation and the effects of endocrine disruptors. It is necessary to increase the responsibility of all generators of medical and PhW, with clear guidelines on how to perform a Toxicological risk assessment, Environmental Risk Assessment and Specific protection goals.

**KEYWORDS:** pharmaceutical waste, management of pharmaceutical waste, environment protection, cytotoxic and cytostatic waste



## IZLOŽENOST ČESTICAMA PM<sub>2,5</sub> I PM<sub>10</sub> NA TERITORIJI GRADA BEOGRADA U ZIMSKOM PERIODU 2022/23. GODINE

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Kvalitet vazduha u urbanim sredinama ima veći uticaj na zdravlje stanovništva nego ostali faktori životne sredine, a jedan od glavnih zagađivača su u vazduhu suspendovane čestice PM<sub>10</sub> i PM<sub>2,5</sub>, koje potiču iz različitih procesa sagorevanja. U ovom radu je praćen kvalitet vazduha u pogledu koncentracije PM<sub>2,5</sub> i PM<sub>10</sub> čestica, za: 6.11.2022, 18.12.2022. i 11.01.2023.godine na teritoriji grada Beograda. Agencija za zaštitu životne sredine vrši monitoring kvaliteta vazduha koji definiše indeksom CAQI (The Common Air Quality Index) u realnom vremenu. Analiza podataka ukazuje da vazduh najčešće može da se okarakteriše kao DOBAR, vrednosti za PM<sub>2,5</sub>: 15,01 µg/m<sup>3</sup> – 30 µg/m<sup>3</sup>, a za PM<sub>10</sub>: 25,01 µg/m<sup>3</sup> – 50 µg/m<sup>3</sup>. U manjem broju slučajeva, za čestice PM<sub>2,5</sub>: 0 µg/m<sup>3</sup> – 15 µg/m<sup>3</sup>, za PM<sub>10</sub> čestice 0 µg/m<sup>3</sup> – 25 µg/m<sup>3</sup>, što se može okarakterisati kao ODLIČAN kvalitet vazduha.

Od 29 tačaka merenja kvalitet vazduha je okarakterisan kao PRIHVATLJIV, obuhvatajući koncentracije za PM<sub>2,5</sub> čestice u rasponu 30,01 µg/m<sup>3</sup> – 55 µg/m<sup>3</sup>, odnosno za PM<sub>10</sub> čestice: 50,01 µg/m<sup>3</sup> – 90 µg/m<sup>3</sup>, na 8 lokacija. Moguće je primenom matematičkih obrazaca izvršiti konverziju koncentracije agensa u vazduhu u ekvivalentnu oralnu dozu:  $EOD = (C \times EL \times MV \times AF_{10-6}) / BW$  (EOD – ekvivalentna oralna doza, C – koncentracija supstance u vazduhu, EL – trajanje ekspozicije, MV – minutni volumen, AF – apsorpcioni faktor, BW – telesna masa). Izloženost PM česticama ima veoma loš uticaj na respiratorni, ali i kardiovaskularni i nervni sistem, uz povećan karcinogeni rizik.

**KLJUČNE REČI:** suspendovane čestice PM<sub>2,5</sub> i PM<sub>10</sub>, zagađenost, vazduh



## EXPOSURE TO PM<sub>2.5</sub> AND PM<sub>10</sub> PARTICLES ON THE TERRITORY OF THE CITY OF BELGRADE IN THE WINTER PERIOD OF 2022/23.

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Air quality in urban areas has a greater impact on the health than other environmental factors, and one of the main pollutants are suspended particles PM<sub>10</sub> and PM<sub>2.5</sub>. The air quality was monitored in terms of the concentration of PM<sub>2.5</sub> and PM<sub>10</sub>, for: 11/6/2022, 12/18/2022 and 11/1/2023 in the territory of the city of Belgrade. The Environmental Protection Agency monitors air quality, defined by the CAQI index (The Common Air Quality Index) in real time. Data indicates that the air can most often be characterized as GOOD, values for PM<sub>2.5</sub>: 15.01 µg/m<sup>3</sup> – 30 µg/m<sup>3</sup>, and for PM<sub>10</sub>: 25.01 µg/m<sup>3</sup> – 50 µg/m<sup>3</sup>. In a smaller number of cases, for PM<sub>2.5</sub> particles: 0 µg/m<sup>3</sup> – 15 µg/m<sup>3</sup>, for PM<sub>10</sub> particles 0 µg/m<sup>3</sup> – 25 µg/m<sup>3</sup>, which can be characterized as EXCELLENT air quality.

From 29 measurement points, the air quality was characterized as ACCEPTABLE, including concentrations for PM<sub>2.5</sub> particles in the range 30.01 µg/m<sup>3</sup> – 55 µg/m<sup>3</sup>, or for PM<sub>10</sub> particles: 50.01 µg/m<sup>3</sup> – 90 µg/m<sup>3</sup>, at 8 locations. It is possible to convert the concentration of the agent in the air into an equivalent oral dose using mathematical formulas:  $EOD = (C \times EL \times MV \times AF_{10-6}) / BW$  (EOD – equivalent oral dose, C – concentration of the substance in the air, EL – duration of exposure, MV – minute volume, AF – absorption factor, BW – body mass). Exposure to PM particles has a very bad effect on the respiratory, cardiovascular and nervous systems, with an increased carcinogenic risk.

**KEYWORDS:** particulate matter PM<sub>2.5</sub>, PM<sub>10</sub>, pollution, air



## SUBAKUTNA TOKSIČNOST FLUORIDA U KALCIFIKOVANIM TKIVIMA WISTAR PACOVA

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Preventivna upotreba fluorida (F<sup>-</sup>) ima pozitivan uticaj na zubna tkiva. Međutim, prekomeran unos F<sup>-</sup> može doprineti nastanku lokalne i/ili sistemske toksičnosti. Cilj studije bio je ispitivanje mehanizama subakutne toksičnosti rastućih koncentracija F<sup>-</sup> kod pacova u tkivima zuba i femura pomoću Benchmark dose (BMD) metodologije. Za potrebe eksperimenta, 30 mužjaka Wistar pacova podeljeno je u 6 grupa (n=5) i tokom 28 dana imali su ad libitum pristup vodi sa česme ili vodi sa rastućim koncentracijama F<sup>-</sup> (10, 25, 50, 100 i 150 mg/l). Sekutići, molari i levi femur izolovani su pomoću odgovarajućih klješta i podeljeni na 3 dela za određivanje koncentracija F<sup>-</sup>, bioelemenata (cinka (Zn), bakra (Cu) i gvožđa (Fe)) i histološke analize. PROASTweb 70.1 softver korišćen je za analizu odnosa doze i efekta F<sup>-</sup>.

Rezultati ove studije pokazali su da subakutna ekspozicija povišenim dozama F<sup>-</sup> dovodi do značajno povišenih vrednosti koncentracija F<sup>-</sup> u femuru i zubima pacova doznih grupa sa 100 i 150 mg/l F<sup>-</sup>. Fluoridi su uticali na disbalans koncentracija Zn i Cu, dok morfološka oštećenja u kalcifikovanim tkivima nisu uočena. Potvrđena je dozna zavisnost za efekat F<sup>-</sup> za smanjenje koncentracija Cu u femuru (BMDL 1,6 mg F<sup>-</sup>/kg t.m.) i smanjenje koncentracija Zn u zubima (BMDL 21,0 mg F<sup>-</sup>/kg t.m.). Na osnovu rezultata ove studije možemo zaključiti da subakutna ekspozicija povišenim dozama F<sup>-</sup> dovodi do disbalansa bioelemenata i taloženja F<sup>-</sup> u zubima i femuru eksperimentalnih pacova. S obzirom da su ljudi i životinje svakodnevno izloženi F<sup>-</sup>, ova studija je značajna za buduću procenu zdravstvenog rizika ekspozicije fluoridima.

**KLJUČNE REČI:** fluoridi, zubi, femur, bioelementi, Benchmark doza



## SUBACUTE FLUORIDE TOXICITY IN CALCIFIED TISSUES OF WISTAR RATS

ECOTOXICOLOGY

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Prophylactic fluoride (F-) use has positive effects on tooth structure. However, excessive F- consumption could cause local and/or systematic intoxication. The present study aimed to examine subacute toxicity of increasing F- concentrations in rats via Benchmark dose (BMD) methodology. The experiment was conducted on 30 male Wistar rats for 28 days, divided into six groups (n=5), and during 28 days animals had ad libitum access to tap water or water with F- solutions (10, 25, 50, 100 and 150 mg/L F-). The teeth and left femur were extracted and processed for the determination of F- tissue concentrations, bioelements (zinc (Zn), copper (Cu), and iron (Fe)), and histological analyses. PROASTweb 70.1 was used for the determination of the dose-response F- relationship.

The results confirmed a significant increase of F- concentrations in the femur and teeth among groups with 100 and 150 mg/L F-. Fluorides caused Zn and Cu disbalance, while histological changes were not observed. The dose-dependent changes were confirmed for decreased Cu concentrations in the femur (BMDL 1.6 mg F-/kg b.w.) and decreased Zn concentrations in the teeth (BMDL 21.0 mg F-/kg b.w.). According to the results of the present study, subacute exposure to increased F- concentrations leads to bioelements disbalance and F- deposition in the teeth and femur of experimental rats. Since human and animal populations are daily exposed to F-, this dose-response study is valuable for future health risk assessment regarding fluoride exposure.

**KEYWORDS:** fluorides, teeth, femur, bioelements, Benchmark dose



## UTICAJ ISPUSTA OTPADNIH VODA NA DVE LOKACIJE NA RECI DUNAV: GENOTOKSIKOLOŠKA PROCENA

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Jedan od najozbiljnijih problema za tok reke Dunav kroz Srbiju je direktno ispuštanje neprečišćenih otpadnih voda. U studijama biomonitoringa, fekalne indikatorske bakterije se koriste za otkrivanje prisustva neprečišćenih komunalnih otpadnih voda, dok odgovor biomarkera u autohtonoj bioti služi kao koristan indikator njihovog štetnog potencijala. Cilj ovog istraživanja bio je da se proceni genotoksični potencijal neprečišćenih komunalnih otpadnih voda na dve lokacije na reci Dunav, Višnjici (Beograd) i Novim Banovcima, korišćenjem in situ pristupa kod krupatice (*Blicca bjoerkna* L.).

Za merenje oštećenja DNK u eritrocitima, ćelijama jetre i škrge odabran je komet test, dok je mikronukleus test primenjen za merenje hromozomskih aberacija u eritrocitima. Pored toga, analiza akumulacije 22 elementa u jetri i mišiću ribe rađeno je ICP-OES metodom. Simultana detekcija ukupnih koliforma i *E.coli* u vodi obavljena je enzimskim Colilert-18 testom (IDEXX). Komet test otkrio je najveći nivo DNK oštećenja u škragama na lokalitetu Novi Banovci, a u krvi na lokalitetu Višnjica. Ukupna učestalost mikronukleusa na obe lokacije je bila niska. Na oba lokaliteta, većina analiziranih elemenata pokazala je viši nivo u jetri u odnosu na mišić. Mikrobiološki indikatori su potvrdili loš kvalitet vode, jer je na oba lokaliteta voda kategorisana kao kritično do jako zagađena. Rezultati ove studije su istakli značaj in situ pristupa u biomonitoringu i potrebu za efikasnijim upravljanjem prirodnim resursima i implementacijom sistema za prečišćavanje otpadnih voda.

**KLJUČNE REČI:** ekogenotoksikologija, reka, monitoring



## IMPACT OF WASTEWATER EFFLUENTS AT TWO SITES AT DANUBE RIVER: GENOTOXICOLOGICAL ASSESSMENT

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One of the most severe threats to the Danube River course through Serbia is the direct discharge of untreated wastewater. In biomonitoring studies, faecal indicator bacteria are used to reveal the presence of untreated communal wastewater, while biomarker response in indigenous biota serves as a useful indicator of their harmful potential. The objective of this study was to estimate the genotoxic potential of untreated communal wastewater at two sites on the Danube River, Višnjica (Belgrade) and Novi Banovci, by using in situ approach in white bream (*Blicca bjoerkna* L.). A comet assay was selected to measure DNA damage in erythrocytes, liver, and gill cells, while the micronucleus test was applied to measure chromosomal aberrations in erythrocytes.

Additionally, the accumulation of 22 elements in fish liver and muscle was analysed by ICP-OES method. Simultaneous detection of total coliforms and *E.coli* in water was performed by enzyme-based Colilert-18 test (IDEXX). Comet assay revealed the highest level of DNA damage in gills at the site Novi Banovci, and in blood at the site Višnjica. The overall frequency of micronucleus at both sites was low. At both localities, the majority of analysed elements showed higher levels in the liver in comparison to muscle. Microbiological indicators confirmed the poor water quality, since at both sites water was categorised as critically to strongly polluted. The results of this study highlighted the importance of in situ biomonitoring approach and the need for more effective management of natural resources and implementation of wastewater treatment systems.

**KEYWORDS:** ecogenotoxicology, river, monitoring



## FITOREMEDIJACIJA OTPADNIH VODA KAO MEHANIZAM ZA SMANJENJE BIOTOKSIČNOSTI ENDOKRINIH DISRUPTORA PREMA *ALIIVIBRIO FISCHERI*

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Eksperiment fitoremedijacije endokrinih ometača, bisfenola A i nonilfenola u model rastvoru, primenom biljne vrste *Eichhornia Crassipes*, sproveden je u laboratorijskim uslovima. Eksperiment je realizovan u vremenskom intervalu od 14 dana, pri čemu je četvrtog i četrnaestog dana uzorkovano po 2 L model rastvora. Biotoksičnost model rastvora sa redukovanim koncentracijama analita nakon fitoremedijacije procenjena je primenom testa detekcije akutne toksičnosti upotrebom Gram-negativnih bioluminiscentnih bakterija *Aliivibrio fischeri*.

Toksičnost je merena primenom liofiliziranog uzorka bakterija *Aliivibrio fischeri* u skladu sa standardom SRPS EN ISO 11348-3. U okviru merenja primenjene su BioFix® Lumi luminiscentne bakterije proizvođača Macherey-Nagel GmbH & Co., Düren, Germany. Primenom kalijum-hidroksida podešena je pH vrednost uzoraka model rastvora na 7,0±0,2. Potom, pomenuti uzorci model rastvora sa analitima razblaženi su na 50, 25, 12,5, 6,25 i 3,25% u odnosu na originalne koncentracije. Nakon toga natrijum hlorid je rastvoren u suspenziji kako bi se ostvarila koncentracija od 2% u odnosu na NaCl. Inokulum luminiscentnih bakterija pripremljen je u skladu sa standardom i dodat svakom uzorku pojedinačno. Nakon inkubacionog perioda od 30 minuta merena je luminiscencija bakterija primenom uređaja BioFix Lumi-10. Ostvareni rezultati su pokazali da je maksimalna inhibicija luminiscentnih bakterija ostvarena 4. dana u iznosu od 99,44% pri razblaženju suspenzije od 50%. Trend opadanja je bio prisutan, te je inhibicija luminiscentnih bakterija u okviru 14. dana iznosila 3,99% pri razblaženju suspenzije od 50%. Na osnovu ovih rezultata može se zaključiti da se smanjena toksičnost model rastvora bisfenola A i nonilfenola može postići nakon dve nedelje primene fitoremedijacije upotrebom biljne vrste *Eichhornia Crassipes*.

**KLJUČNE REČI:** biotoksičnost, *Aliivibrio fischeri*, endokrini ometači, fitoremedijacija otpadnih voda





## PHYTOREMEDIATION OF WASTEWATER AS A TECHNIQUE FOR REDUCING THE BIOTOXICITY OF ENDOCRINE DISRUPTORS TO *ALIIVIBRIO FISCHERI*

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A phytoremediation experiment using the plant species *Eichhornia Crassipes* in a model solution containing bisphenol A and nonylphenols was conducted in laboratory conditions over 14 days. Two liters of the model solution were collected on the fourth and fourteenth days. An acute toxicity detection test utilizing the Gram-negative bioluminescent bacteria *Aliivibrio fischeri* was used to evaluate the biotoxicity of the model solution samples during the phytoremediation method. Toxicity measurements were performed according to SRPS ISO 11348-3 standard using freeze-dried *Aliivibrio fischeri* bacteria. The bacteria used in the assays were BioFix® Lumi luminescent bacteria acquired from Macherey-Nagel GmbH & Co., Düren, Germany.

The pH of the model solution samples was adjusted to  $7.00 \pm 0.2$  using potassium hydroxide. The model solution samples were diluted to 50, 25, 12.5, 6.25, and 3.25% of the initial concentration. Then, sodium chloride was dissolved in the aftermentioned solutions to obtain a 2% NaCl concentration. The luminescent bacteria inoculum was prepared and added to each sample. After 30 min of incubation, the luminescence of the bacteria was measured by a BioFix Lumi-10 toxicity analyzer. The results showed that the maximum inhibition of luminous bacteria was attained on the fourth day and equaled 99.44%, at 50% dilution. The inhibition of luminous bacteria showed a declining trend and on the 14th day reached 3.99%, at 50% dilution. According to these results, two weeks of phytoremediation utilizing the plant species *Eichhornia Crassipes* can reduce the toxicity of the model solution of bisphenol A and nonylphenols.

**KEYWORDS:** biotoxicity, *Aliivibrio fischeri*, endocrine disruptors, phytoremediation of wastewater



## ODREĐIVANJE PRISUSTVA MIKROCISTINA U SLATKOVODNIM AKUMULACIJAMA SRBIJE

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Mikrocistini (MCs) predstavljaju grupu toksičnih monocikličnih peptida koje proizvode cijanobakterije. Ovi sekundarni metaboliti mogu dostići koncentracije opasne po zdravlje i kontaminirati površinske akumulacije, a nakon toga dospeti i u vodu za piće i na taj način povećati rizik po korisnike.

Mikrocistini uneti putem kontaminirane vode mogu izazvati različite štetne efekte kod čoveka, životinja, biljaka. Izloženost ljudi mikrocistinima najčešće uzrokuje hepatoksičnost i neurotoksičnost. Takođe, opisani su i poremećaji učenja i pamćenja, anksioznost, depresija, glavobolja, mučnina, inflamacija i neurodegenerativnih bolesti kao posledice izloženosti. Kongeneri mikrocistina su značajna grupa toksina cijanobakterija, i njihova kvantifikacija je od izuzetnog značaja da bi se procenio uticaj na zdravlje u slučaju korišćenja vode za piće ili rekreativnog korišćenja voda koje ih sadrže. Voda za analizu uzorkovana je tokom perioda jun-novembar koji obuhvata celokupni životni ciklus algi, sa posebnim osvrtom na pik sezone cvetanja.

Uzorci voda koncentrovani su i prečišćeni ekstrakcijom na čvrstoj fazi (SPE), nakon čega su određivani tečnom hromatografijom spregnutom sa elektrosprej jonizacijom masenom spektrometrijom (LC-ESI-MS). Prema smernicama Svetske Zdravstvene Organizacije (WHO), toksične koncentracije ukupnih mikrocistina jesu 1µg/L za pijaće vode i 1.5µg/L za rekreativne vode. Detektovane koncentracije toksina različite su u zavisnosti od akumulacije i perioda uzorkovanja. Opseg nađenih koncentracija iznosi 0.05 – 2.27µg/L. Tokom jula i avgusta dostignute su najviše koncentracije – toksične vrednosti koje utiču na ljude i životinje. Skrining slatkovodnih akumulacija na teritoriji Srbije nije dovoljno zastupljen. Neophodno je unaprediti rutinsku kontrolu i podići svest o važnosti praćenja ovih toksina u rezervoarima u kojima žive vrste koje mogu da ih proizvode.

**KLJUČNE REČI:** slatka voda, cvetanje algi, mikrocistin, LC-ESI-MS



## DETECTION AND OCCURRENCE OF MICROCYSTINS IN FRESHWATER OF SERBIA

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Microcystins (MCs) are toxic monocyclic peptides produced by cyanobacteria in fresh water. These secondary metabolites can reach hazardous concentrations, and contaminate surface drinking water supplies and thereby increase the risk of exposure to consumers.

Microcystins can create a variety of adverse health effects in humans, animals, and plants through contaminated water. MCs human exposure most commonly cause hepatotoxicity and neurotoxicity. Also, learning memory dysfunction, anxiety, depression, headaches, nausea, inflammation, and neurodegenerative diseases have been described. Microcystin congeners are significant group of cyanobacterial toxins and their quantification is essential in order to assess the health risk when such waters are used for drinking purpose or in recreational manner. Water samples, from accumulation and recreational lakes in Serbia, were collected in a period June–November, during whole algae lifecycle, especially referring to algal bloom peak. Determination of MCs is done using liquid chromatography with electrospray ionization mass spectrometry (LC-ESI-MS), which preceded solid phase extraction (SPE) of raw water sample in manner to clean up and concentrate sample. World Health Organization (WHO) guidelines are 1 and 1.5 µg/L concentration of total MCs in drinking and recreational water respectively.

Toxin concentration was different depending of accumulation and period of sampling. Concentration range was 0.05–2.27 µg/L. During July and August MCs concentration were highest, reaching it hazardous values that effects people and animals. Freshwater screening for MCs in our country is insufficient, so it is necessary to improve routine control and raise awareness about monitoring of these toxins in reservoirs, where species which produce them exist.

**KEYWORDS:** freshwater, algae bloom, microcystyn, LC-ESI-MS



## PROCENA IZLOŽENOSTI HRVATSKIH DIVLJIH ZAŠTIĆENIH FELIDA ( *LYNX LYNX* I *FELIS SILVESTRIS SILVESTRIS* ) METAL(OID)IMA IZ OKOLIŠA

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Kopnene zveri na vrhu lanca ishrane su sklone kumulaciji perzistentnih neorganskih zagađujućih supstanci u bubrezima i jetri zbog trofičkog nivoa. Toksični potencijal metal(oid)a, koje predator unosi konzumirajući plen, nosi rizik od štetnih efekata na zdravlje predatorske vrste. Evroazijski ris (*Lynx lynx*) i evropska divlja mačka (*Felis silvestris silvestris*) strogo su zaštićene zveri koje delimično dele stanište, ali nešto manje plen (glodari). Toksikološki podaci o nivoima metal(oid)a u jetri evropske divlje mačke ne postoje, dok su podaci za evroazijskog risa vrlo retki. Kako bi procenili efekte izloženosti zagađujućim supstancama iz životne sredine na hrvatsku populaciju divljih felida, uzorkovali smo jetre od osam risova (svih starosnih grupa) i 22 odrasle divlje mačke između 1998. i 2022. godine i u njima utvrdili potencijalno toksične i esencijalne metal(oid)e, metodom induktivno spregnute plazme masene spektrometrije.

Nijedna životinja nije imala nivoe metal(oid)a iznad poznatih granica toksičnosti za sisare. Komponente ishrane, tj. životinjske vrste koje čine plen (srna i sivi puh za risa, a glodari za divlju mačku) verovatno su najvažniji faktori koji utiču na razlike u nivoima metal(oid)a između dve istraživane vrste. Kod risa se akumulira više bakra (59,3 µg/kg suve mase), cinka (403 µg/kg), cezijuma (1973 µg/kg), žive (338 µg/kg), talijuma (7,46 µg/kg) i olova (257 µg/kg) u jetri nego kod divlje mačke (23,5; 91,1; 88,2; 70,6; 2,52; odnosno 34,7 µg/kg). Suprotno ovome, kod divlje mačke se akumulira više arsena (62,0 µg/kg), molibdena (1627 µg/kg) i antimona (24,5 µg/kg) u jetri u poređenju s risom (22,5; 1212, odnosno 4,05 µg/kg). Generalno, obe istraživane vrste felida iz dinarske populacije sadržale su nivoe metal(oid)a u okviru podataka objavljenih za iberijskog risa, a značajno niže koncentracije kadmijuma i olova, nego što su nađene u dinarskoj populaciji smeđeg medveda i sivog vuka.

**KLJUČNE REČI:** ris, divlja mačka, jetra, granice toksičnosti u jetri



# METAL(LOID) EXPOSURE ASSESSMENT FROM LIVER OF CROATIAN WILD PROTECTED FELIDS

[ *LYNX LYNX AND FELIS SILVESTRIS SILVESTRIS* ]

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Terrestrial top carnivores are susceptible to renal and hepatic accumulation of persistent inorganic pollutants due to their trophic position. Toxic potential of metal(loid)s taken up by prey consumption carries the risk of adverse effects for exposed predator. Eurasian lynx (*Lynx lynx*) and European wildcat (*Felis silvestris silvestris*) are strictly protected carnivores partly sharing their habitat with marginal trophic niche overlap (rodents). Toxicological data of hepatic metal(loid) burden in European wildcat do not exist, while data from Eurasian lynx are very scarce. In order to assess the impact of environmental pollution exposure of Croatian wild felid populations, livers of eight lynx (all age classes) and 22 adult wildcats were collected in 1998-2022 period, and both potentially toxic and essential metal(loid)s were quantified by inductively coupled plasma spectrometry.

None of the animals had hepatic metal(loid)s levels crossing known toxicity thresholds for mammals. Dietary components, i.e. prey species (roe deer and edible dormouse for lynx and rodents for wildcat), were the most probable factor influencing differences in metal(loid) levels between the two investigated species. Lynx accumulated more copper (median 59.3 µg/kg dry mass), zinc (403 µg/kg), caesium (1973 µg/kg), mercury (338 µg/kg), thallium (7.46 µg/kg) and lead (257 µg/kg) in the liver than wildcat (23.5, 91.1, 88.2, 70.6, 2.52 and 34.7 µg/kg, respectively). On the contrary, wildcat had higher hepatic arsenic (62.0 µg/kg), molybdenum (1627 µg/kg) and antimony (24.5 µg/kg) levels compared to lynx (22.5, 1212 and 4.05 µg/kg, respectively). Generally, both investigated felids from Dinaric population had hepatic metal(loid) levels in the range of previously reported for Iberian lynx, and notably lower cadmium and lead than Dinaric brown bear and grey wolf.

**KEYWORDS:** lynx, wildcat, metal contamination, hepatic toxicity threshold



## NIVO KONTAMINACIJE KRVI OLOVOM KOD KVARNERSKE POPULACIJE ( HRVATSKA ) LEŠINARA BELOGLAVOG SUPA ( *GYPVS FULVUS* )

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Supovi su dugoživuće velike ptice grabljivice, koje se hrane leševima velikih i srednjih sisara te često mogu biti izložene olovu (Pb) poreklom iz municije. Uz pesticide i nesteroidne antiinflamatorne lekove, Pb je najčešća antropogena hemikalija za koju je u svetu utvrđeno da dovodi do smrti i bolesti kod slobodnoživućih supova. Posebna fiziološka stanja, kao što su neuhranjenost, nezrelost i istovremena izloženost drugim štetnim hemikalijama mogu pogoršati štetne efekte Pb. Oni se mogu ispoljiti na hematopoetskom, nervnom, kardiovaskularnom, bubrežnom, jetrenom, imunološkom i reproduktivnim sistemima organa. Hrvatska populacija beloglavih supova (*Gyps fulvus*) danas se još jedino gnezdi na kvarnerskim ostrvima, na stenama iznad mora.

Trenutni naponi da se populacija očuva usmereni su ka smanjenju broja trovanja i istraživanju uzroka trovanja supova. Cilj ove studije bio je da se procene nivoi Pb metodom induktivno spregnute plazme masene spektrometrije (ICP-MS). Uzorak za analizu je puna krv, većinom mladih beloglavih supova, kvarnerske populacije, koja je uzimana u periodu 2018.-2022. godine, tokom oporavka u Centru za posetitelje i oporavištu za beloglave supove "Beli". Prosečan nivo Pb u krvi (KPb) iznosio je  $20,9 \pm 10,5$   $\mu\text{g/dL}$  (N=25) i odgovara uobičajenim nivoima za red Accipitriformes ( $< 20$   $\mu\text{g/dL}$ ) što je za 16-51% niže od vrednosti (KPb) kod populacije supova s Pirinejskog poluostrva. S druge strane u 8 od 25 ptica, koncentracija KPb bila je iznad granice za subkliničku toksičnost, dok je jedna ptica (53,0  $\mu\text{g/dL}$ ) čak imala koncentraciju Pb ( $> 50$   $\mu\text{g/dL}$ ), koja je ukazivala na kliničko trovanje. Za beloglave supove se smatra da su otporniji na Pb od drugih ptica grabljivica, te su za donošenje konačnih zaključaka o toksičnosti Pb neophodna dodatna istraživanja uticaja na njihovo zdravlje.

**KLJUČNE REČI:** Pb, krv, municija, granica toksičnosti kod ptica



## LEAD CONTAMINATION IN BLOOD OF APEX SCAVENGER GRIFFON VULTURE (*GYPS FULVUS*) FROM KVARNER ARCHIPELAGO POPULATION (CROATIA)

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Vultures are long-lived large raptors whose feeding on carcasses of large and medium-sized mammals exposes them often to lead (Pb) from spent ammunition. In addition to pesticides and non-steroidal anti-inflammatory drugs, Pb is the most frequent anthropogenic chemical reported to cause death and disease in free-living vultures in the world. Specific physiological conditions like malnutrition or immaturity and co-exposure to other toxicants may aggravate the Pb-related adverse effects comprising hematopoietic, nervous, cardiovascular, renal, hepatic, immune and reproductive systems. Croatian population of griffon vultures (*Gyps fulvus*) has shrank to Kvarner area where they nest on cliffs above the sea. Ongoing efforts in maintaining that population stabile are directed at reduction of vulture poisoning and studies of poisoning causes.

This study aimed at assessing whole blood Pb by inductively coupled plasma mass spectrometry (ICP-MS) in Kvarner population of mainly juvenile griffon vultures sampled in the period 2018-2022 during their rehabilitation at Beli. Visitor Centre and Rescue Centre for Griffon Vultures. On average (mean  $\pm$  SD, 20.9 $\pm$ 10.5  $\mu$ g/dL, N=25), Croatian vultures had blood Pb (BPb) levels in the range of background Pb level for Accipitriformes (<20  $\mu$ g/dL) and 16-51% lower mean BPb than reported for populations from Iberian Peninsula. However, Pb level in 8 of 25 birds (23.0-45.0  $\mu$ g/dL) was above threshold for subclinical toxicity, with one bird (53.0  $\mu$ g/dL) even crossing threshold indicative of clinical poisoning (>50  $\mu$ g/dL). Griffon vultures are assumed to be more tolerant to Pb than other raptors, thus effect studies are essential before strong conclusions about toxicity are drawn.

**KEYWORDS:** Pb, blood, metal, ammunition, bird toxicosis threshold



## ZAGAĐENJE VODE ARSEKOM I POTENCIJALNO REŠENJE ZA ČISTU VODU U SRPSKOM SELU SKORENOVAC

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U selu Skorenovac izvori vode za piće su nebezbedni za korišćenje od 2015. godine. Zbog toga je Eko česma 2019. godine napravljena kao alternativni način vodosnabdevanja. Studija je imala za cilj da proceni nivo zagađenja vode za piće sela Skorenovac arsenom i da ispita korisnost Eko česme kao rešenje za zagađenje vode arsenom. Studija je ispitivala period od protekle četiri godine (2019-2022) tokom kojih su uzeta tri do četiri mesečna uzorka vode za piće u okviru monitoringa i to sa izvorišta, iz distributivne mreže i sa Eko česme u selu Skorenovac.

Uzorak je testiran na arsen i izmeren je njegov nivo. Dobijeni nalazi su statistički analizirani. U protekle četiri godine u okviru monitoringa uzeta su 173 uzorka vode za piće, od kojih su 139 (80,3%) sa izvorišta i iz distributivne mreže i 34 (19,7%) uzorka vode za piće sa Eko česme. Nivoi arsena u uzorcima vode kretali su se od <0,01 do 0,076 mg/l (srednja vrednost  $\pm$  sd = 0,041  $\pm$  0,071 mg/l). Nivoi arsena u vodi za piće nisu se razlikovali između ispitivanih godina ( $p=0,333$ ) i meseci uzorkovanja ( $p=0,674$ ). Međutim, u uzorcima sa izvorišta i iz distributivne mreže registrovano je značajno više arsena od onih sa Eko česme ( $p=0,003$ ). U svim uzorcima sa Eko česme nivoi arsena su bili ispod 0,01 mg/l. U slučaju zagađenja vode arsenom Eko česme sa predstavljaju dobro rešenje za dobijanje čiste vode za piće.

**KLJUČNE REČI:** arsen, zagađenje vode, Eko česma





## ARSENIC WATER POLLUTION AND POTENTIAL SOLUTION FOR CLEAN WATER IN SERBIAN SKORENOVAC VILLAGE

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In the Skorenovac village regular drinking water sources have been unsafe for use since 2015. That is why the Eco fountain was created in 2019 as an alternative way of water supply. Study aimed to assess the level of Arsenic contamination water of Skorenovac village and to investigate the usefulness of the Eco fountain as a solution for Arsenic water contamination. Study investigated the period of the past four years (2019-2022) during which three to four monthly samples water were taken from as part of monitoring from the source, from the distribution network and from the Eco fountain in the village of Skorenovac.

The sample was tested for Arsenic and its levels were measured. The obtained findings were statistically analyzed. In the past four years, 173 samples of drinking water were taken as part of the monitoring, of which 139 (80.3%) were from the source and from the distribution network and 34 (19.7%) were samples water from the Eco fountain. Arsenic levels in water samples ranged from <0.01 to 0.076 mg/l (mean  $\pm$  sd = 0.041 $\pm$ 0.071 mg/l). Arsenic levels in drinking water did not differ between the years ( $p=0.333$ ) and months of sampling ( $p=0.674$ ). However, significantly more arsenic was registered in the samples from the source and from the distribution network than those from the Eco fountain ( $p=0.003$ ). In all samples from the Eco fountain, arsenic levels were below 0.01 mg/l. In case of water contamination with arsenic, Eco fountains are a good solution for obtaining clean drinking water.

**KEY WORDS:** arsenic, water pollution, Eco fountain



## ZNAČAJ ODREĐIVANJA SUMPORA PRI KARAKTERIZACIJI OTPADA KOJI SE MOŽE ISKORISTITI ZA POTREBE DOBIJANJA ENERGIJE

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Spaljivanje (insineracija) otpada je jedna od tehnički najrazvijenijih opcija upravljanja otpadom koja je raspoloživa danas, a primenjuje se u cilju smanjivanja njegove količine i dobijanja energije. Karakterizacija otpada, odnosno utvrđivanje njegovog sastava i osobina predstavlja obavezan kontrolni postupak pri svakom preuzimanju otpada u postrojenja za insineraciju, sa ciljem smanjenja emitovanja toksičnih supstanci u životnu sredinu. Sadržaj sumpora je obavezan parameter koji izveštaj o ispitivanju otpada mora da sadrži. Sadržaj ukupnog sumpora određuje se na Thermo Scientific FlashSmart elementarnom analizatoru metodom totalnog sagorevanja na temperaturi od 950°C pri parcijalnom pritisku kiseonika od 15 bara i dodavanjem V2O5 kao dodatnog donora kiseonika. Nastali gasovi nošeni helijumom prolaze kroz reaktor od elektrolitičkog bakra i bakar-oksida. Na ovaj način sav sumpor prisutan u uzorku prevodi se u SO<sub>2</sub>, koji se od ostalih nastalih gasova razdvaja na koloni za gasnu hromatografiju, a zatim detektuje na TCD detektoru. Ovom tehnikom moguće je odrediti sadržaj sumpora u širokom opsegu koncentracija i različitim matriksima: čvrstim, viskoznim, tečnim, organskim i neorganskim. Minimalan sadržaj sumpora koji se može odrediti ovom tehnikom je 0,1%.

Grafična vrednost za sumpor u otpadu koji se spaljuje kao alternativno gorivo iznosi 3%, što obezbeđuje mogućnost efikasnog prečišćavanja nastalih štetnih gasova prilikom insineracije i da njihov nivo ne pređe dozvoljene granične vrednosti emisija u vazduh, koja za SO<sub>2</sub> na nivou dnevnog proseka iznosi 50mg/m<sup>3</sup>. Nepravilni tretman otpada predstavlja izvor zagađenja vazduha, vode i zemljišta. Republika Srbija poseduje adekvatan zakonodavni okvir rešavanja problema otpada, ali njegova primena zahteva značajno unapređenje.

**KLJUČNE REČI:** otpad, insineracija, ukupan sumpor



## THE IMPORTANCE OF TOTAL SULFUR DETERMINATION IN THE CHARACTERIZATION OF WASTE THAT CAN BE USED FOR THE PURPOSES OF OBTAINING ENERGY

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Burning (incineration) of waste is one of the most technically developed waste management option available today, and it is applied in order to reduce its amount and obtain energy. Characterization of waste (determination of its composition and properties) is a mandatory control procedure for every waste intended for incineration, with the aim of reducing the emission of toxic substances into the environment.

Sulfur content is a mandatory parameter that waste test report must contain. Total sulfur content is determined on ThermoScientific FlashSmart elemental analyzer by total combustion method at a temperature of 950°C and oxygen pressure of 15bar and by adding V2O5 as additional oxygen donor. Created gases carried by helium pass through reactor made of copper and copper-oxide. In this way, all the sulfur present in the sample is converted into SO<sub>2</sub>, which is separated from the other gases on gas chromatography column, and then detected on TCD detector. With this technique, it is possible to determine sulfur content in wide range of concentrations and in different matrices: solid, viscous, liquid, organic and inorganic. Minimum sulfur content that can be determined with this technique is 0.1%. Limit value for sulfur in waste as an alternative fuel is 3%, which ensures possibility of efficient purification of harmful gases generated during incineration and that their level does not exceed permitted limit values of emissions into the air, which for SO<sub>2</sub> at the daily average is 50mg/m<sup>3</sup>. Improper waste treatment is a source of air, water and soil pollution. Republic of Serbia has an adequate legislative framework for solving waste problem, but its implementation requires significant improvement.

**KEYWORDS:** waste, incineration, total sulfur



## KVALITET VAZDUHA GRADA NOVOG SADA - GASOVITI POLUTANTI

ECOTOXICOLOGY

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Gasoviti polutanti vazduha potiču kako iz prirodnih tako i antropogenih izvora, koji prevladavaju u urbanoj sredini. Cilj ove studije je sagledavanje koncentracija SO<sub>2</sub>, NO<sub>x</sub>, NO, NO<sub>2</sub>, CO i O<sub>3</sub> u vazduhu na teritoriji Novog Sada. Koncentracije su praćene na urbano-saobraćajnoj lokaciji tokom 2018. i osnovnoj ruralnoj tokom 2019-2022. godine, korišćenjem automatske merne stanice.

Na urbano-saobraćajnoj lokaciji zabeležene su sledeće prosečne godišnje (2018) i maksimalne koncentracije (g/m<sup>3</sup>): SO<sub>2</sub> 7.9 i 33.4, NO<sub>x</sub> 52.5 i 213.8, NO 12.5 i 127.9, NO<sub>2</sub> 33.3 i 134.1, CO 0.4 i 1.4, O<sub>3</sub> 66.6 i 379.5. Zakonski maksimalni dnevni nivoi su bili prevaziđeni u slučaju NO<sub>2</sub> i O<sub>3</sub>, tokom 17 i 6 dana, redom. Takođe je prekoračena prosečna godišnja koncentracija NO<sub>x</sub> (40 nasuprot zabeleženih 52.2 g/m<sup>3</sup>). Uzastopne prosečne godišnje (2019-2022) i maksimalne koncentracije (g/m<sup>3</sup>) na urbano-saobraćajnoj lokaciji iznosile su: SO<sub>2</sub> 7.8/7.8/6.7/6.6 i 36.7 (2021), NO<sub>x</sub> 29.6/27.1/24.0/24.9 i 142 (2019 i 2022), NO 5.6/6.5/5.8/6.0 i 63.0 (2022), NO<sub>2</sub> 20.9/17.3/15.6/16.2 i 78.7 (2019), CO 0.3/0.3/0.3/0.4 i 1.8 (2019 i 2020), O<sub>3</sub> 67.9/63.0/67.0/67.1 i 133.3 (2022).

Samo je O<sub>3</sub> prekoračio maksimalni dnevni nivo, tokom 2/0/7/6 dana (ukupno 15 dana tokom 4 godine), što je usaglašeno sa zakonom (do 25 dana tokom 3 godine). Uspostavljeni godišnji limiti (O<sub>3</sub> je isključen) nisu dostignuti, a najviše se približila koncentracija NO<sub>x</sub> (60.0-74.0%). Povišene koncentracije gasovitih polutanata u vazduhu mogu dovesti do brojnih negativnih zdravstvenih ishoda. Stoga je od izuzetne važnosti obezbediti odgovarajući kvalitet vazduha koji podržava zdrav život i dugovečnost populacije.

**KLJUČNE REČI:** kvalitet vazduha, monitoring, Novi Sad



## AIR QUALITY IN THE CITY OF NOVI SAD - GASEOUS POLLUTANTS

ECOTOXICOLOGY

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Gaseous pollutants in the air can originate from various natural and anthropogenic sources, the latter prevailing in urban environment. The current study aimed to investigate concentrations of SO<sub>2</sub>, NO<sub>x</sub>, NO, NO<sub>2</sub>, CO and O<sub>3</sub> in the air in Novi Sad. Gases' concentrations were monitored on urban-traffic site during 2018, and basic rural site during 2019-2022, using automatic measurement station.

Annual (2018) mean and maximum concentrations (g/m<sup>3</sup>) recorded on the urban-traffic site were: SO<sub>2</sub> 7.9 and 33.4, NO<sub>x</sub> 52.5 and 213.8, NO 12.5 and 127.9, NO<sub>2</sub> 33.3 and 134.1, CO 0.4 and 1.4, O<sub>3</sub> 66.6 and 379.5. Regulatory maximum daily levels were surpassed in case of NO<sub>2</sub> and O<sub>3</sub>, in 17 and 6 days respectively. Regarding annual limits, the one for NO<sub>x</sub> was by far exceeded (40 versus 52.2 g/m<sup>3</sup>). Consecutive annual (2019-2022) mean and overall maximum concentrations (g/m<sup>3</sup>) recorded on the basic urban site were: SO<sub>2</sub> 7.8/7.8/6.7/6.6 and 36.7 (2021), NO<sub>x</sub> 29.6/27.1/24.0/24.9 and 142 (2019 and 2022), NO 5.6/6.5/5.8/6.0 and 63.0 (2022), NO<sub>2</sub> 20.9/17.3/15.6/16.2 and 78.7 (2019), CO 0.3/0.3/0.3/0.4 and 1.8 (2019 and 2020), O<sub>3</sub> 67.9/63.0/67.0/67.1 and 133.3 (2022). Only O<sub>3</sub> exceeded maximum daily level, in 2/0/7/6 days (15 days over 4 years), which was acceptable according to the regulation (up to 25 days over 3 years).

Established annual limits (O<sub>3</sub> excluded) were unattainable, with NO<sub>x</sub> reaching closest proximity of its respective limit (60.0-74.0%). Elevated concentrations of gaseous pollutants in the air can result in various adverse health outcomes. Therefore, it is of utmost importance to secure proper air quality, supporting healthy living and longevity of population.

**KEYWORDS:** air quality, monitoring, Novi Sad



## EFEKAT VISOKIH DOZA MIKOTOKSINA ZEARALENONA NA LARVE BRAŠNENOG CRVA *TENEbrio MOLITOR* L.

ECOTOXICOLOGY

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*Tenebrio molitor* se smatra štetočinom uskladištenih žitarica i proizvoda od žitarica, ali i kao važan dodatak u ishrani životinja i ljudi. Važno je istaći da su *T. molitor* larve osjetljive na prisustvo mikotoksina u okruženju, menjajući ponašanje i vrednosti biohemijskih parametara. Fungalni toksin zearalenon (ZEA) je korišćen za procenu uticaja na oksidativni stres i lokomotornu aktivnost larvi brašnenog crva, *T. molitor*. Cilj ovog istraživanja je bio da se ispita uticaj visokih doza mikotoksina ZEA (10 i 20 mg/kg) u hrani na aktivnost enzima superoksid dismutaze (SOD), katalaze (CAT) i glutation S-transferaze (GST), kao i na parametre lokomotorne aktivnosti: pređeni put, vreme kretanja i prosečnu brzinu kretanja larvi *T. molitor*.

Rezultati ovog istraživanja su pokazali da prisustvo ZEA u hrani u koncentracijama od 10 i 20 mg/kg značajno povećava specifičnu aktivnost SOD, CAT i GST. Takođe, uticalo je i na lokomotornu aktivnost larvi tj. pređeni put i vreme kretanja su bili značajno niži kod larvi izloženih 10 i 20 mg/kg ZEA, dok se prosečna brzina kretanja nije značajno menjala.

Prisustvo ZEA dovodi do značajnih promena u fiziologiji i ponašanju larvi *T. molitor* i može biti korisno u ranom otkrivanju kontaminacije hrane ovim opasnim mikotoksinom.

**KLJUČNE REČI:** *T. molitor*, mikotosin ZEA, antioksidativni



## THE EFFECT OF HIGH DOSES OF THE MYCOTOXIN ZEARELENONE ON *TENEBRIO MOLITOR* L. LARVAE

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*Tenebrio molitor* is considered a pest of stored grains and grain-based products, but also as an important supplement in animal and human nutrition. It is important to point out that *T. molitor* larvae are sensitive to the presence of mycotoxins in the environment, changing the behavior and some biochemical parameters. Mycotoxin zearalenone (ZEA) was used to assess the effect on the induction of oxidative stress and behavior in *T. molitor* larvae. The aim of this research was to examine the effect of high doses of mycotoxin ZEA (10 and 20 mg/kg) in feed on the activity of the enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST), as well as on the parameters of locomotor activity: travel distance, time in movement, and average speed while in motion in *T. molitor* larvae.

The results of this study showed that the presence of ZEA in feed at concentrations of 10 and 20 mg/kg significantly increased the specific activity of SOD, CAT, and GST. It also affected the locomotor activity of the larvae, i.e. travel distance and time in movement were significantly lower in larvae exposed to 10 and 20 mg/kg ZEA, while average speed did not change significantly. The presence of ZEA leads to significant changes in the physiology and behavior of *T. molitor* larvae and can be useful in the early detection of food contamination with this dangerous mycotoxin.

**KEYWORDS:** *T. molitor*, mycotoxin ZEA, antioxidant



## HRONIČNI EFEKAT ALUMINIJUMA U HRANI NA OSOBINE FITNESA KOD LARVI *LYMANTRIA DISPAR* L. ( *EREBIDAE* ).

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Aluminijum (Al) je najzastupljeniji metal u Zemljinoj kori. Ovaj metal ima različite primene u mnogim granama industrije, uključujući hranu, medicinu, farmaciju i kozmetiku. Mehanizmi toksičnosti ovog metala još uvek nisu dovoljno razjašnjeni. Do sada su najčešće ispitivani efekti pesticida koji sadrže aluminijum, dok su rezultati koji opisuju dejstvo različitih koncentracija ovog metala na fitofagne insekte retki, posebno u Srbiji. U našem eksperimentu, larve gubara (*Limantria dispar* L., *Erebidae*) bile su izložene hroničnom dejstvu aluminijuma (tretman, T) u hrani (T1=50; T2=250, T3=500 i T4=1000 microg/g suve hrane), od izleganja do trećeg dana petog larvenog stupnja (V3). Praćene su osobine fitnesa (relativna brzina rasta - RGR, masa larve (V3) i vreme razvića - DT), kao prvi pokazatelji uticaja aluminijuma na rast i razvoj.

Statistički značajna razlika je otkrivena kod svih osobina fitnesa na T2 i T4. Primećene su značajne razlike: između kontrole - i tretmana (T1 i T2) za masu larve i DT; kontrole - i tretmana (T3 i T4) za RGR i DT; kao i između T1 i T4 za RGR i masu larve. Na tretmanima T1, T2, T3 postoje značajne pozitivne korelacije između mase larvi i RGR, dok na T2, T3 i T4 postoje značajne negativne korelacije između RGR i DT. Promene osobina fitnesa insekata mogu biti prvi signal upozorenja na prisustvo aluminijuma u hrani.

**KLJUČNE REČI:** aluminijum, hronični efekat, osobine fitnesa, gubar





## CHRONIC EFFECT OF DIETARY ALUMINUM ON FITNESS TRAITS IN

### *LARVAE LYMANTRIA DISPAR L. (EREBIDAE)*

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Aluminum (Al) is the most abundant metal in the Earth's crust. It has a variety of applications in many branches of industry, medicine, pharmacy and cosmetics. The mechanisms of toxicity of this metal have not yet been elucidated. So far, the effects of pesticide containing Al have been investigated, while the results describing the effect of different concentrations of this metal on phytophagous insects are rare, especially in Serbia. In our experiment, larvae of gypsy moth (*Lymantria dispar* L., Erebidae) were exposed to a chronic effect of dietary aluminum (treatment, T) (T1=50; T2 =250, T3=500 and T4=1000 microg/g of dry food), from hatching until the third day of the fifth larval stage (V3). Fitness traits (relative growth rate – RGR, larval mass at V3, and development time – DT) were monitored, as the first indicators of aluminum influence on growth and development.

Statistically significant differences were detected in all fitness traits between T2 and T4. In more detail, differences were noticed between: Control – and treatments (T1 and T2) for larval mass and DT; Control – and treatments (T3 and T4) for RGR and DT; as well as between T1 and T4 for RGR and larval mass. On treatments T1, T2, T3, there are significant positive correlations between larval mass and RGR, while on T2, T3 and T4, there are significant negative correlations between RGR and DT. Changes in insect fitness traits can be the first warning signal of the aluminum presence in food.

**KEYWORDS:** aluminum, chronic effect, fitness traits, gypsy moth



# EKOTOKSIKOLOŠKA ISPITIVANJA BISFENOLA A I NJEGOVIH DERIVATA U VODI I MULJU, IZAZOVI RAZLIČITIH KONCENTRACIJA IZLOŽENOSTI ENDOKRINIH OMETAČA NA ŽIVOTNU SREDINU I ZDRAVLJE LJUDI

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U ovom radu će biti ispitana ekotoksičnost različitih koncentracija Bisfenola A, njegovih derivata Bisfenola F i Bisfenola S, kao i smeša koje oni grade, a mogu se naći u mikroplastici u vodi i mulju, kao i istaloženi na dimetildioksiran koji predstavlja veliki izazov za njihovo uklanjanje. Ova istraživanja smo vršili na različitim mikrobiološkim kulturama, na tri gram-pozitivnih i gram-negativnih, kao i izolovanih mikrobioloških kultura iz zagađene vode i mulja koja u sebi sadrže ove endokrine ometače u realnim uslovima.

Bisfenol A predstavlja veliki izazov za našu životnoj sredinu – posebno pijaću vodu i zdravlje ljudi, sastavni je deo i mikroplastike koju usled mikroskopski sitnih čestica nije moguće ukloniti sistemima i tehnologijama za prečišćavanje otpadnih i pijaćih voda. Pred ispitivanja efikasnosti biodegradacije izabranih mikrobioloških kultura ispitivali smo i antioksidativnu aktivnost vitamina C i drugih značajnih antioksidanasa koje smo ekstrahovali iz svežeg voća i povrća, u odnosu na oksidativnu aktivnost Bisfenola A i njegovih derivata i DMDO, i obogatili izabrane mikrobiološke kulture sa ciljem stvaranja otporne biote na ove endokrine ometače koji su bili predmet istraživanja. Time očekujemo da ćemo doći do najotpornije biote obogaćene različitim kombinacijama antioksidativnih supstanci i njihovih smeša, čime ćemo doći do potencijalnih doza koje bi mogle nakon dodatnih istraživanja i testiranja na drugim ćelijskim organizmima moći predstavljati antioksidativnu odbranu naše populacije na endokrine ometače kao zagađujuće materije, koje različitim načinima unosa mogu dospeti u ljudski organizam.

**KLJUČNE REČI:** ekotoksikološka studija, endokrini ometači, Bisfenol A, Bisfenol F, Bisfenol S, mikrobiološke kulture, antioksidansi, mikroplastika, voda i mulj



## ECOTOXICOLOGICAL STUDIES OF BISPHENOL A AND THEIR DERIVATIVES IN WATER AND SLUDGE, CHALLENGES OF DIFFERENT CONCENTRATIONS OF EXPOSURE TO ENDOCRINE DISRUPTORS ON THE ENVIRONMENT AND HUMAN HEALTH

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This paper presents an Ecotoxicological study of endocrine disrupting chemicals with the examples of Bisphenol A and the derivatives that can be found in micro-plastics in water and sludge. We are researching different concentrations of Bisphenol A and their derivatives: Bisphenol F and Bisphenol S., including the mixtures these can be found in micro-plastics in water and sludge and precipitated at Dimethyl-dioxirane (DMDO), as great challenge for their removal. We carried out these researches on various microbiological cultures, on three gram positive and three gram negative, as well as on isolated microbiological cultures from such polluted water and sludge that contain these endocrine disruptors in real conditions. Bisphenol A represents a great challenge for our environment - especially for drinking water and human health, as it is also an integrated part of micro-plastics. The microscopically small particles cannot be removed by systems and technologies usually applied for purifying waste and drinking water.

Before testing the biodegradation efficiency of the selected microbiological cultures, we also tested the antioxidant activity of vitamin C and other important antioxidants that we extracted from fresh fruits and vegetables, in relation to the oxidative activity of Bisphenol A, its derivatives and DMDO, and enriched the selected microbiological cultures with the aim of creating a resistant biota to these endocrine disruptors that were the subject of the research. Finally, we will reach the most resistant biota enriched with different combinations of antioxidant substances and their mixtures; so it will lead us to potential doses that could, after additional research and testing on other cellular organisms, be able to represent the antioxidant defense of our population against endocrine disruptors such as pollutants that can reach us through different ways of intake into the human organism.

**KEYWORDS:** ecotoxicological study, endocrine disruptors, Bisphenol A, Bisphenol F, Bisphenol S, microbial cultures, antioxidants, micro-plastics, water and sludge



# BIOINFORMATIČKI ALATI ZA ISPITIVANJE TOKSİGENOG POTENCIJALA CIJANOBAKTERIJSKOG SOJA *OSCILLATORIA NIGRO-VIRIDIS* K3

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U ovom radu je primenjen set bioinformatičkih alata kako bi se ispitaio toksični potencijal cijanobakterijskog soja *Oscillatoria nigro-viridis* K3 na osnovu prisustva genskih klastera odgovornih za biosintezu najznačajnijih grupa cijanotoksina. Toksičnost ekstrakata ispitivanog soja je prethodno potvrđena in vivo, na osnovu detekcije subletalnih promena u eseuju sa embrionima vrste *Danio rerio* (zebrica). Najveći broj promena, među kojima su najizraženije deformiteti kičme i repa, edemi srčane komore i nepravilan razvoj očiju, je primećen kod grupa izloženih višim dozama ekstrakata (160, 200 i 250 µg/ml). Dodatno, zabeležen je porast u broju i intenzitetu zabeleženih malformacija sa povećanjem vremena izlaganja jedinki dejstvu ekstrakata.

Kako bi se obezbedile dodatne informacije o metaboličkom “repertoaru” ispitivanog soja i potencijalno identifikovalo jedinjenje ili grupa jedinjenja odgovornih za zabeležene toksične efekte, sekvenciran je i analiziran celokupan genom ovog soja. Hibridni pristup sekvenciranja koji kombinuje metode kojima se dobijaju duga (Oxford nanopore) i kratka (Illumina) očitavanja je primenjen kako bi se postiglo preciznije i kompletnije sastavljanje genoma de novo. Prokka i antiSMASH softveri su upotrebljeni za anotaciju genoma i detekciju genskih klastera za biosintezu sekundarnih metabolita. Rezultati su ukazali na prisustvo 15 biosintetskih klastera, među kojima je otkriven klaster uključen u proizvodnju cilindrospersina, potentnog alkaloidnog cijanotoksina. Dodatno, detektovani su klasteri za biosintezu bioaktivnih peptida (nostoficin, anabenopeptin, nostopeptolid i cijanobaktin) koji su smatrani manje toksičnim ili u nekim slučajevima netoksičnim, međutim, poznato je da u smešama njihove interakcije mogu dovesti do povećanja toksičnog dejstva. Rezultati ovog istraživanja ukazuju na značaj primenjenih bioinformatičkih alata u analizi i karakterizaciji cijanobakterijske toksičnosti.

**KLJUČNE REČI:** cijanobakterije, cilindrospersin, *Danio rerio*, bioinformatika, komparativna genomika



## USING BIOINFORMATICS TOOLS FOR EVALUATE THE TOXIGENIC POTENTIAL OF THE CYANOBACTERIA L STRAIN *OSCILLATORIA NIGRO-VIRIDIS* K3

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In this work, a set of bioinformatics tools was applied to examine the toxigenic potential of the cyanobacterial strain *Oscillatoria nigro-viridis* K3, based on the presence of gene clusters responsible for the biosynthesis of the most important groups of cyanotoxins. The toxicity of the tested strain's extracts was confirmed in vivo through the detection of sublethal changes in an assay with *Danio rerio* (zebrafish) embryos. The observed alterations included spine and tail deformities, pericardial edema and irregular eye development, with most pronounced effects seen in groups exposed to higher doses (160, 200, and 250 µg/ml). Additionally, there was an increase in the number and intensity of recorded malformations with prolonged exposure. To provide additional information on the metabolic "repertoire" of the tested strain and potentially identify the compound or group of compounds responsible for the recorded toxic effects, this strain's genome was sequenced and analyzed. A hybrid sequencing approach combining long (Oxford nanopore) and short (Illumina) reads was applied to achieve more accurate and complete de novo genome assembly.

Prokka and antiSMASH software were used for genome annotation and detection of gene clusters for the biosynthesis of secondary metabolites. The results indicated the presence of 15 biosynthetic clusters, among which was a cluster involved in the production of cylindrospermopsin, a potent alkaloid cyanotoxin. Additionally, clusters responsible for the biosynthesis of bioactive peptides, including nostophycin, anabenopeptin, nostopeptolide, and cyanobactin, were detected. The results of this research highlight the value of applied bioinformatics tools in the analysis and characterization of cyanobacterial toxicity.

**KEYWORDS:** cyanobacteria, cylindrospermopsin, *Danio rerio*, bioinformatics, comparative genomics



## ODREĐIVANJE POLICIKLIČNIH AROMATIČNIH UGLJOVODONIKA U SUSPENDOVANIM ČESTICAMA PM<sub>10</sub> U VAZDUHU NA TERITORIJI GRADA NOVOG SADA

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Frakcije u vazduhu suspendovanih čestica koje mogu imati najveći efekat na zdravlje ljudi su fine čestice PM<sub>10</sub> i PM<sub>2,5</sub>, čiji su konstituenti i policiklični aromatični ugljovodonici (PAH). Hronična izloženost PAH-ovima može dovesti do negativnih zdravstvenih efekata, uključujući povećan rizik od karcinoma. U okviru monitoringa kvaliteta vazduha na teritoriji Grada Novog Sada, u periodu oktobar 2021.–mart 2022. god. kontinualno su uzorkovane suspendovane čestice PM<sub>10</sub> na odabranim fiksnim lokacijama: prigradsko-saobraćajna, osnovna urbana, urbano-saobraćajna, osnovna ruralna i industrijska stanica. Za određivanje koncentracije 16 EPA PAH primenjena je GC/MS tehnika nakon ultrazvučne ekstrakcije PM<sub>10</sub> smešom aceton:heksan. Koncentracije ukupnih PAH-ova u suspendovanim česticama PM<sub>10</sub> kretale su se od 18 ng/m<sup>3</sup> (novembar 2021.) na mernom mestu osnovne urbane stanice, do 35 ng/m<sup>3</sup> (januar 2022.) na mernom mestu prigradsko saobraćajne stanice.

Maksimalne prosečne koncentracije pojedinačnih PAH kongenera u analiziranom vremenskom periodu na osnovnoj urbanoj i osnovnoj ruralnoj stanici dostigle su vrednosti od 2.5-3 ng/m<sup>3</sup>, na urbano saobraćajnoj i industrijskoj do 3-4 ng/m<sup>3</sup>, dok su na prigradskoj saobraćajnoj stanici prelazile 4 ng/m<sup>3</sup>. Na osnovu dominantnog prisustva PAH-ova sa 4 i 5 prstenova (BaA, Flu, CHR, suma BbkFA) zaključeno je da primarni izvor emisije PAH-ova u Gradu Novom Sadu čini saobraćaj. Primenom TEF metodologije izračunat je toksični potencijal kvantifikovanih 16 EPA PAH i utvrđeno da najveći doprinos ukupnoj karcinogenoj aktivnosti smeše PAH daje benzo(a)piren, sa udelom od 69% do 73%. Procena izloženosti benzo(a)pirenu (ILCR= 9.0x10<sup>-8</sup> - 3.6x10<sup>-7</sup>) pokazala je da je rizik od karcinoma usled inhalatorne izloženosti odrasle populacije BaP-u u vazduhu na teritoriji Grada Novog Sada zanemarljiv.

**KLJUČNE REČI:** monitoring vazduha, policiklični aromatični ugljovodonici, procena rizika, Novi Sad



## DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SUSPENDED PARTICLES PM<sub>10</sub> IN THE AIR ON THE TERRITORY OF THE CITY OF NOVI SAD

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The fractions of air suspended particles that could have the greatest effect on human health are fine particles PM<sub>10</sub> and PM<sub>2.5</sub>, and polycyclic aromatic hydrocarbons (PAHs) are their ubiquitous constituents. Chronic exposure to PAHs consequently causes a series of adverse health effects, including increased risk of cancer. As part of air quality monitoring in the City of Novi Sad, in the period October 2021 - March 2022, suspended PM<sub>10</sub> were continuously sampled at selected fixed locations: suburban-traffic, basic urban, urban-traffic, basic rural and industrial stations.

Concentrations of 16 EPA PAHs were determined using GC/MS, after ultrasonic extraction with acetone:hexane mixture. The concentrations of total PAHs ranged from 18ng/m<sup>3</sup> (November 2021) at the basic urban station, to 35ng/m<sup>3</sup> (January 2022) at the suburban traffic station. The maximum average concentrations of individual PAH congeners at the basic urban and basic rural stations reached 2.5-3ng/m<sup>3</sup>, at the urban traffic and industrial stations 3-4ng/m<sup>3</sup>, and exceeded 4ng/m<sup>3</sup> at the suburban traffic station. Based on the dominant presence of 4 and 5 rings PAHs (BaA, Flu, CHR, sum BbkFA), the primary source of PAH emission was traffic. The toxic potential of 16 EPA PAHs was calculated using the TEF methodology. Benzo(a)pyrene exhibited the greatest contribution to the total carcinogenic activity of the PAH mixture, with a share of 69% to 73%. Assessment of exposure to benzo(a)pyrene (ILCR= 9.0x10<sup>-8</sup> - 3.6x10<sup>-7</sup>) showed that risk of cancer due to inhalation exposure of the adult population to BaP in the City of Novi Sad was negligible.

**KEYWORDS:** air monitoring, polycyclic aromatic hydrocarbons, risk assessment, Novi Sad



## VETERINARSKI LEKOVI KAO MOGUĆI ZAGAĐIVAČI ŽIVOTNE SREDINE

ECOTOXICOLOGY

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Veterinarski lekovi su namenjeni pre svega za preveniranje i lečenje bolesti, odnosno za zaštitu zdravlja životinja. Osim toga, neki ove lekove, ilegalno koriste i za stimulaciju rasta, odnosno kao aditive u hrani. Pokazalo se da široka, a ponekad i neracionalna primena veterinarskih lekova ima za posledicu ne samo razvoj rezistencije (kada su u pitanju antimikrobni lekovi), već se isti (u manjim ili većim koncentracijama) mogu utvrditi i u životnoj sredini. U zemljištu, površinskim i podzemnim vodama najčešće se mogu detektovati antimikrobni lekovi, antiparazitici i antiinflamatorni lekovi. Svi oni u manjem ili većem stepenu mogu delovati štetno na brojne organizme u životnoj sredini.

U životnu sredinu oni dospevaju različitim putevima: tokom proizvodnje, u toku procesa primene, odnosno lečenja, ili u toku uklanjanja neiskorišćenih lekova, odnosno ambalaže. Potencijalni izvor kontaminacije životne sredine veterinarskim lekovima predstavlja svakako otpadni materijal (sa farmi životinja), koji se skuplja i potom (u obliku stajskog đubriva) nanosi na njive na kojima su zasejane određene poljoprivredne kulture. Uticaj veterinarskih lekova na životnu sredinu zavisi od brojnih faktora, uključujući fizičko-hemijska svojstva, korišćenu količinu i način primene, vrstu tretmana i dozu, način sakupljanja, deponovanja i rukovanja stajnjakom, metabolizma unutar životinje, te brzine degradacije u stajnjaku. Podaci o ekotoksičnosti danas su dostupni za veliki broj veterinarskih lekova. Osim direktnog delovanja na organizme u zemljištu (mikroorganizmi, gliste) i vodi (alge, bakterije, biljke), poznato je da neki od ovih lekova mogu delovati indirektno i na ptice grabljivce i slepe miševe.

**KLJUČNE REČI:** veterinarski lekovi, antimikrobni lekovi, antiparazitici, antiinflamatorni lekovi, štetni efekti, životna sredina





## VETERINARY DRUGS AS POSSIBLE CONTAMINANTS OF ENVIRONMENT

ECOTOXICOLOGY

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Veterinary medicines are primarily intended for the prevention and treatment of diseases, i.e. for the protection of animal health. In addition, these drugs are used illegally for growth stimulation, as food additives. It has been shown that the wide and sometimes irrational application of veterinary drugs results not only in the development of resistance (in the case of antimicrobial drugs), but also in the environmental contamination (in lower or higher concentrations). Antimicrobials, antiparasitics and anti-inflammatory drugs can most often be detected in soil, surface and underground water. All of them, to a lesser or greater degree, can have a harmful effect on numerous organisms in the environment.

They reach the environment in different ways: during production, during the process of application, i.e. treatment, or during the removal of unused medicines, i.e. packaging. A potential source of environmental contamination with veterinary drugs is definitely waste material (from animal farms), which is collected and then applied (in the form of manure) to the fields where certain agricultural crops are sown. The impact of veterinary drugs on the environment depends on a number of factors, including physico-chemical properties, the amount used and the method of application, the type of treatment and dose, the method of collecting, depositing and handling manure, metabolism inside the animal, and the rate of degradation in manure. Ecotoxicity data are now available for a large number of veterinary drugs. In addition to direct action on organisms in the soil (microorganisms, earthworms) and water (algae, bacteria, plants), it is known that some of these drugs can act indirectly on birds of prey and bats.

**KEYWORDS:** veterinary drugs, antimicrobial drugs, antiparasitic drugs, anti-inflammatory drugs, adverse effects, environment



## MONITORING SADRŽAJA ARSENA U ARTESKIM BUNARIMA U SREMU I SEVERNOJ MAČVI OD 2017. DO 2022.

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Arsen je jedan od faktora rizika odgovoran za nastanak različitih oboljenja i dokazan je kao karcinogen kod ljudi (IARC, Grupa 1). Izloženost arsenu putem vode za piće može dovesti do hiperpigmentacije i keratoza (sadržaj manji od 50 µg/L) ali i do karcinoma kože, a dovodi se u vezu i sa povećanim rizikom od raka pluća, jetre i mokraćne bešike. Neorganski arsen može povećati i rizik od pojave dijabetes melitusa tip 2. Određivanje sadržaja arsena u vodama za piće na teritoriji Srema i Mačve vrši se dve decenije, a od 2017. sprovodi se kontinuirani monitoring sadržaja arsena u arteskim bunarima u svim naseljima u Sremu i severnom delu Mačve. Sadržaj arsena određuje se metodom atomske apsorpcione spektrometrije (tehnika hidriranja).

Ukupan broj arteskkih bunara u kojima se vrše analize sadržaja arsena u naseljenim mestima Srema i Mačve je 67. Ukupan godišnji broj uzoraka na pomenutim lokacijama je 268. Od ukupnog broja lokacija arteskkih bunara 18% ima sadržaj arsena u vodi iznad maksimalno dozvoljene vrednosti koja iznosi 10 µg/L. Potrebno je prekinuti ekspoziciju stanovništva vodom sa lokacija sa povišenim vrednostima arsena i nastaviti sa monitoringom sadržaja arsena u vodama za piće. Preporuka za naredni period je da se uz monitoring sadržaja arsena u vodama za piće vrši i praćenje trendova oboljevanja stanovništva u mestima sa visokim vrednostima sadržaja arsena.

**KLJUČNE REČI:** arsen, voda za piće, karcinom kože



## MONITORING OF ARSENIC CONTENT IN ARTERIAL WELLS IN SREM AND NORTHERN MAČVA FROM 2017 TO 2022.

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Arsenic is one of the risk factors responsible for the occurrence of various diseases and has been proven to be a human carcinogen (IARC, Group 1). Exposure to arsenic through drinking water can lead to hyperpigmentation and keratoses (content less than 50 µg/L), as well as skin cancer, and is associated with an increased risk of lung, liver and bladder cancer. Inorganic arsenic can also increase the risk of type 2 diabetes mellitus. Determining the content of arsenic in drinking water on the territory of Srem and Mačva has been carried out for two decades, and since 2017, continuous monitoring of the content of arsenic in artesian wells has been carried out in all settlements in Srem and the northern part of Mačva.

The content of arsenic is determined by the method of atomic absorption spectrometry (hydride technique). The total number of artesian wells in which arsenic content is analyzed in the settlements of Srem and Mačva is 67. The total annual number of samples at the mentioned locations is 268. Of the total number of locations of artesian wells, 18% have arsenic content in water above the maximum allowed value of 10 µg/L. It is necessary to stop the exposure of the population to water from locations with elevated arsenic values and to continue monitoring the content of arsenic in drinking water. The recommendation for the next period is that, in addition to monitoring the arsenic content in drinking water, trends in the population's illness in places with high arsenic content should also be monitored.

**KEYWORDS:** arsenic, drinking water, skin cancer



## ENDOKRINI OMETAČI / ENDOCRINE DISRUPTORS

### ENDOKRINO-DISRUPTIVNI UČINCI PIRETROIDNOG INSEKTICIDA NA REPRODUKCIJU I RAZVOJ WISTAR ŠTAKORA IZLOŽENIH TIJEKOM GESTACIJE

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Izloženost endokrinim disruptorima tijekom razdoblja in utero predstavlja posebno osjetljivo razdoblje ranjivosti i može utjecati na zdravstvene ishode kasnije u životu. Piretroidi su jedna od najčešće korištenih skupina insekticida u svijetu, s dokazanim endokrino-disruptivnim učincima. Cilj ovog istraživanja bio je procijeniti učinke izloženosti piretroidnom insekticidu  $\alpha$ -cipermetrinu tijekom gestacije na parametre endokrine disrupcije reprodukcije majki i razvoja potomaka Wistar štakora. Skotne Wistar štakorice bile su izložene  $\alpha$ -cipermetrinu per os u dozama od 1, 10 i 19 mg/kg tm/dan, dietilstilbestrolu (pozitivna kontrola), kukuruznom ulju (kontrola otapala) i vodi (negativna kontrola) od 6. dana gestacije do okota. Tijekom gestacije izmjerene su tjelesne mase majki i potrošnja hrane. Parametri endokrine disrupcije praćeni su u majki štakora pri okotu i u potomcima do puberteta, sukladno odgovarajućim OECD protokolima. Izloženost  $\alpha$ -cipermetrinu blago je smanjila postotke prirasta tjelesnih masa majki i nije utjecala na potrošnju hrane.

Nisu pronađene razlike u parametrima endokrine disrupcije reprodukcije majki među skupinama. Prenatalna izloženost  $\alpha$ -cipermetrinu utjecala je na vrijednosti anogenitalnog razmaka, bez promjena u vremenu spolnog sazrijevanja (otvaranja vagine) u ženskih potomaka. Vrijednosti anogenitalnog razmaka u muških potomaka bile su promijenjene, ali nisu nađene razlike između skupina u prisutnosti bradavica i vremenu spolnog sazrijevanja (odvajanja prepucija). Ovi nalazi upućuju da izloženost  $\alpha$ -cipermetrinu u primijenjenim uvjetima ne utječe na reprodukciju majke uz male promjene u razvoju potomaka.

**KLJUČNE REČI:**  $\alpha$ -cipermetrin, izloženost in utero, endokrina disrupcija, pokusni štakori



## ENDOCRINE DISRUPTIVE EFFECTS OF PYRETHROID INSECTICIDE ON REPRODUCTION AND DEVELOPMENT OF WISTAR RATS EXPOSED DURING GESTATION

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Exposure to endocrine disruptive chemicals during in utero period presents a critical sensitive window of vulnerability and can influence health outcomes later in life. Pyrethroids are one of the most used groups of insecticides worldwide, with proven endocrine-disruptive effects. The aim of this investigation was to evaluate the effects of exposure to pyrethroid insecticide  $\alpha$ -cypermethrin during gestation on endocrine disruptive parameters of reproduction in mothers and development in offspring of Wistar rats. Pregnant Wistar rats were exposed per os to  $\alpha$ -cypermethrin at 1, 10 and 19 mg/kg bw/day, diethylstilbestrol (positive control), corn oil (solvent control) and water (negative control) from the 6th day of gestation until delivery. During gestation, maternal body mass and food consumption were recorded. The parameters of endocrine disruption in mother rats were monitored at delivery and in offspring until puberty, according to adequate OECD protocols.

Exposure to  $\alpha$ -cypermethrin slightly reduced the percentages of maternal body mass gain and did not affect food consumption. No differences in maternal endocrine disruptive parameters of reproduction between the groups were found. Prenatal exposure to  $\alpha$ -cypermethrin affected the values of anogenital distance, with no changes in the timing of sexual maturation (vaginal opening) in female offspring. The values of anogenital distance in male offspring were changed, but no differences between groups were found in the presence of nipples and the timing of sexual maturation (prepubertal separation). These findings suggest that exposure to  $\alpha$ -cypermethrin in applied scenarios does not affect maternal reproduction, although slight changes in offspring development were observed.

**KEYWORDS:**  $\alpha$ -cypermethrin, in utero exposure, endocrine disruption, experimental rats



## EFEKAT INTOKSIKACIJE KADMIJUMOM NA NIVOE BIELEMENATA U TKIVU TESTISA PACOVA

ENDOCRINE  
DISRUPTORS

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Endokrini ometači, u koje se između ostalih ubrajaju i toksični metali poput kadmijuma (Cd), se dovode u vezu sa sve češćom pojavom neplodnosti i poremećajima reproduktivnog zdravlja. Kako je jedan od najznačajnijih mehanizama toksičnosti Cd njegova interakcija sa bioelementima, dalje ispitivanje ovog mehanizma moglo bi da doprinese rasvetljavanju mehanizama toksičnosti ovog metala u testisima, kao i uloge izloženosti ovom metalu u reproduktivnom zdravlju. Cilj ovog rada bio je određivanje nivoa bioelemanata Cu, Fe, Mn i Zn u kontrolnom tkivu i tkivu testisa pacova koji su oralno tretirani različitim dozama Cd odabranim tako da odgovaraju dozama profesionalne izloženosti i izloženosti ovom metalu iz životne sredine.

Pacovi su podeljeni u jednu kontrolnu i šest tretiranih grupa od po sedam životinja u svakoj. Životinje su svakodnevno tokom 28 dana oralnom gavažom primale vodeni rastvor CdCl<sub>2</sub>·H<sub>2</sub>O u dozama: 0,3; 0,6; 1,25; 2,5; 5 i 10 mg Cd/kg/dan. Nakon žrtvovanja, izolovani testisi su mineralizovani i koncentracije bioelemanata određene su atomskom apsorpcionom spektrofotometrijom uz sistem za atomizaciju u plamenu. Nivoi Cu i Fe nisu se statistički značajno promenili u odnosu na kontrolnu grupu. Nivo Mn u grupi koja je primila 5 mg/kg/dan bio je značajno viši u poređenju sa kontrolnom grupom ( $p=0,017$ ). Takođe, nivo Zn u grupi koja je tretirana sa 5 mg/kg/dan bio je značajno viši u odnosu na kontrolu ( $p=0,023$ ). Ova studija je pokazala da Cd može značajno da poremeti homeostazu Mn i Zn u tkivu testisa, kao i to da ovaj efekat zavisi od primenjene doze Cd.

**KLJUČNE REČI:** kadmijum, endokrini ometači, homeostaza bioelemanata, Wistar pacovi



## EFFECT OF CADMIUM INTOXICATION ON THE BIOELEMENTS LEVELS IN TESTICULAR TISSUE OF RATS

ENDOCRINE  
DISRUPTORS

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Endocrine disruptors, which include, but are not limited to, toxic metals such as Cd, have been linked to the increasing incidence of infertility and reproductive health disorders. As one of the most important mechanisms of Cd toxicity is its interaction with bioelements, further investigation of this mechanism could contribute to a better understanding of mechanisms of endocrine-disrupting Cd activities in testes and its role in human reproductive health. The aim of this study was to determine the levels of bioelements Cu, Fe, Mn and Zn in the control tissue and testicular tissue of rats treated orally with different doses of Cd representing occupational and environmental human exposure.

Rats were randomly divided into one control, an untreated group, and six treated groups of seven animals each. Animals from the treated groups received an aqueous solution of CdCl<sub>2</sub>·H<sub>2</sub>O in doses: 0.3; 0.6; 1.25; 2.5; 5 and 10 mg Cd/kg b.w./day for 28 days and then sacrificed. After the collection and mineralization of testicular tissue, the concentration of bioelements was determined by flame atomic absorption spectrometry. Cu and Fe levels did not significantly change when compared to the control group. The Mn level in the group receiving 5 mg/kg/day was significantly higher than the control level (p=0.017). Also, the level of Zn in the group treated with 5 mg/kg/day was significantly higher than the control level (p=0.023). This study showed that Cd can significantly disrupt the homeostasis of Mn and Zn in testicular tissue and that this effect is dose-dependent.

**KEYWORDS:** cadmium, endocrine disruptors, homeostasis of bioelements, Wistar rats



## IZLOŽENOST NIKLU KAO ZAGAĐIVAČU ŽIVOTNE SREDINE KOD PACIJENATA IZ VOJVODINE SA ADENOKARCINOMOM PLUĆA

ENDOCRINE  
DISRUPTORS

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Međunarodna agencija za istraživanje raka klasifikovala je jedinjenja nikla (Ni) kao Grupu 1 kancerogena za ljude, dok je metalni nikl u Grupu 2B, verovatno kancerogen za ljude. Jedinjenja nikla se koriste u proizvodnji nerđajućeg čelika i drugih legura nikla, katalizatora, baterija, pigmenata i keramike. Pošto je nikl veoma rasprostranjen industrijski zagađivač, opšta populacija je izložena niklu kroz kontaminirani vazduh, zemljište, hranu i vodu za piće. Izloženost prašini nikla iz rafinerije i prašini sup-sulfida nikla prepoznata je kao faktor rizika za razvoj karcinoma pluća. Cilj ovog rada bio je odrediti izloženost niklu među muškim pacijentima sa uznapredovalim adenokarcinomom pluća. U ovom istraživanju prve jutarnje uzorke urina dalo je 36 pacijenata muškog pola (39-84 godine) sa inoperabilnim IIIB i IV stadijumom adenokarcinoma pluća, dijagnostikovanih na Institutu za plućne bolesti Vojvodine, Srbija. Nikl je određen ICP-MS u uzorcima. U 69,44% (25/36) uzoraka urina Ni je detektovan iznad granice kvantifikacije. Izmereni nivoi nikla bili su iznad referentnih nivoa u urinu od 1-3 µg/L za zdrave odrasle osobe koje je definisao ToxGuide Američke agencije za toksične supstance i registar bolesti. Koncentracije nikla u urinu kod posmatranih pacijenata bile su čak 446,50 µg/L. Iako je glavni neželjeni efekat izloženosti niklu kontaktni dermatitis, nijedan od pacijenata izloženih Ni nije prijavio kontaktni dermatitis. Skoro 70% pacijenata sa adenokarcinomom pluća (sa neoperabilnim IIIB i IV stadijumom) iz Vojvodine je izloženo niklu iznad nivoa za zdrave odrasle osobe.

**KLJUČNE REČI:** zagađenje, adenokarcinom pluća, epidemiološka studija, nikl endokrini ometač

**ZAHVALNICA:** Ovaj rad je podržao Pokrajinski sekretarijat za visoko obrazovanje i naučnoistraživačku delatnost, AP Vojvodina, Republika Srbija [broj granta 142-451-3120/2022-01] Ključne reči: zagađenje; adenokarcinom pluća; epidemiološka studija; nikl; endokrini ometači





## EXPOSURE TO NICKEL AS ENVIRONMENTAL POLLUTANT AMONG LUNG ADENOCARCINOMA PATIENTS IN VOJVODINA

ENDOCRINE  
DISRUPTORS

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The International Agency for Research on Cancer has classified nickel (Ni) compounds as Group 1 carcinogenic to humans while metallic nickel as Group 2B, possibly carcinogenic to humans. Nickel compounds are used in the production of stainless steel and other nickel alloys, catalysts, batteries, pigments, and ceramics. Since nickel is widespread used industrial pollutant, the general population is exposed to nickel through contaminated air, soil, food and drinking water. Exposure to nickel refinery dusts and nickel subsulfide was reported as risk factor for lung cancer.

The aim of this study was to determine the exposure to nickel among advanced lung adenocarcinoma male patients. In this research 36 male patients (39-84 years old) with inoperable IIIB and IV stadium of lung adenocarcinoma, diagnosed in the Institute for Pulmonary Diseases of Vojvodina, Serbia provided their first morning urine samples. Nickel was determined by ICP-MS in the samples. In 69.44% (25/36) urine samples Ni was detected above the limit of quantification. In all analysed samples the nickel levels were above urinary reference levels 1-3 µg/L for healthy adults defined by the ToxGuide of the US Agency for Toxic Substances and Disease Registry. The nickel urinary concentrations in the observed patients were up to 446.50 µg/L. Although the main adverse health effect of nickel exposure is contact dermatitis, none of the Ni exposed patients reported contact dermatitis. Almost 70% of lung adenocarcinoma patients (with inoperable IIIIB and IV stadium) from Vojvodina are exposed to nickel above levels for healthy adults.

**KEYWORDS:** pollution, lung adenocarcinoma, epidemiological study, nickel, endocrine disruptor

**ACKNOWLEDGEMENT:** This work was supported by the Provincial Secretariat for Higher Education and Scientific Research, AP Vojvodina, Republic of Serbia [grant number 142-451- 3120/2022-01]



## ŠTETNI EFEKTI ENDOKRINIH DISRUPTORA I NEIZVESNI REPRODUKTIVNI ISHODI

ENDOCRINE  
DISRUPTORS

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Stalno interesovanje za izloženost životne sredine i reproduktivno zdravlje dovelo je do sve većeg broja studija koje se fokusiraju na endokrine disruptore (EDCs). Oni su deo našeg svakodnevnog života zbog njihove široke upotrebe. Na primer, plastifikatori, koji uključuju bisfenol A (BPA) i ftalate, prisutni su u hrani, ambalaži i potrošačkim proizvodima kao što su medicinski uređaji, kozmetika, farmaceutske proizvodi i igračke. Godišnja svetska proizvodnja plastike koja sadrži EDCs porasla je sa 50 miliona tona na 300 miliona tona od 1970-ih i još uvek raste.

Raniji pubertet kod devojčica, opadanje broja spermatozoida i genitalne malformacije otkriveni su širom sveta, što se danas najčešće povezuje sa izloženošću EDCs. Ove supstance se oslobađaju u životnu sredinu u nivoima koji možda ne predstavljaju značajne rizike, ali su efekti hronične izloženosti zabrinjavajući i mogu biti vrlo opasni. Povećani nivoi BPA i ftalata u urinu su povezani sa smanjenim brojem oocita, poremećenom folikulogenezom, pojavom fibroida i endometrioze kod žena. Takođe, pokazalo se da izlaganje BPA ugrožava implantaciju embriona, povećava rizik od sindroma policističnih jajnika i ponavljajućih pobačaja. Sa druge strane, smanjenje kvaliteta sperme, u smislu broja, zapremine i pokretljivosti sperme, kao i karcinom prostate zabeleženi su kod muškaraca izloženih BPA i ftalatima. Utvrđeno je da uobičajeni metaboliti ftalata izazivaju kriptorhizam, hipospadiju i sindrom disgenetze testisa, što dovodi do tumora testisa. Razumevanje efekata EDCs na reproduktivno zdravlje će pomoći u razvoju smernica i za pojedince i za industriju, i podstaknuti inovacije alternativnih jedinjenja koja ne utiču na plodnost i reproduktivno zdravlje, generalno.

**KLJUČNE REČI:** bisfenol A, ftalati, endokrini disruptor, reproduktivna toksikologija

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**KLJUČNE REČI:** bisfenol A, ftalati, endokrini ometači, reproduktivna toksičnost



## UNCERTAIN REPRODUCTIVE OUTCOME AND DETRIMENTAL EFFECT OF ENDOCRINE- DISRUPTING CHEMICALS

ENDOCRINE  
DISRUPTORS

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Endocrine-disrupting chemicals (EDCs) are now part of our daily life due to their widespread usage. For example, plasticizers, which include bisphenol A (BPA) and phthalates, are found in food, packaging, and consumer products such as medical devices, cosmetics, pharmaceuticals, and toys. Annual global production of plastics containing EDCs has increased from 50 to 300 million tons since the 1970s and it continues to grow. The risen earlier puberty in girls, the drop in sperm counts, and genital malformations have been found worldwide, which are linked now mainly to the EDCs exposure. These substances are released into the environment at levels that may not pose substantial risks, but the effects of chronic exposure are of concern and they can be in some way even deleterious.

The increased urinary BPA and phthalate concentrations have been associated with decreased oocyte yield, impaired folliculogenesis, fibroids, and endometriosis in women. In addition, BPA exposure has been shown to impair embryo implantation, increase the risk of polycystic ovary syndrome, and lead to repeated miscarriages. On the other hand, the deterioration of sperm quality, in terms of sperm count, volume and motility, as well as prostate cancer have been found in men exposed to both BPA and phthalates. Common phthalate metabolites were found to cause cryptorchidism, hypospadias, and testicular dysgenesis syndrome, leading to testicular cancer. Understanding the impact of EDCs on reproductive outcome will help developing the guidelines for both individuals and industry, and hopefully encourage innovations in alternative compounds that do not affect reproductive health.

**KEYWORDS:** bisphenol A, phthalates, endocrine disruptor, reproductive toxicology

**ACKNOWLEDGEMENT:** This work was supported by the Provincial Secretariat for Higher Education and Scientific Research, AP Vojvodina, Republic of Serbia [grant number 142-451- 3120/2022-01]



## KADMIJUM I OLOVO KAO FAKTORI RIZIKA ZA NASTANAK HORMON ZAVISNIH KARCINOMA

ENDOCRINE  
DISRUPTORS

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Iako se poslednjih godina beleži pad koncentracija Cd i Pb u životnoj sredini, istraživanja nedvosmisleno potvrđuju da i izloženost niskim dozama može dovesti do oštećenja različitih organskih sistema. Imajući u vidu metaloestrogena svojstva, posebno činjenicu da deluju kao endokrini ometači i oponašaju dejstvo estrogena, njihov uticaj na reprodukciju i uloga u etiologiji hormon zavisnih karcinoma se ne sme zanemariti. Studija je imala za cilj da ispita nivoe Cd i Pb u krvi, zdravim i tumorskim tkivima pacijenata obolelih od raka dojke, prostate i testisa. Istraživanje je obuhvatilo 55 pacijentkinja sa karcinomom dojke, 41 pacijenta sa karcinomom prostate i 52 pacijenta sa karcinomom testisa, dok su kontrolne grupe bile sastavljene od zdravih dobrovoljaca.

Uzorci krvi i tkiva su mineralizovani, a za analiziranje metala korišćena je atomska apsorpciona spektrofotometrija. Koncentracije Cd i Pb u krvi nisu se značajno razlikovale u populaciji žena, dok su kod obe grupe obolelih muškaraca utvrđene značajno više koncentracije Cd u krvi u odnosu na kontrolu. Cd u krvi je prepoznat i kao značajan prediktor za nastanak karcinoma prostate (OR=1,18) i testisa (OR=1,98). U populaciji žena, koncentracija Cd u tumorskom tkivu je bila značajno viša od koncentracije Cd u zdravom tkivu, dok kod pacijenata sa karcinomom testisa nije bilo statistički značajne razlike između ispitivanih tkiva. Rezultati ove studije su potvrdili da Cd i Pb kao dvovalentni joni prolaze gotovo sve membrane i akumuliraju se u tkivu dojke, prostate i testisa. Nivo kadmijuma u krvi je prepoznat kao značajan prediktor u nastanku hormon zavisnih karcinoma.

**KLJUČNE REČI:** kadmijum, olovo, endokrini ometači, hormon zavisni karcinomi



## CADMIUM AND LEAD AS RISK FACTORS IN DEVELOPMENT OF HORMONE RELATED CANCER

ENDOCRINE  
DISRUPTORS

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Regardless of decreased environmental Cd and Pb levels in recent decades, research unequivocally confirms that exposure to low levels can damage numerous organs. Considering their metaloestrogen properties, especially the fact that they act as an endocrine disruptor and mimic the effects of estrogen, their potential role on the reproduction as well in the etiology of hormone related cancers must not be neglected. The study aimed to investigate levels of Cd and Pb in blood, healthy and tumor tissues of breast, prostate and testis cancer patients. The study enrolled 55 breast (BC) female, 41 prostate (PC) and 52 testis (TC) cancer patients, while control groups were encompassed by healthy ones. Blood and tissue samples were mineralized, prior to graphite furnace atomic absorption spectrophotometry of Cd and Pb determination.

Blood Cd and Pb levels did not differ significantly among female groups, while both male cohorts with cancers had higher blood Cd levels in comparison to control. Blood Cd was recognized as a significant predictor of PC (OR=1.18) and TC (OR=1.98). In the BC group, Cd level in tumor tissue was significantly higher than Cd level in healthy tissue, while in TC patients statistical significance difference was not reached between examined tissues. Results of the present study confirmed that there are no obstacles to Cd and Pb as bivalent ions cross almost all barriers of the organism and accumulate in breast, prostate and testis tissue. Cd has been recognized as significant factor in prediction of hormone related cancers.

**KEYWORDS:** cadmium, lead, endocrine disruptors, hormone related cancers



## INVESTIGATION OF PERFLUOROCTANE SULFONIC ACID AND PERFLUOROCTANOIC ACID EXPOSURE ON THE INTERLEU KIN-17 SIGNALING AND INDUCED LUNG DAMAGE IN *BALB/C* MICE

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The aim of our study is to examine the effects of co-exposure of Perfluorooctane-Sulfonic-Acid (PFOS) and Perfluorooctanoic-Acid (PFOA), which are per/polyfluoroalkyl compounds in the group of endocrine disrupting chemicals, on the IL-17 pathway in Balb/c male mice. In addition, to investigate the relationship of these effects with the severity of lung damage induced by exposure to polyinosinic:polycytidylic acid (poly I:C), which mimics the viral response in the organism. In our study, 9 weeks old adult male Balb/c mice weighing 18-25 grams are studied. A total of 11 groups were formed, with 6 mice in each group.

These groups are: Control groups (sham control, vehicle control, intratracheal poly I:C control, vehicle + intratracheal poly I:C control), experimental groups in which the effects of PFOS+PFOA on the basal IL-17 signaling pathway will be studied (1, 3, 9mg/kg/day PFOS+PFOA), experimental groups to investigate the effects of PFOS+PFOA on the severity of poly I:C-induced lung injury (1, 3, 9mg/kg/day PFOS+PFOA+intratracheal poly I:C). In order to examine the effect of PFOS+PFOA on the severity of lung injury, lung injury is induced by intratracheal administration of poly I:C after oral exposure to PFOS+PFOA in mice.

In order to determine these effects, quantitative analyzes of cytokines related to each other in the IL-17 signaling pathway will be performed in bronchoalveolar lavage samples and the percentages of T helper (Th) lymphocytes will be determined by flow cytometry. In addition, gene expressions related to Th lymphocytes and IL-17 in lung tissue will be examined and myeloperoxidase, which is formed as a result of neutrophil activation, will be analyzed. Currently, studies on control groups and experimental groups in which the effects of PFOS+PFOA on the basal IL-17 signaling pathway will be studied are continuing.



The results of our data obtained from our ongoing studies so far are as follows: no statistically significant difference was found in lung weights, a significant decrease in total kidney and spleen weights in the 9 mg/kg/day PFOS+PFOA dose group compared to the sham control group, a significant increase in liver weights in the 1, 3 and 9mg/kg/day PFOS+PFOA dose groups compared to the sham control group, a decrease was determined in the mean terminal body weights in the 9mg/kg/day PFOS+PFOA dose group compared to the sham control group.

It was observed that edema in the lung tissue increased in the 3 and 9mg/kg/day PFOS+PFOA dose groups compared to the sham control group. In histological results, it was determined that PFOS+PFOA exposure caused an increase in fibrous tissue formation and alveolar histiocytosis in the lung tissue. Although our studies have not been completed yet, according to the data we have obtained, it is seen that PFOS+PFOA exposure causes toxic effects on the lung tissue.

When our study is completed, it is planned to reveal the effects on the basal IL-17 pathway in the organism as a result of co-exposure to PFOS+PFOA and to reveal the effects of exposure to PFOS+PFOA on the severity of poly I:C-induced lung injury. It is thought that evaluations can be made about the mechanism of many different diseases with the data we will obtain as a result of the study.

**KEYWORDS:** perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), lung injury, IL-17 signaling pathway, toxicity



## MOŽE LI BISFENOL A DOPRINETI GOJAZNOSTI KOD PCOS?

ENDOCRINE  
DISRUPTORS

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Danas sve veću pažnju privlače efekti bisfenola A (BPA) na ženski reproduktivni sistem. Cilj istraživanja bio je da se ispita prisustvo BPA kod žena sa sindromom policističnih jajnika (PCOS) i potencijalni uticaj BPA na povećanje telesne mase. Sprovedena studija preseka uključivala je 29 žena u reproduktivnom periodu sa dijagnostifikovanim PCOS. Prisustvo bilo koje druge endokrine ili autoimune bolesti je isključeno. Nivoi BPA su određeni u jutarnjem urinu gasnom hromatografijom sa masenom spektrometrijom.

BPA je detektovan u polovini ispitivanog uzorka u koncentraciji do 39 ng/μg Cr. PCOS BPA+ žene su imale povećan indeks telesne mase (BMI) i obim struka (WC) u odnosu na PCOS BPA- žene (28,65 prema 20,1 kg/m<sup>2</sup> i 90,5 prema 72 cm, respektivno). Polovina žena sa BMI preko 30 imala je BPA u urinu. Pored toga, PCOS BPA+ žene su imale skoro 7 puta veći rizik za WC iznad 80 cm (granična vrednost za prekomernu telesnu masu) i 4 puta veću verovatnoću da će imati BMI veći od 30 (granična vrednost za gojaznost). Statistički značajno veće vrednosti količnika obima struka i visine (WtHR) su zabeležene kod PCOS BPA+ žena u poređenju sa PCOS BPA- (0,55 u odnosu na 0,42, p=0,046). Takođe, PCOS BPA+ žene imale su veći rizik za WtHR iznad 0,5. Iako je gojaznost uobičajena kod PCOS, dobijeni rezultati sugerišu da su žene izložene BPA u većem riziku da imaju prekomernu telesnu masu ili visceralnu gojaznost. Neophodna su dalja istraživanja kako bi se bolje istražili efekti BPA kod PCOS žena.

**KLJUČNE REČI:** bisfenol A, PCOS, endokrini disruptori, urin, gojaznost





## MAY BISPEHOL A INDUCE OBESITY IN PCOS?

ENDOCRINE  
DISRUPTORS

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Nowadays, bisphenol A (BPA) effects on female reproductive system attract growing attention. The aim of the research was to investigate the BPA occurrence in women with polycystic ovary syndrome (PCOS) and its possible influence on the increase of body weight. This cross-sectional study was performed on 29 PCOS women in reproductive period without any other endocrine or autoimmune disease. BPA levels were determined in morning spot urine by gas-chromatography coupled with mass spectrometry.

BPA was detected in almost half of the examined sample in concentration up to 39 ng/μg Cr. PCOS BPA+ women had increased body mass index (BMI) and waist circumference (WC) in comparison to non-exposed ones (28.65 versus 20.1 kg/m<sup>2</sup> and 90.5 versus 72 cm, respectively). Half of PCOS women with BMI over 30 had BPA in their urine. Additionally, PCOS BPA+ women had almost 7 times higher risk for WC above 80 cm (cut-off for overweight) and 4 times are more likely to have BMI higher than 30 (cut-off for obesity). Considering the waist-to high-ratio (WtHR), the statistically significant higher values were observed in PCOS BPA+ women in comparison to PCOS BPA- (0.55 versus 0.42, p=0.046). Also, PCOS BPA+ women are at higher risk to have WtHR more than 0.5. Although obesity is generally common among PCOS women, the obtained results suggest that BPA exposed women are at higher risk to be overweight, obese or with visceral obesity. Further research is needed in order to better explore the BPA effects on exacerbating and potentiating some PCOS features.

**KEYWORDS:** bisphenol A, PCOS, endocrine disruptors, urine, obesity



## DA LI SU ADOLESCENTI U VOJVODINI IZLOŽENI MEŠAVINI KANCEROGENIH ELEMENATA?

ENDOCRINE  
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Arsen i teški metali, kao što su hrom, nikl i kadmijum, su klasifikovani kao kancerogeni elementi. Hronična izloženost ovim elementima povezana je sa povećanjem oksidativnog stresa, inflamacijom i narušavanjem biohemijskih procesa kod ljudi. Naročito vulnerabilna su deca i mlade odrasle osobe. Cilj pilot studije je bio da se utvrdi izloženost adolescenta sa teritorije Vojvodine arsenu, hromu, niklu i kadmijumu.

Metodom slučajnog izbora prikupljeni su uzorci jutarnjeg urina 54 adolescenata oba pola, starosti od 11 do 21 godine. Sadržaj hroma, nikla, arsena i kadmijuma određen je u 1 ml urina, nakon mikrotalasne digestije, indukovano kuplovanom plazmom sa masenom spektrometrijom (ICP-MS). Najčeće detektovan element u analiziranim uzorcima bio je arsen (98,15%), dok je najniža frekvencija detektovanja zabeležena za kadmijum (29,63%). U više od polovine analiziranih uzoraka izmereni su hrom (55,55%) i nikl (51,85%) iznad limita kvantifikacije. Kako bi se utvrdilo da li postoje razlike između dečaka i devojčica u pogledu frekvencije detektovanja analiziranih elemenata, utvrđeno je da su devojčice više izložene hromu (60,00%) i niklu (62,86%) u odnosu na dečake (47,37% i 31,58%, respektivno).

Međutim, kod dečaka, arsen je detektovan u svim uzorcima (100%) a kadmijum u 36,56% uzoraka urina, u poređenju sa devojčicama (97,14% i 28,57% uzoraka urina, respektivno). Dobijeni rezultati ukazuju da su adolescenti sa teritorije Vojvodine izloženi smeši kancerogenih elemenata. S obzirom na to da je arsen sveprisutan u proučavanoj populaciji, neophodne je preduzeti adekvatne mere za njegovu redukciju u životnoj sredini.

**KLJUČNE REČI:** arsen, hrom, nikl, kadmijum, ICP-MS



## ARE ADOLESCENTS IN VOJVODINA EXPOSED TO MIXTURE OF CARCINOGENIC ELEMENTS?

ENDOCRINE  
DISRUPTORS

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Arsenic and heavy metals such as chromium, nickel and cadmium are classified as carcinogenic elements. Chronic exposure to those elements is associated with increased oxidative stress, inflammation and impairment of normal biochemical mechanism in humans. Children and young adults are more vulnerable to adverse effects of toxic elements than adults. Therefore, the goal of this pilot study was to identify if adolescents in Vojvodina are exposed to arsenic, chromium, nickel and cadmium. This cross-sectional study was conducted on 54 healthy adolescents of both genders, aged from 11-21. Chromium, nickel, arsenic and cadmium were determined in 1 mL of morning urine by inductively coupled plasma-mass spectrometry (ICP-MS) after the microwave system digestion.

The highest frequency of the detection was obtained for arsenic (98.15%) while cadmium was presented with the lowest incidence in analysed urine samples (29.63%). Chromium and nickel were found in more than half of the collected samples with 55.55% and 51.85% frequency of the detection, respectively. Regarding the gender differences, girls were more exposed to chromium (60.00%) and nickel (62.86%) in comparison with boys (47.37% and 31.58%, respectively). However, in boys, arsenic and cadmium were detected in 100% and 36.56% urine samples, respectively, in compared with girls (97.14% and 28.57% urine samples, respectively). The obtained results confirmed that adolescents are ubiquitously exposed to mixture of carcinogenic elements. Considering that arsenic is omnipresent in the studied population, there is an urgent need for intervention.

**KEYWORDS:** arsenic, chromium, nickel, cadmium, ICP-MS



## INFORMISANA MAMA, ZDRAVA BEBA - KAKO BEZBEDNO ŽIVETI SA HEMIKALIJAMA

ENDOCRINE  
DISRUPTORS

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Poslednjih decenija prošlog veka naučnici su utvrdili da pojedine hemikalije, mogu uticati na brojne sisteme organa i izazvati štetne efekte po naše zdravlje. Među hemikalijama sa kojima dolazimo u kontakt gotovo svakodnevno, posebno mesto zauzimaju hemikalije koje mogu delovati na endokrini sistem. Izloženost ovim supstancama tokom trudnoće se u nekim istraživanjima dovodi u vezu sa malom telesnom masom pri rođenju, prevremenim porođajem, nižim koeficijentom inteligencije, problemima u rastu i razvojem astme kod dece. Ipak, jasne smernice o tome kako možemo smanjiti izloženost hemikalijama tokom osetljivog perioda trudnoće i dojenja, kao i o potencijalnim rizicima koje neke od ovih hemikalija mogu predstavljati za potomstvo ne postoje.

Glavni cilj ovog projekta bio je podizanje svesti, informisanje i edukacija žena o značaju koji ima izloženost hemikalijama u periodu trudnoće i tokom dojenja, kao i o aktivnostima koje svaka buduća mama može preduzeti da smanji izloženost ploda, a kasnije i bebe, ovim štetnim supstancama. Kroz brojne društvene kanale komunikacije (instagram, fejsbuk, mediji) glavna ciljna grupa projekta, trudnice i dojilje, bile su upoznate sa osnovnim naučnim činjenicama, predstavljenim na jasan i razumljiv način, o zagađivačima poput bisfenola, parabena, ftalata, usporivača gorenja, toksičnih metala, pesticida i mikroplastike.

Poseban akcenat stavljen je na definisanje jasnih preporuka o malim promenama u stilu života koje mogu dovesti do pozitivnih efekata na zdravlje bebe i cele porodice. Kampanja je rezultirala kreiranjem informatora koji obuhvata pouzdane i naučno zasnovane informacije o potencijalnom štetnom dejstvu hemikalija u periodu trudnoće i dojenja, i koji je omogućio upoznavanje ciljne grupe sa bezbednijim alternativama i zdravim izborima.

**KLJUČNE REČI:** endokrini ometači, bezbednije alternative, javno zdravlje



## INFORMED MOM, HEALTHY BABY - HOW TO LIVE SAFELY WITH CHEMICALS

ENDOCRINE  
DISRUPTORS

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In the last decades of the twentieth century, scientists have observed that exposure to certain, human-made chemicals can affect numerous organ systems and cause harmful effects on our health. Among the chemicals hiding in everyday products, substances of special concern are those that can affect the endocrine system. Exposure to these potentially toxic substances during pregnancy has been linked in some studies to low birth weight, premature birth, lower IQ, growth problems, and the development of asthma in children. However, there are no clear guidelines on how we can reduce exposure to chemicals during the sensitive period of pregnancy and breastfeeding, as well as on the potential risks that some of these chemicals may pose to the offspring. The main goal of this project was to raise awareness, inform and educate women about the importance of exposure to chemicals during pregnancy and breastfeeding, as well as about the activities that every expectant mother can undertake to reduce the exposure of the fetus, and later the baby, to these harmful substances.

Through numerous social channels of communication (Instagram, Facebook, media) the main target group of the project, pregnant and lactating women, were introduced to basic scientific facts, presented in a clear and comprehensible way, about pollutants such as bisphenols, parabens, phthalates, flame retardants, toxic metals, pesticides, and microplastics. Special emphasis is placed on defining clear recommendations for small lifestyle changes that can lead to positive effects on the health of the baby and the entire family. The campaign resulted in the creation of an Informative Brochure that includes reliable and scientifically based information about the potentially harmful effects of chemicals during pregnancy and breastfeeding, and which enabled the target group to be introduced to safer alternatives and healthy choices.

**KEYWORDS:** endocrine disruptors, safer alternatives, public health



## FORENZIČKA TOKSIKOLOGIJA / FORENSIC TOXICOLOGY

### SMRTNI SLUČAJEVI U VEZI SA TROVANJEM AMFETAMINIMA - FORENZIČKE KARAKTERISTIKE I POSTMORTALNA HEMIJSKO-TOKSIKOLOŠKA ANALIZA

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Smrt u vezi sa unošenjem amfetamina posledica je trovanja, ali može biti uslovljena i promenom ponašanja, te fatalnom samopovređivanju ili zadesnim izlaganjem opasnim situacijama. Istovremeno uzimanje drugih droga i/ili lekova, kao i smrt pri niskim koncentracijama amfetamina u krvi, mogu otežati interpretaciju hemijsko-toksikološke analize.

Cilj ovog rada bio je utvrditi učestalost i forenzičke karakteristike slučajeva obdukovanih osoba sa intoksikacijom amfetaminima u trenutku smrti. Uključene su sve osobe obdukovane u Institutu, u čijim je telesnim tečnostima identifikovao prisustvo amfetamina i/ili njemu sličnih jedinjenja (MDMA, MDA), tokom četvorogodišnjeg perioda (2019-2022.). Utvrđene su koncentracije amfetamina u telesnim tečnostima, postojanje istovremene intoksikacije drugim drogama, lekovima i etil-alkoholom, uzrok i poreklo smrti. U posmatranom periodu urađene su 3682 sudskomedicinske obdukcije, a u 1378 (37,4%) učinjena je hemijsko-toksikološka analiza. U 22 (1,6%) slučaja identifikovani su amfetamini (18 muškaraca, 4 žene).

Medijana starosne dobi bila je 34,5 godine (opseg 21-46). U 19 slučajeva identifikovan je amfetamin (medijana koncentracija: 0,21 mg/l, 0,19 mg/l i 2,67 mg/l u krvi, tečnosti staklastog tela i urinu, tim redom), a u 3 samo MDMA/MDA. U 13 (59%) od 22 osobe identifikovano je prisustvo i drugih psihoaktivnih supstanci i/ili psihotropnih lekova. Etil-alkohol identifikovan je u njih petoro. U 16 od 22 osobe, smrt jeste nastupila usled direktnog dejstva droge. U po dva se slučaja radilo o samoubistvu i zadesnom povređivanju u saobraćaju, zatim o jednoj žrtvi ubistva i u jednom slučaju je smrt bila posledica postojeće bolesti. U smrtnim slučajevima sa akutnom intoksikacijom amfetaminima često je istovremeno unošenje drugih psihoaktivnih supstanci, a smrt najčešće nastupa direktno usled trovanja.

**KLJUČNE REČI:** amfetamini, forenzička toksikologija, postmortalna dijagnostika, trovanje



## AMPHETAMINE RELATED DEATHS - FORENSIC CHARACTERISTICS AND POSTMORTEM TOXICOLOGY ANALYSIS

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Death associated with amphetamine are consequence of intoxication, but it can also be caused by behavioral change, fatal self-harm, or accidental exposure to dangerous situations. Simultaneous use of other drugs, as well as death at low blood concentrations of amphetamines, can make interpretation of toxicology analysis difficult. The aim of this study was to determine the frequency and forensic characteristics of autopsied cases with amphetamine intoxication at the time of death. The study included subjects autopsied at the Institute over a four-year period (2019-2022), in whose body fluids amphetamines and/or related compounds (MDMA, MDA) were detected.

The concentrations of amphetamines in body fluids, the simultaneous intoxication with other psychoactive substances and ethyl-alcohol, the cause and manner of death were determined. In the observed period, 3682 forensic autopsies were performed, in 1378 (37.4%) toxicology analysis was performed. In 22 (1.6%) cases, amphetamines (18 men, 4 women) were identified. The median age was 34.5 years (range 21-46). Amphetamine was identified in 19 cases (median concentration: 0.21 mg/l, 0.19 mg/l, and 2.67 mg/l in blood, vitreous humor, and urine, respectively), and in 3 only MDMA/MDA. In 13 (59%) of 22 people, other drugs were identified.

Ethyl-alcohol was identified in five subjects. In 16 of 22 subjects deaths were due to direct drug effects. There were two suicides and two accidental traffic injury cases, one murder victim and in one case death was due to an illness. In deaths with amphetamine intoxication, simultaneous use of other psychoactive substances is common, and death most often occurs directly due to poisoning.

**KEYWORDS:** amphetamines, forensic toxicology, postmortem diagnostics, poisoning



## SALIVA I GUMA ZA ŽVAKANJE - ALTERNATIVNI UZORAK I PREDMET U FORENZIČKOJ TOKSIKOLOGIJI - PRIKAZ SLUČAJA

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Saliva kao biološki uzorak zauzima sve značajnije mesto u toksikološkoj analizi, naročito u kontroli korišćenja psihoaktivnih supstanci. Nivoi lekova i droga u salivi reflektuju nivo slobodnih formi tih supstanci u krvi i ukazuju na njihovu nedavnu upotrebu. Žvakaće gume, obično po sastavu gumeni polimeri sa aditivima, nakon upotrebe sadrže izvesnu količinu salive.

Cilj ovog ispitivanja bio je utvrditi da li guma za žvakanje može da se koristi kao alternativni uzorak za detekciju supstanci koje su prisutne u krvi. U toksikološku laboratoriju Instituta za sudsku medicinu stigli su postmortem uzorci krvi, urina i tečnosti staklastog tela kao i žvakaća guma, nađena u usnoj duplji muškarca, starog 30 godina. Žvakaća guma je bila čvrste konzistencije, suva i bez vidljivih tragova tečnosti. Obdukcija je vršena 24 h nakon ustanovljenja smrti. Uzorci bioloških tečnosti su pripremani tečno-tečnom ekstrakcijom, a žvakaća guma ispiranjem u metanolu.

Metodom LC-MS/MS u krvi je identifikovano prisustvo fentanila (10,6 µg/L), zolpidema (0,02 mg/L), i bupropiona i hidroksibupropiona koji nisu kvantifikovani. U urinu, tečnosti staklastog tela, kao i u metanolnom ekstraktu žvakaće gume identifikovano je prisustvo fentanila, zolpidema, bupropiona i hidroksibupropiona. U urinu je detektovan i norfentanil, metabolit fentanila. Metanolni ekstrakt gume za žvakanje sadržao je iste supstance identifikovane u krvi, i u drugim biološkim tečnostima, osim norfentanila koji je detektovan samo u urinu. Guma za žvakanje je pogodan medijum za inkorporaciju lekova i droga iz salive te može biti značajan uzorak za hemijsko-toksikološku analizu i naći primenu kako u forenzičkoj tako i u kliničkoj toksikologiji.

**KLJUČNE REČI:** saliva, guma za žvakanje, psihoaktivne supstance





## SALIVA AND CHEWING GUM - AN ALTERNATIVE SAMPLE AND SUBJECT OF FORENSIC TOXICOLOGY - CASE REPORT

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Saliva as a biological sample is gaining more importance in toxicological analyses, especially in the control of the psychoactive substances use. Levels of drugs in saliva reflect the levels of their free forms in the blood and indicate their recent use. Chewing gums, usually composed of rubber polymers with additives, contain a certain amount of saliva after use.

The aim of this study was to determine whether chewing gum can be used as an alternative sample for the detection of substances present in the blood. The toxicological laboratory of the Institute of Forensic Medicine received post-mortem samples of blood, urine and vitreous fluid, as well as chewing gum found in the mouth of a 30-year-old man. The autopsy was performed 24 hours after the death was established. The chewing gum was firm, dry, without visible liquid traces. Liquid-liquid and methanol extractions were performed for biological fluids and chewing gum, respectively. Using LC-MS/MS, fentanyl (10.6 µg/L), zolpidem (0.02 mg/L), and bupropion and hydroxybupropion (not quantified) were identified in the blood.

Fentanyl, zolpidem, bupropion and hydroxybupropion were identified in urine, vitreous humor, and in the methanol extract of chewing gum. Norfentanil, a metabolite of fentanyl, was also detected in the urine. The chewing gum methanol extract contained the same substances identified in the blood and other biological fluids, except for norfentanil. Chewing gum is a suitable medium for the incorporation of drugs from saliva. It can be an important sample for chemical-toxicological analysis and find application in both forensic and clinical toxicology.

**KEYWORDS:** saliva, chewing gum, psychoactive substances



## ANALIZA SINTETIČKIH KANABINOIDA U BILOŠKIM UZORCIMA - IZAZOVI I MOGUĆNOSTI

FORENSIC  
TOXICOLOGY

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Kontinuirana pojava novih psihoaktivnih supstanci (NPS), razvoj njihove proizvodnje na ilegalnom tržištu narkotika i raznovrsnost bioloških materijala, rezultirali su razvojem brojnih analitičkih tehnika za identifikaciju različitih NPS i njihovih metabolita u biološkim uzorcima. Tokom 2022. godine Odeljenje za fizičko-hemijska i toksikološka veštačenja, Nacionalnog centra za kriminalističku forenziku, Ministarstva unutrašnjih poslova Republike Srbije, dostavljeno je ukupno 66 različitih uzoraka u čvrstom stanju ovakvih supstanci, ukupne mase 12029,94 g, dok je ukupna zapremina uzoraka u tečnom stanju iznosila 2,25 mL u 3 uzorka.

Najzastupljenije NPS čvrstog oblika bile su sintetički kanabinoidi: 5-FLUORO-MDMB-PICA i 5F-MDMB-PINACA, dok je tečni uzorak sadržao N,N-DIMETIL-TRIPTAMIN (DMT). Sintetički kanabinoidi su agonisti kanabinoidnih receptora, dizajnirani sa ciljem da oponašaju željene efekte kanabisa. Imaju karakterističnu složeniju strukturu, te identifikacija ovih jedinjenja i njihovih metabolita zahteva brižljiv pristup u pripremi bioloških uzoraka usled složenosti matriksa, kao primenu različitih instrumentalnih tehnika. Izbor analitičkih tehnika zavisi od vrste supstance koja se analizira, potrebne osetljivosti i selektivnosti samih tehnika. Rad prikazuje pogodne analitičke tehnike koje se primenjuju u analizi sintetičkih kanabinoida u biološkim uzorcima, upućujući na njihove prednosti i ograničenja.

**KLJUČNE REČI:** nove psihoaktivne supstance, biološki materijali, sintetički kanabinoidi, analitičke tehnike



# ANALYSIS OF SYNTHETIC CANNABINOIDS IN BIOLOGICAL SAMPLES - CHALLENGES AND OPPORTUNITIES

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The continuous appearance of new psychoactive substances (NPS), the development of their production on the illegal drug market and the diversity of biological materials, resulted in the development of numerous analytical techniques for the identification of different NPS and their metabolites in biological samples. During 2022, a total of 66 different samples in the solid state of such substances, with a total weight of 12,029.94 g, were delivered to the Department for Physical, Chemical and Toxicological Expertise, National Center for Criminal Forensics, Ministry of Internal Affairs of the Republic of Serbia, while the total volume of samples in the liquid state was 2.25 mL in 3 samples. The most abundant NPS in solid form were synthetic cannabinoids: 5-FLUORO-MDMB-PICA and 5F-MDMB-PINACA, while the liquid sample contained N,N-DIMETHYL-TRYPTAMINE (DMT).

Synthetic cannabinoids are cannabinoid receptor agonists, designed to mimic the desired effects of cannabis. They have a characteristic more complex structure and the identification of these compounds and their metabolites requires a careful approach in the preparation of biological samples due to the complexity of the matrix, as well as the application of various instrumental techniques. The choice of analytical techniques depends on the type of substance to be analyzed, the required sensitivity and selectivity of the techniques themselves. The paper presents suitable analytical techniques applied in the analysis of synthetic cannabinoids in biological samples, pointing out their advantages and limitations.

**KEYWORDS:** new psychoactive substances, biological materials, synthetic cannabinoids, analytical techniques



## ASFISIJA IZAZVANA INHALACIJOM TEČNOG NAFNOG GASA - PRIKAZ SLUČAJA

FORENSIC  
TOXICOLOGY

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Propan i butan jesu alifatični zasićeni ugljovodonici koji ulaze u sastav tečnog naftnog gasa (TNT). Butan postoji u obliku dva izomera I to n-butan i izobutan. Upotrebljavaju se u industriji i domaćinstvu, ali i zloupotrebljavaju zbog narkotičkog dejstva. Lako se distribuiraju u tkiva bogata lipidima i imaju sedativni efekat na centralni nervni sistem. Cilj ovog rada je prikaz smrtnog slučaja zadesne asfiksije usled inhalacije TNT-a.

Prema podacima istražnih organa, muškarac, star 34 godine, nastradao je na benzinskoj pumpi na kojoj je radio na kontroli rezervoara i instalacija za TNT. Muškarac je ušao u šahtu da pričvrsti crevo, ustao, zateturao i izgubio svest. Na obdukciji su uočeni znakovi udušenja, tačkasti krvni podlivi na vežnjačama i plućnicama, zastoj krvi u svim organima, otok mozga i pluća. Toksikološkom analizom uzoraka krvi, urina i tečnosti staklastog tela, primenom metoda headspace gasne hromatografije sa plameno-jonizujućim detektorom, (HS-GC/FID), gasne hromatografije sa masenim detektorom (GC/MS) i tečne hromatografije sa photodiode array detektorom (HPLC/PDA), nije dokazano prisutvo etanola, lekova, droga i psihoaktivnih supstanci. Spektrofotometrijskim metodama u uzorku krvi utvrđeno je prisustvo 1,29 % MetHb i 6% COHb.

Primenom metoda HS-GC/FID i HS-GC/MS, u uzorcima krvi, želudačnom sadržaju i tkivu mozga je dokazano prisusvo butana i izobutana, dok u uzorcima urina i tečnosti staklastog tela nije dokazano prisustvo alkana. Na osnovu predloženih okolnosti slučaja, obdukcionog nalaza, nalaza na histološkim preparatima, a naročito toksikoloških analiza, zaključeno je da je smrt nasilna i da je nastupila kao posledica asfiksije usled trovanja organizma gasovitim otrovima iz grupe alkana - butanom i izobutanom.

**KLJUČNE REČI:** butan, asfiksija, HS-GC/FID, HS-GC/MS



## SUDDEN DEATH CAUSED BY INHALATION OF LIQUID PETROLEUM GAS - CASE REPORT

FORENSIC  
TOXICOLOGY

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Propane and butane are aliphatic saturated hydrocarbons that are a part of liquid petroleum gas (LPG). Butane exist in the form of two isomers, n-butane and isobutane. They are used in industry and households but are also abused due to their narcotic effect. They are very easily distributed in lipid-rich tissues and have a sedative effect on the central nervous system. This paper aims to describe a fatal case of accidental asphyxia due to inhalation of LPG. According to the investigation by the authorities, a 34-year-old man was killed at the gas station where he worked on the control of tanks and installations for LPG.

The man climbed down a shaft to attach a hose, stood up, staggered, and lost consciousness. At the autopsy, there were signs of suffocation, spot hemorrhages on the conjunctivae and lungs, blood stagnation in all organs, and swelling of the brain and lungs. Toxicological analysis of blood, urine, and vitreous humor samples, using the "head space" gas chromatography with a flame ionization detector (HS-GC/FID), gas chromatography with a mass detector (GC/MS), and liquid chromatography with a photodiode array detector (HPLC/PDA), did not identify presence of ethanol, medicaments, drugs and psychoactive substances. Spectrophotometric methods revealed the presence of 1.29% MetHb and 6% COHb in the blood sample.

The presence of butane and isobutane, using the HS-GC/FID and HS-GC/MS, was detected in the blood samples, stomach contents, and brain tissue, while the presence of alkanes was not detected in the urine and vitreous fluid samples. Based on the presented circumstances of the case, autopsy findings, findings on histological preparations, and especially toxicological analyses, it was concluded that the death was violent and that it occurred as a result of suffocation due to poisoning with gas from the alkane group - butane and isobutane.

**KEYWORDS:** butane, asphyxia, HS-GC/FIS, HS-GC/MS



## IBUPROFENOM IZAZVANE KOMPLIKACIJE SA LETALNIM ISHODOM - PRIKAZ SLUČAJA

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Ibuprofen, iz grupe nesteroidnih anti-inflamatornih lekova, najčešći je među korišćenim lekovima u terapiji akutnih i hroničnih bolova i febrilnih stanja. Predoziranje ibuprofenom obično nemaju ozbiljnije posledice, ali mogu dovesti do teške hipotenzije, oštećenja bubrega, gastrointestinalnog krvarenja, ali i letalnog ishoda. Opseg terapijskih koncentracija ibuprofena u krvi je od 15-30 mg/L, nekad i do 50 mg/L, a toksične  $\geq 100$  mg/L. Primena ibuprofena (2,4 g/dan) povezuje se sa povećanim rizikom od trombotičnih događaja.

Predstavljamo slučaj 41-godišnjeg muškarca, dugogodišnjeg alkoholičara, koji je zatečen mrtav u krevetu, u sedećem položaju. Pored njega nađen je prazan blister leka Bensedin® i blister leka Brufen® sa samo dve tablete. Nekoliko dana pre smrti žalio se na bolove u stomaku. Obdukcijom je uočen ka-tranast sadržaj u želucu i crevima, a mikroskopskim pregledom, između ostalog, i nekroza želudačne sluzokože, masno izmenjena jetra i tromboza grana vene porte.

Metodom gasne hromatografije sa masenom spektrometrijom nakon tečno-tečne ekstrakcije i derivatizacije sa bis-(trimetilsilil) trifluoroacetamidom sa 1% trimetilhlorsilana identifikovan je ibuprofen u koncentracijama: 76,35 mg/L u krvi, 2201,40 mg/L u urinu, 676,20 mg/kg u želudačnom sadržaju, a u tečnosti staklastog tela u tragu. U dostavljenim tabletama potvrđeno je prisustvo ibuprofena. U krvi je dokazano prisustvo diazepama 0,467 mg/L i alkohola 0,38 mg/mL. Zaključeno je da je smrt nastupila neposredno usled gubitka veće količine krvi u želudac i creva zbog izumiranja želudačne sluzokože, a zbog tromboze portne vene. Neselektivna primena ibuprofena jedan je od faktora koji su doprineli nastanku tromboze portne vene. Nekritična primena leka može imati neželjene posledice.

**KLJUČNE REČI:** ibuprofen, tromboza, gasna hromatografija



## IBUPROFEN-INDUCED COMPLICATIONS WITH A FATAL OUTCOME - CASE REPORT

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Ibuprofen is a non-steroidal anti-inflammatory drug, widely used in treatment of acute and chronic pain and fever. Ibuprofen overdose usually results in mild symptoms, but sometimes can lead to severe hypotension, kidney damage, gastrointestinal bleeding, even death. The therapeutic range is usually 15 to 30 mg/L up to 50 mg/L, and toxic concentrations  $\geq 100$  mg/L. The consumption of ibuprofen (2.4 g/day) is associated with an increased risk of thrombotic events.

Herein we present a case of a 41-year-old man, a long-term alcoholic, found dead in a sitting position in bed. Empty containers of Bensedin® and Brufen® with two tablets were found at the scene. In days prior to death, he complained of abdominal pain. Autopsy revealed tarry contents in the stomach and intestines, while microscopic examination revealed necrosis of the gastric mucosa, fatty liver disease and thrombosis of the portal vein branches. Ibuprofen was identified by gas chromatography with mass spectrometry after liquid-liquid extraction and derivatization with bis-(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane. The ibuprofen concentrations were: 76.35 mg/L, 2201.40 mg/L, 676.20 mg/kg in blood, urine and stomach content, respectively, and trace amounts in the vitreous humor. Ibuprofen was confirmed in the tablets. Also, diazepam 0.467 mg/L and alcohol 0.38 mg/mL were detected in blood. The cause of death was declared to be the loss of a large amount of blood down the intestinal tract from necrotic gastric musosa consequential to thrombosis of the portal vein. Uncritically used ibuprofen can lead to unwanted consequences.

**KEYWORDS:** ibuprofen, thrombosis, gas chromatography



## UČESTALOST SAOBRAĆAJNIH NESREĆA POD DEJSTVOM PSIHOAKTIVNIH SUPSTANCI NA TERITORIJI BEOGRADA

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Korišćenje psihoaktivnih supstanci (PAS) koje ispoljavaju efekte na centralni nervni sistem utiče na sposobnost vozača da upravlja vozilom i dovodi do povećanja rizika od saobraćajnih nezgoda (SN). Pored testiranja učesnika SN na prisustvo alkohola u krvi, u Nacionalnom centru za kontrolu trovanja (NCKT) se vrši testiranje učesnika SN i na prisustvo PAS (heroin, kokain, jedinjenja amfetaminske strukture, kanabinoidi). Cilj rada bio je prikazati učestalost zloupotrebe PAS kod učesnika SN registrovanih u NCKT u 2021 i 2022. godini. U istraživanju su korišćeni podaci NCKT dobijeni analizom urina na prisustvo PAS, nakon intervencije saobraćajne policije.

Detekcija PAS vršena je primenom imunohromatografskih test traka, a potvrda prisustva primenom tačne hromatografije sa UV skenirajućim i maseno-spektrometrijskim detektorom. U 2021 i 2022. godini, skrining na PAS bio je zahtevan za 103 učesnika SN (55 u 2021. godini i 48 u 2022. godini). Oko 60% analiziranih uzoraka bilo je negativno na prisustvo PAS. Psihostimulansi, kokain i jedinjenja amfetaminske strukture, potvrđeni su u 18% analiza.

Posmatrano pojedinačno, najčešće korišćena PAS bila je marihuana u 19% slučajeva i kokain u 12% slučajeva, Registrovan je po jedan slučaj korišćenja MDMA, metadona i buprenorfina. Upotreba samo jedne PAS bila je zabeležena kod 31 ispitanika, dok je kod 4 ispitanika zabeležena kombinacija kanabinoida sa kokainom (3 slučaja) i amfetaminom (1 slučaj). Testiranje učesnika SN na prisustvo PAS od strane saobraćajne policije se vrši kada postoji sumnja na njihovo korišćenje. Prikazani podaci ukazuju na usku povezanost vožnje pod dejstvom PAS i saobraćajnih nezgoda.

**KLJUČNE REČI:** psihoaktivne supstance, saobraćajne nezgode, vožnja pod dejstvom PAS





## FREQUENCY OF TRAFFIC ACCIDENTS UNDER THE INFLUENCE OF PSYCHOACTIVE SUBSTANCES IN THE TERRITORY OF BELGRADE

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Use of psychoactive substances (PAS) that have effects on central nervous system and affect the driver's ability to drive and leads to risk increase of traffic accidents (TA). Besides testing TA participants for alcohol presence in their blood, National Poison Control Center (NPCC) also tests TA participants for presence of PAS (heroin, cocaine, compounds of amphetamine structure, cannabinoids). The aim of this study was show frequency of PAS abuse in traffic accidents (TA) participants, registered in NPCC in 2021 and 2022.

This research used NPCC data obtained after urine analysis for PAS presence, after traffic police intervention. Detection of PAS presence was performed using immuno-chromatographic test strips, and confirmation was performed using a liquid chromatography with UV scanning and mass spectrometric detector. In 2021 and 2022, PAS screening was required at 103 TA participants (55 in 2021 and 48 in 2022). About 60% of analyzing samples were negative on PAS. Psychostimulants, cocaine and amphetamines confirmed in 18% of samples. Considered individually, most frequently used PAS was marijuana in 19% of cases, followed by cocaine in 12% of cases. One case of using MDMA, methadone and buprenorphine was registered. The use of only one PAS was recorded in 31 respondents, while in four respondents, combination of cannabinoids and cocaine (three cases) and amphetamine (one case) was recorded. Testing of TA participants for the presence of PAS by the traffic police is carried out when there is suspicion of their use. The presented data indicate a close connection between driving under influence of PAS and road traffic accidents

**KEYWORDS:** psychoactive substances, traffic accidents, driving under the influence of PAS



## SMRT U EKSCITACIONOM DELIRIJUMU - PRIKAZ SLUČAJA

FORENSIC  
TOXICOLOGY

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Stanje ekscitacionog delirijuma karakteriše iznenadni psihomotorni nemir, praćen psihičkom i fizičkom ekscitacijom i hiperaktivnošću osobe, tako da ona postaje konfuzna, iracionalnog ponašanja i nasilna. Prisustvo psihoaktivnih supstanci (PAS) koje je osoba prethodno konzumirala značajno potencira štetne posledice ovakvog stanja i doprinosi nastanku smrti.

Prikazujemo slučaj iznenadne smrti mladića starog 35 godina, posle faze ekscitacionog delirijuma. Prema podacima o okolnostima slučaja, mladić je neposredno pre smrti bio veoma nasilan, izrazito agitiran i uznemiren. Smrtnom ishodu je prethodio verbalni i fizički sukob sa članovima porodice, nakon čega je iz kuće izašao polomivši staklena vrata (kroz koja je prošao dok su bila zatvorena), a zatim i kroz živu ogradu. Sudskomedicinskom obdukcijom su ustanovljene povrede lica i udova nastale od stakla i šiblja (krvni podlivi, oguljotine). U analiziranim uzorcima bioloških tečnosti i briseva sluzokoža nosnih hodnika identifikovani su kokain i njegovi metaboliti (benzoilekgonin, metilekgonin). Posle potpune analize slučaja (obdukcija, hemijsko-toksikološki i mikroskopski pregled organa, podaci o okolnostima slučaja i dr.), ustanovljeno je da je smrt bila u vezi s uzimanjem droge, a ne posledica zadobijenih povreda.

Prikaz slučaja potencira moguće patofiziološke mehanizme umiranja u stanju ekscitacionog delirijuma sa posebnim osvrtom na činjenicu da ne postoje medicinski parametri na osnovu kojih bi se moglo ustanoviti da li je i u kojoj meri neželjeno dejstvo unetih PAS bilo potencirano i okolnostima slučaja.

Upoznavanje obducenta sa specifičnim okolnostima pod kojima se smrt dogodila, uz obdukciju, hemijsko-toksikološku analizu i mikroskopski pregled organa, neophodno je za donošenje adekvatnog zaključka o uzroku i poreklu smrti.

**KLJUČNE REČI:** ekscitacioni delirijum, obdukcija, uzrok smrti, hemijsko-toksikološka analiza



## DEATH IN EXCITED DELIRIUM SYNDROME - CASE REPORT

FORENSIC  
TOXICOLOGY

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The state of excited delirium is characterized by sudden psychomotor agitation, mental and physical excitement and hyperactivity, so that the person becomes confused, irrational and violent. The presence of previously consumed psychoactive substances (PAS) significantly enhances the harmful consequences of this condition and contributes to the occurrence of death.

We present a case of a 35-year-old man sudden death, after a phase of excited delirium. Immediately before death the man was very violent and agitated. He had verbal and physical conflict with family members, after which he left the house by breaking a glass-door (through which he passed while it was closed) and then through a hedge. Autopsy revealed injuries caused by glass and bushes (bruises, lacerations).

Cocaine and its metabolites were identified in the analyzed samples. Complete analysis of the case (autopsy, toxicological analyses and microscopic organ examination, along with the circumstances of the case) showed that the death was related to drug use, and not a consequence of the sustained injuries.

The case emphasizes the possible pathophysiological mechanisms of dying in a state of excited delirium, with special reference to the fact that there are no medical parameters on which it would be possible to establish whether and to what extent the adverse effect of administered PAS was potentiated by the circumstances of the case. Death circumstances, autopsy, toxicological analysis and microscopic organ examination is necessary to make an adequate conclusion about the cause of death.

**KEYWORDS:** excited delirium, autopsy, cause of death, toxicological analysis



## POVEZANOST STEPENA ATEROSKLEROZE KORONARNIH ARTERIJA I STAROSTI OSOBE SA TEŽINOM TROVANJA UGLJENMONOKSIDOM KOD ŽRTAVA POŽARA U ZATVORENOM PROSTORU

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Ateroskleroza koronarnih arterija jeste faktor rizika za povećanu osetljivost na trovanje ugljen-monoksidom (CO). Produkcija CO uglavnom je značajna u požarima u zatvorenom prostoru. Cilj rada bio je analizirati stepen trovanja CO u odnosu na težinu ateroskleroze koronarnih arterija i starost osoba, koje su stradale bez opsežnih opekotina u požaru u zatvorenom prostoru. Korišćen je autopsijski materijal Instituta za sudsku medicinu u Beogradu.

Uzorak je činilo 17 osoba stradalih u požaru usled trovanja CO, sa opečenošću do 20% površine tela. Podeljen je u dve grupe, prema stepenu ateroskleroze koronarnih arterija, na sledeći način: 1- blaga do umerena ateroskleroza, bez kalcifikovanih plakova; 2 – teška ateroskleroza sa kalcifikovanim plakovima. Nivoi karboksihemoglobina (COHb) upoređeni su između grupa. Uzorak je činilo 14 muškarca i 3 žene. Medijana starosne dobi bila je 68 godina (opseg (24-89)). Medijana nivoa COHb bila je 70% (30%-80%). Blagu do umerenu aterosklerozu imalo je 10 osoba, a tešku 7 osoba. Osobe sa teškom aterosklerozom nisu imale značajno niže nivoe COHb u odnosu na osobe sa blagom do umerenom aterosklerozom (medijana 70%, opsega 30%-75% naspram medijane 75%, opsega 30%-80%;  $p>0,05$ ) i nisu bile značajno starije ( $p>0,05$ ).

Postojanje kalcifikovanih plakova nije bilo povezano sa vrednostima COHb<50%. Međutim, životna dob značajno je negativno korelirala sa vrednostima COHb ( $\rho = -0.58$ ,  $p<0,05$ ). Osobe sa COHb<50% bile su značajno starije od onih sa višim vrednostima COHb (79 godina (73-89)), naspram 60 godina (24-87);  $p<0,05$ ). Starija životna dob, ali ne nužno i teška ateroskleroza koronarnih arterija, jeste povezana sa smrtnim trovanjem ugljen-monoksidom pri nižim koncentracijama.

**KLJUČNE REČI:** ugljen-monoksid, trovanje, ateroskleroza, koronarne arterije, požar u zatvorenom prostoru



## THE ASSOCIATION BETWEEN THE CORONARY ATHEROSCLEROSIS SEVERITY, AGE, AND SEVERITY OF CARBON MONOXIDE POISONING IN VICTIMS OF INDOOR FIRES

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Coronary artery atherosclerosis is a risk factor for higher susceptibility to carbon monoxide (CO) poisoning. CO production is usually significant in indoor fires. The study aimed to evaluate the severity of CO poisoning regarding the severity of coronary atherosclerosis and age in subjects who died without extensive burns in indoor fires.

We used autopsy records from the Institute of Forensic Medicine, Belgrade. Seventeen subjects who died of CO poisoning at the scene, with <20% of body surface area burned, were included. Subjects were assigned into two groups based on the severity of coronary atherosclerosis: 1 – minimal/moderate, without plaque calcification; 2 – severe, with plaque calcifications. The carboxyhemoglobin (COHb) levels were compared between the groups. Fourteen men and three women were included.

The median age was 68 years (range 24-89). The median COHb level was 70% (30%-80%). Ten subjects had minimal/moderate atherosclerosis, and 7 had severe atherosclerosis. Subjects with severe atherosclerosis did not have significantly lower COHb levels compared to the other group (median 70%, range 30%-75% vs. median 75%, range 30%-80%;  $p>0.05$ ) and were not significantly older ( $p>0.05$ ). Plaque calcifications were not associated with COHb<50% levels ( $p>0.05$ ). However, age significantly negatively correlated with COHb levels ( $\rho = -0.58$ ,  $p<0.05$ ). Subjects with COHb<50% were significantly older than those with higher COHb levels (79 (73-89) vs. 60 (24-87) years;  $p<0.05$ ). Our results show that older age, and not necessarily severe coronary atherosclerosis, is associated with lethal outcomes at lower concentrations of CO in indoor fires.

**KEYWORDS:** carbon monoxide, poisoning, atherosclerosis, coronary arteries, indoor fire



## GENOTOKSIKOLOGIJA I KARCINOGENOST / GENOTOXICOLOGY AND CARCINOGENICITY

### DETEKCIJA DVOLANČANIH OŠTEĆENJA DNK MOLEKULA $\gamma$ H2AX TESTOM I ANALIZA ĆELIJSKOG CIKLUSA NAKON DEJSTVA POTENCIJALNIH ANTITUMORSKIH AGENASA TBQ I NJEGOVIH ALKILTIO I ARILTIO DERIVATE

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2-terc-butil-1,4-benzohinon (TBQ) i njegovi alkiltio i ariltio derivati: 2-terc-butil-5-(izopropiltio)-1,4-benzohinon, 2-terc-butil-5-(propiltio)-1,4-benzohinon, 2-terc-butil-5,6-(etileneditio)-1,4-benzohinon, 2-terc-butil-5-(feniltio)-1,4-benzohinon i 2-terc-butil-6-(feniltio)-1,4-benzohinon su sintetisani kao analozi biološki aktivnih jedinjenja prirodnog porekla sa antitumorskim delovanjem: hinona avarola/avarona. Za detekciju potencijalnog genotoksičnog efekta TBQ i njegovih derivata u HepG2 ćelijskoj liniji korišćen je  $\gamma$ H2AX test. Pored toga, na istom HepG2 model sistemu praćen je i efekat ovih supstanci na ćelijski ciklus. Budući da fosforilacija histona H2AX predstavlja rani događaj u ćelijskom odgovoru na dvolančane prekide DNK molekula (DSBs), analiza koja se zasniva na detekciji fosforilisanih histona H2AX ( $\gamma$ H2AX) može se koristiti kao biomarker genotoksičnosti i genomske nestabilnosti. U ovom radu, primenjeni su  $\gamma$ H2AX test i protočna citometrija za analizu genotoksičnog potencijala i uticaja na ćelijski ciklus TBQ i njegovih derivata.



## GENOTOKSIKOLOGIJA I KARCINOGENOST / GENOTOXICOLOGY AND CARCINOGENICITY

Obeleženim antitelima specifičnim za  $\gamma$ H2AX, detektuju se dvolančana oštećenja DNK molekula (DSBs), a protočnom citometrijom se analizira ćelijski ciklus u HepG2 ćelijskoj liniji. Na osnovu dobijenih rezultata samo je 2-terc-butil-5,6-(etileneditio)-1,4-benzohinon indukovao povećano formiranje DSBs.

Takođe, isti derivat, izazvao je i značajno veće zaustavljanje ćelija u G2/M fazi ćelijskog ciklusa u odnosu na TBQ sa oko 27% (TBQ) na 34% ukupne populacije sa smanjenjem ćelijske populacije S faze. Formiranjem DSBs, 2-terc-butil-5,6-(etileneditio)-1,4-benzohinon dovodi do genomske nestabilnosti HepG2 ćelijske linije što kao posledicu ima zaustavljanje ćelijskog ciklusa u G2/M fazi.

**KLJUČNE REČI:** TBQ,  $\gamma$ H2AX esej, DSBs, ćelijski ciklus



## DETECTION OF DOUBLE-STRAND BREAKS IN DNA MOLECULES BY THE $\gamma$ H2AX ASSAY AND ANALYSIS OF THE CELL CYCLE AFTER TREATMENT WITH POTENTIAL ANTITUMOR AGENTS TBQ AND ITS ALKYLTHIO AND ARYLTHIO DERIVATIVES

GENOTOXICOLOGY  
AND CARCINOGENICITY

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2-tert-butyl-1,4-benzoquinone (TBQ) and its alkylthio and arylthio derivatives: 2-tert-butyl-5-(isopropylthio)-1,4-benzoquinone, 2-tert-butyl-5-(propylthio)-1,4-benzoquinone, 2-tert-butyl-5,6-(ethylenedithio)-1,4-benzoquinone, 2-tert-butyl-5-(phenylthio)-1,4-benzoquinone and 2-tert-butyl-6-(phenylthio)-1,4-benzoquinone were synthesized as analogs of biologically active compounds of natural origin with antitumor activity: quinone avarol/avarone.

The  $\gamma$ H2AX test was used to detect the potential genotoxic effect of TBQ and its derivatives in the HepG2 cell line. In addition, the effect of these substances on the cell cycle was monitored on the same HepG2 model system. Since the phosphorylation of histone H2AX is an early event in the cellular response to DNA double-strand breaks (DSBs), an assay based on the detection of phosphorylated histone H2AX ( $\gamma$ H2AX) can be used as a biomarker of genotoxicity and genomic instability. In this work, the  $\gamma$ H2AX test and flow cytometry were used to analyze the genotoxic potential and the effect on the cell cycle of TBQ and its derivatives.





Double-strand breaks (DSBs) in DNA are detected with  $\gamma$ H2AX-specific antibodies, and the cell cycle in the HepG2 cell line is analyzed by flow cytometry. Based on the obtained results, only 2-tert-butyl-5,6-(ethylenedithio)-1,4-benzoquinone induced increased formation of DSBs.

Also, the same derivative caused a significantly greater arrest of cells in the G2/M phase of the cell cycle compared to TBQ from about 27% (TBQ) to 34% of the total population with a decrease in the S phase cell population. By forming DSBs, 2-tert-butyl-5,6-(ethylenedithio)-1,4-benzoquinone leads to genomic instability of the HepG2 cell line, which results in cell cycle arrest in the G2/M phase.

**KEYWORDS:** TBQ,  $\gamma$ H2AX assay, DSBs, cell cycle



# PARAMETRI OKSIDATIVNOG STRESA KAO ALTERNATIVNI BIOMARKERI EKSPOZICIJE GENOTOKSIČNIM AGENSIMA

GENOTOXICOLOGY  
AND CARCINOGENICITY

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Jonizujuće zračenje je potentan genotoksični agens koji uzrokuje oksidativna oštećenja stvaranjem reaktivnih kiseoničnih radikala. Studija je imala za cilj da vrednuje komponente redoks statusa u odgovoru na izlaganje zračenju i proceni njihov značaj kao alternativnih biomarkera ekspozicije, pored dobro utvrđenih citogenetičkih markera kao što su mikronukleusi.

Uzorci krvi 52 zdrava donora ozračeni su poznatim dozama X zračenja (0 Gy; 0.75 Gy; 1,5 Gy i 3Gy) nakon čega su analizirani citogenetički i biohemijski. Uočavaju se povećanja vrednosti svih analiziranih parametara u zavisnosti od doze. Nekoliko parametara oksidativnog stresa pokazalo je nižu interindividualnu varijabilnost kao odgovor na delovanje zračenja nego mikronukleusi. Dozna zavisnost između učestalosti mikronukleusa i pojedinačnih biohemijskih parametara otkrio je značajan paralelizam uz najveću uočenu varijabilnost u neozračenim uzorcima.

Uzimajući u obzir sve rezultate dobijene u ovom eksperimentu, preporuka je dopuniti mikronukleus test dodatnim biohemijskim analizama, pre svega utvrđivanjem vrednosti proteinskih proizvoda napredne oksidacije (AOPP), malondialdehida (MDA) i ukupnog oksidativnog statusa (TOS), kako bi se umanjila visoka interindividualna varijabilnost u vrednostima mikronukleusa na dozama od 0 Gy i 3 Gy i postigao bolji uvid u oštećenja izazvana jonizujućim zračenjima.

**KLJUČNE REČI:** jonizujuće zračenje, mikronukleusi, parametri oksidativnog stresa



## OXIDATIVE STRESS PARAMETERS AS ALTERNATIVE MARKERS OF GENOTOXIC EXPOSURE

GENOTOXICOLOGY  
AND CARCINOGENICITY

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Ionizing radiation (IR) is a potent genotoxic agent that can cause oxidative damage by producing reactive oxygen species. The aim of this study was to evaluate the potential of redox status components to become alternative or surrogate markers of IR-induced DNA damage, besides well-established cytogenetic biomarkers, such as micronuclei (MN). Blood samples from 52 healthy donors were subjected to four doses of IR (0, 0.75, 1.5 and 3 Gy) followed by cytogenetic and biochemical analyses. The results showed clear dose-dependent increases in all analyzed parameters.

Several oxidative stress parameters exhibited lower interindividual variability in response to IR damage than MN. The dose dependent ratio between MN frequency and individual biochemical parameters and MN distribution revealed significant parallelism with the highest variability in unirradiated samples. Considering all the results obtained in this experiment, we propose that the well-established biodosimetry cytokinesis block-micronucleus (CBMN) test should be supplemented with estimation of advanced oxidation protein products (AOPP) and/or malondialdehyde (MDA) and/or total oxidant status (TOS), in order to diminish the high interindividual variability of MN values at 0 Gy and 3 Gy and to achieve better insight into IR-induced damage.

**KEYWORDS:** ionizing radiation, micronuclei, oxidative stress parameters



## UTJECAJ RAZLIČITIH VREMENSKIH OKVIRA IZLOŽENOSTI NA DESKRIPTORE KOMET TESTA: SLUČAJ ONEČIŠĆENJA ZRAKA

GENOTOXICOLOGY  
AND CARCINOGENICITY

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Onečišćenje zraka predstavlja veliki okolišni rizik za zdravlje, koji doprinosi razvoju mnogih bolesti. Učinke izloženosti onečišćenju zraka teško je odrediti jer različite kemijske vrste u njemu nije lako definirati ili izmjeriti te mogu izazvati aditivne, sinergističke ili antagonističke učinke. Komet test je metoda koja se uspješno koristi u praćenju genotoksičnosti onečišćenja zraka kod izloženih populacija.

Stoga smo željeli istražiti moguće učinke onečišćenja zraka na integritet DNA s pomoću alkalnog komet testa na ljudskim perifernim krvnim stanicama. Istraživanje je provedeno tijekom hladnijeg razdoblja 2021. godine i uključilo je 60 zdravih ispitanika (34 žene i 26 muškaraca), starosti 36,4±9,6 godina i BMI<30 kg/m<sup>2</sup> koji žive u Zagrebu (Hrvatska). Kako bi se procijenio potencijalni utjecaj onečišćenja zraka na deskriptore komet testa, korelacija je napravljena korištenjem različitih vremenskih okvira izloženosti onečišćenju zraka prije uzorkovanja krvi (1, 3 i 7 dana). Svi izmjereni parametri onečišćenja vanjskog zraka bili su u skladu s prethodno prijavljenim vrijednostima, pri čemu je udio čestica PM<sub>10</sub> (čestice aerodinamičkog promjera manjeg od 10 μm) i benzo[a]piren vezan za PM<sub>10</sub> povremeno prelazio regulatorne granične vrijednosti.

Nije bilo jasne povezanosti između izmjerenih parametara onečišćenja vanjskog zraka i deskriptora komet testa (duljina repa, intenziteta ili momenta). Statistička analiza provedena je korištenjem Spearmanove korelacije i modeliranja linearne regresije. Buduća istraživanja uključivat će isti dizajn studije za stanovništvo koje živi u drugim hrvatskim gradovima, gdje očekujemo izloženost različitim onečišćivačima.

**KLJUČNE REČI:** onečišćenje zraka, komet test, biomonitoring ljudi

**ZAHVALNICA:** Financirala Hrvatska zaklada za znanost: #1192 (HUMNap) i "Projekt razvoja karijera mladih istraživača – izobrazba doktoranada".



## THE IMPACT OF DIFFERENT EXPOSURE TIME FRAMES ON THE COMET ASSAY DESCRIPTORS: A CASE OF AIR POLLUTION

GENOTOXICOLOGY  
AND CARCINOGENICITY

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Air pollution represents a major environmental risk to health, facilitating development of many diseases. The effects of air pollution exposure are difficult to determine because the different chemical species in it are not easily definable or measurable, and they can induce additive, synergistic or antagonistic effects. The comet assay is a method successfully used in monitoring air pollution genotoxicity in exposed populations.

Hence, we aimed to investigate possible effects of air pollution on DNA integrity using the alkaline comet assay in human peripheral blood cells. The study was conducted during the colder period of the year 2021 and involved 60 healthy subjects (34 females and 26 males), aged 36.4±9.6 years and BMI<30 kg/m<sup>2</sup> living in Zagreb (Croatia). To evaluate potential impact of air pollution on comet assay descriptors, association was made using different time windows of exposure to air pollution prior to blood sampling (1, 3, and 7 days). All measured outdoor air pollution parameters were in agreement with previously reported values, with PM<sub>10</sub> particle fraction (particles with aerodynamic diameter less than 10 μm) and benzo[a]pyrene bound to PM<sub>10</sub>, that occasionally exceeded regulatory limit levels. There was no clear association between measured outdoor air pollution parameters and comet assay descriptors (tail length, intensity, or moment). Statistical analysis was done using Spearman's correlation and Linear regression modelling. Future research will include the same study design for populations living in other Croatian cities, where we expect exposure to different set of air pollutants.

**KEYWORDS:** air pollution, comet test, human biomonitoring

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## ODLOMAK IZ META-ANALIZE: DA LI JE IZLOŽENOST ZAGAĐIVAČIMA IZ ŽIVOTNE SREDINE POVEZANA SA KARCINOMOM PANKREASA?

GENOTOXICOLOGY  
AND CARCINOGENICITY

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Istraživanje u oblasti karcinoma pankreasa i zagađivača iz životne sredine sve je intenzivnije. Za neke od ovih zagađivača pretpostavlja se da su povezani sa nastankom ili progresijom karcinoma pankreasa.

Nijedna meta-analiza o povezanosti navedenog nije sprovedena, dok bi rezultati iste mogli pomoći u tumačenju dostupnih podataka i usmeravanju budućeg istraživanja. Cilj ovog rada je da pruži zbirni prikaz osnovnih podataka o povezanosti ekspozicije zagađivačima iz životne sredine sa karcinomom pankreasa. Protokol meta-analize razvijen je u skladu sa MOOSE i PRISMA preporukama. Izvršena je pretraga PROSPERO baze kako bi se pronašli slični protokoli.

Literatura je pretražena koristeći PubMed i primarna, recenzirana, originalna, opservaciona istraživanja kod ljudi su razmatrana, ukoliko je u njima kvantitativno okarakterisana povezanost ekspozicije zagađivačima iz životne sredine sa karcinomom pankreasa. Razmatrane su samo publikacije na engleskom jeziku. Odnos šansi (eng. odds ratio, OR) i, ukoliko je primenjivo, drugi odnosi su analizirani koristeći SPSS verziju 29.0.0.0, i dodeljena im je težina metodom inverzne varijanse.



## GENOTOXICOLOGY AND CARCINOGENICITY

Kvalitet i pristrasnost istraživanja procenjeni su koristeći Newcastle-Ottawa i ROBINS-E skale. Slični protokoli nisu pronađeni u PROSPERU. Ukupni OR (95% CI) iznosio je 1.00 (0.87-1.16). Stratifikovano po glavnim zagađivačima, dobijeni su OR (95% CI) od 0.82 (0.61-1.10) za pesticide, 1.42 (0.36-5.62) za p,p'-DDT, 1.24 (0.32-4.76) za p,p'-DDE, 1.17 (0.30-4.63) za PCB 138, 1.27 (0.31-5.16) za PCB 153, 1.12 (0.28-4.54) za PCB 180, 1.09 (0.25-4.86) za HCB, 1.16 (0.27-5.01) za  $\beta$ -HCH i 1.16 (0.27-5.01) za metale.

Brojniji i/ili bolje opisani podaci neophodni su da bi se iz meta-analize moglo izvući više zaključaka, što je osnovano očekivati s obzirom na dostupnost smernica i intenzivno istraživanje.

**KLJUČNE REČI:** karcinom pankreasa, zagađivači iz životne sredine, meta-analiza, izloženost, karcinogeneza



## A META-ANALYSIS EXCERPT: IS EXPOSURE TO ENVIRONMENTAL POLLUTANTS ASSOCIATED WITH PANCREATIC CANCER?

GENOTOXICOLOGY  
AND CARCINOGENICITY

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There is a rise in research on the connection between pancreatic cancer and environmental pollutants. Some of these pollutants are assumed to be associated with the onset or progression of pancreatic cancer. No meta-analyses summarizing the association between these two have been conducted yet, while the results of such an analysis could help interpret current and guide future research.

The aim of this work is to provide a basic summary of the current data concerning the association between exposure to environmental pollutants and pancreatic cancer. A protocol for a meta-analysis compliant with MOOSE and PRISMA guidelines was developed. PROSPERO registry was searched for similar protocols. A literature search was conducted using PubMed and primary, peer-reviewed, original, observational research in humans was considered on the condition it quantitatively assessed the association between exposure to environmental pollutants and pancreatic cancer. Only publications in English were considered. Odds ratios (ORs) and, if appropriate, other ratios were analyzed using SPSS Version 29.0.0.0 and weighted using the inverse variance method. Study quality and bias were assessed using Newcastle-Ottawa and ROBINS-E scales.





No similar protocols were found in PROSPERO. A summary OR (95% CI) of 1.00 (0.87-1.16) was obtained. Stratified by main pollutants, obtained ORs (95% CI) were 0.82 (0.61-1.10) for pesticides, 1.42 (0.36-5.62) for p,p'-DDT, 1.24 (0.32-4.76) for p,p'-DDE, 1.17 (0.30-4.63) for PCB 138, 1.27 (0.31-5.16) for PCB 153, 1.12 (0.28-4.54) for PCB 180, 1.09 (0.25-4.86) for HCB, 1.16 (0.27-5.01) for  $\beta$ -HCH, and 1.16 (0.27-5.01) for metals.

More and/or better reported data is needed to draw more reliable conclusions from the meta-analysis, a feasible prospect with guidelines available and research growing.

**KEYWORDS:** pancreatic cancer, environmental pollutants, meta-analysis, exposure, carcinogenesis



## KLOTHO GENE POLYMORPHISM AS A SUSCEPTIBILITY FACTOR FOR OXIDATIVE DNA DAMAGE IN CORONARY ARTERY DISEASE

GENOTOXICOLOGY  
AND CARCINOGENICITY

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Klotho protein has been associated with protective effects that contribute to cardiovascular health maintenance due to its ability to increase resistance to oxidative stress. The Klotho gene polymorphisms are suspected to have a potential role in the regulation of human aging and age-related diseases, including coronary artery disease, chronic kidney disease, osteoporosis, and stroke.

The goal of our study was to examine the correlation between rs9527025 (Cys370Ser) polymorphism of the klotho gene and coronary artery calcification, as well as the relationship between rs9527025 polymorphism and oxidative DNA damage. For this purpose, 90 individuals who had undergone coronary angiography participated in the study. The genotyping of Cys370Ser in exon 2 of the Klotho gene was performed using the polymerase chain reaction.

The oxidative DNA damage was assessed by the alkaline comet assay. The results indicated that the allelic distributions of Klotho gene polymorphism were not significantly related with the presence of coronary artery diseases (CAD). In the meantime, the total comet score (TCS) frequency was significantly associated with the rs9527025 polymorphism. TCS frequency tends to increase in the presence of the rs9527025 missense variant. Our findings showed that Klotho gene variants might influence oxidative DNA status in age-related diseases. In consequence, larger studies are required to confirm the association between Klotho deficiency and the progression of cardiovascular disease in order to elucidate risk factors for CAD and develop potential therapeutic strategies.

**KEYWORDS:** cardiovascular disease, coronary artery disease (CAD), genetic polymorphism, Klotho, oxidative DNA damage



## FRANGULA ALNUS EKSTRAKT I EMODIN KAO POTENCIJALNI ANTIKANCERSKI AGENSI

GENOTOXICOLOGY  
AND CARCINOGENICITY

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Hepatocelularni i kolorektalni karcinom se sve češće javlja i ima visoku stopu smrtnosti. Postojanje aktivnih mehanizama hemorezistencije otežava korisnost hemoterapije. Upravo je pojava hemorezistencije podstakla opsežna istraživanja za razvoj novih terapijskih agenasa i strategija za lečenje ovih vrsta karcinoma.

Prirodni proizvodi biljnog porekla (sekundarni metaboliti) su bogat izvor potencijalnih lekova. Zbog toga smo u istraživanju usmerili pažnju na etil-acetatni ekstrakt biljke *Frangula alnus* (FA) i njegovu dominantnu komponentu emodin (E) i ispitali njihovo antikancerski potencijal na ćelijama hepatocelularnog (HepG2) i kolorektalnog (HCT116) karcinoma i citotoksičnost na normalnim MRC-5 ćelijama. Pokazan je snažan antioksidativni potencijal ekstrakta (DPPH i TBA test). Citotoksičnost ispitana MTT testom pokazala je jaku toksičnost obe supstance na ćelije HepG2 i HCT116, ali bez uticaja na MRC-5 ćelije.

Dalje je analiza ćelijskog ciklusa pokazala da su i FA i E izazvali zaustavljanje u G1 fazi i blago nakupljanje ćelija u G2/M fazi. Pored toga, obe supstance su uvele ćelije u apoptozu i nekrozu i uticale su na mitohondrijalni membranski potencijal. Dalje, izazvali su značajan genotoksični efekat u kometnom testu na svim ćelijskim linijama. Može se zaključiti da su FA i E dobri kandidati za nove antikancerogene agense prirodnog porekla. Međutim, neophodna su dodatna istraživanja, posebno kada je u pitanju citotoksična aktivnost i bezbednost primene ovih supstanci.

**KLJUČNE REČI:** *Frangula alnus*, emodin, ćelijski ciklus, mitohondrijalni membranski potencijal, genotoksičnost



## FRANGULA ALNUS EXTRACT AND EMODIN AS POTENTIAL ANTICANCER AGENTS

GENOTOXICOLOGY  
AND CARCINOGENICITY

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Hepatocellular and colorectal carcinoma is swiftly increasing alongside a high mortality rate. The existence of active mechanisms of chemoresistance hampers the usefulness of chemotherapy. The emergence of chemoresistance encouraged extensive research to develop new therapeutic agents and strategies for the treatment of these types of cancer. Natural products of plant origin (secondary metabolites) have been the most successful source of potential drug leads. Therefore, we turned our attention to the *Frangula alnus* ethyl-acetate extract (FA) and its dominant constituent emodin (E) and explored them for their anticancer potential on hepatocellular (HepG2) and colorectal (HCT116) carcinoma cells, and cytotoxicity on normal MRC-5 cells.

Strong antioxidant potential of the extract was demonstrated (DPPH and TBA test). Cytotoxicity investigated by the MTT assay showed strong cell toxicity of both substances on HepG2 and HCT116 cells, but without affecting MRC-5 cells. Next, the analysis of cell cycles exhibited that both FA and E induced arrest in the G1 phase and slight accumulation of cells in the G2/M phase. In addition, both substances introduced cells into apoptosis and necrosis and modulated mitochondrial membrane potential. Further on, they caused significant genotoxic effect in comet assay applied in all cell lines. It can be concluded that FA and E are good candidates for new anticancer agents of natural origin. However, additional studies are necessary, especially when it comes to the cytotoxic activity and safety of the application of these substances.

**KEYWORDS:** *Frangula alnus*, emodin, cell cycle, mitochondrial membrane potential, genotoxicity



## EKSTRAKTI *GENTIANA LUTEA* I NJIHOV ANTIGENOTOKSIČNI I ANTIOKSIDATIVNI POTENCIJAL KOJI BI MOGLI BITI ISKORIŠĆENI U ZAŠTITI OD UV ZRAČENJA

GENOTOXICOLOGY  
AND CARCINOGENICITY

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Zbog snažnog genotoksičnog dejstva koje se pretežno ostvaruje kroz direktno formiranje pirimidinskih dimera i oksidativno oštećenje DNK, ultraljubičasto zračenje (UV) predstavlja značajni mutageni agens koji doprinosi razvoju karcinoma kože. Shodno tome, postoji rastuće interesovanje za istraživanje prirodnih antigenotoksičnih i antioksidativnih agenasa koji bi se mogli koristiti u UV zaštiti.

Ciljevi istraživanja su bili hemijska karakterizacija 50%-etanolnih ekstrakata korena i lista *Gentiana lutea*, kao i ispitivanje njihovog antigenotoksičnog (u odnosu na UV-indukovana oštećenja) i antioksidativnog potencijala. Hemijska analiza je vršena pomoću UPLC-PDA MS/MS, antioksidativni potencijal je detektovan u DPPH, TBA, FIC, FRAP i CUPRAC testovima, dok je antigenotoksičnost prema UV-C i UV-A-indukovanim DNK oštećenjima praćena primenom alkalnog komet testa na ćelijama humanog melanoma (Hs 294T) i normalnim fetalnim fibroblastima (MRC-5). Hemijska analiza je ukazala da su dominantni sastojci ekstrakta korena bili genciopikrozid i sverozid, a lista homoorientin i izoviteksin. Svi testovi antioksidativnosti osim TBA su ukazali na dobar efekat ekstrakta lista, dok je ekstrakt korena bio aktivan samo u najosetljivijem DPPH testu (IC50 vrednosti su bile 335,0±7,1 za ekstrakt korena, i 27,5±3,5 µg mL<sup>-1</sup> za ekstrakt lista).

Komet test je ukazao na snažni i dozno-zavisni potencijal ekstrakata da redukuju genotoksičnost indukovanu UV-C i UV-A zračenjem u obe ćelijske linije. Najveća inhibicija je detektovana u slučaju tretmana MRC-5 ćelija UV-C zračenjem (inhibicija DNK oštećenja 78% u slučaju ekstrakta korena, odnosno 66% za ekstrakt lista). Dobijeni rezultati su pokazali da se ekstrakti *G. lutea* mogu smatrati izvorom UV-protektivnih agenasa i antioksidanata.

**KLJUČNE REČI:** ekstrakti *Gentiana lutea*, hemijska karakterizacija, UV zračenje, antigenotoksičnost, antioksidativna aktivnost



## GENTIANA LUTEA EXTRACTS AND THEIR ANTIGENOTOXIC AND ANTIOXIDATIVE POTENTIAL THAT COULD BE UTILIZED IN UV PROTECTION

GENOTOXICOLOGY  
AND CARCINOGENICITY

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Due to strong genotoxic effect that is mainly realized through direct formation of pyrimidine dimers and oxidative DNA damage, ultraviolet radiation (UV) is an important mutagenic and skin-carcinogenic agent. Consequently, there is a growing interest to search for natural antigenotoxins and antioxidants which could be used as UV-protective agents. The aim was to chemically characterize and investigate antigenotoxic (against UV-induced DNA damage) and antioxidative properties of *Gentiana lutea* root and leaf 50%-ethanolic extracts. Chemical characterization was performed by UPLC-PDA MS/MS analysis, antioxidant effect was screened in DPPH, TBA, FIC, FRAP and CUPRAC assays, while antigenotoxicity against UV-C and UV-A was monitored in the alkaline comet assay performed on human melanoma cells (Hs 294T) and normal fetal fibroblasts (MRC-5). Chemical analysis showed gentiopicroside and swerozide, and homoorientin and isovitexin, as the dominant constituents of root and leaf extract, respectively.

All antioxidative tests except TBA demonstrated notable effect of leaf extract, while root extract was active only in the most sensible DPPH test (determined IC<sub>50</sub> values were 335.0±7.1 and 27.5±3.5 µg mL<sup>-1</sup>, for root and leaf extract, respectively). Comet test demonstrated high and dose-dependable potential of the extracts to reduce UV-C- and UV-A-induced genotoxicity in both cell lines. The highest inhibitions of DNA damage were determined for UV-C treatment in MRC-5 fibroblasts (78% and 66% for the root and leaf extract, respectively). Obtained results demonstrated that *G. lutea* extracts could be used as a source of potent UV-protective agents and antioxidants.

**KEYWORDS:** *Gentiana lutea* extracts, chemical characterization, UV irradiation, antigenotoxicity, antioxidative activity



# PROCENA ANTIOKSIDATIVNOG, CITOTOKSIČNOG I GENOTOKSIČNOG EFEKTA ETARSKOG ULJA CIMETA I NJEGOVE EMULZIJE

GENOTOXICOLOGY  
AND CARCINOGENICITY

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Cimet je jedna od najpoznatijih aromatičnih biljaka, koje je najčešće korišćena kao začim. Prepoznat je kao potencijalni terapeutik u narodnoj medicini, zbog čega se dugo koristi za lečenje različitih infekcija. Etarska ulja su sekundarni metaboliti koje sintetišu aromatične biljke. Za etarsko ulje cimeta (CEO) su definisane različite biološke aktivnosti, međutim fizičko-hemijske karakteristike ulja su slaba rastvorljivost u vodi i laka isparljivost, što ograničava njihovu primenu. Iz tog razloga, formulacija emulzija etarskih ulja predstavlja dobro rešenje za prevazilaženje problema njihove primene. Iako se CEO smatra bezbednim za upotrebu, rezultati istraživanja variraju, ukazujući na neophodnost daljih toksikoloških istraživanja.

Cilj ovog rada je bila komparativna analiza biološke aktivnosti i in vitro toksičnosti CEO i njegove emulzije (EM). Antioksidativni potencijal je ispitivan DPPH testom. MTT test je primenjen u cilju procene citotoksičnog efekta CEO i EM na fibroblastima pluća fetusa (MRC-5). Na istoj ćelijskoj liniji, ispitivan je nivo oštećenja molekula DNK, primenom alkalnog komet testa. Na osnovu rezultata DPPH testa, IC50 vrednost za CEO je bila 320 µg/mL, dok je za EM bila 54 µg/mL. Na ćelijskoj liniji MRC-5, IC50 vrednosti dobijene MTT testom su iznosile 0,13 mg/mL za CEO i 0,025 mg/mL za EM. CEO i EM nisu pokazali genotoksični potencijal. Uzimajući sve prethodno navedeno u obzir, CEO i EM, kao jaki antioksidanti koji pokazuju odsustvo genotoksičnosti i slabu citotoksičnost.

**KLJUČNE REČI:** etarsko ulje cimeta, emulzija, citotoksičnost, genotoksičnost, antioksidativna aktivnost



## EVALUATION OF ANTIOXIDATIVE, CYTOTOXIC AND GENOTOXIC EFFECT OF CINNAMON ESSENTIAL OIL AND ITS EMULSION

GENOTOXICOLOGY  
AND CARCINOGENICITY

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Cinnamon is among most popular aromatic plants, generally used as a spice. Over the centuries it was recognized as a potential therapeutic for different kind of infections. Essential oils are aromatic plants' secondary metabolites. Cinnamon essential oil (CEO) possess many favorable biological activities, but its physicochemical properties, as an oil, are poor solubility in water and high volatility, which limits its application. For that reason, preparation of essential oils' emulsions seems to be solution to overcome applicability problems. Even though CEO is one of the oils recognized as safe to be used, the results from different studies vary, indicating necessity to further study its toxicological properties. The aim of this study was to comparatively analyze CEO and its emulsion (EM) biological activity and in vitro toxicity. Antioxidative potential was investigated using DPPH assay.

The MTT assay was applied for the estimation of cytotoxic effects of CEO and EM on fetal fibroblasts (MRC-5) cell line. On the same cell line, level of DNA damage was evaluated, using alkaline comet assay. According to the DPPH assay results, IC<sub>50</sub> value for CEO was 320 µg/mL, for EM was 54 µg/mL. On MRC-5 cell line, IC<sub>50</sub> values were 0.13 mg/mL for CEO and 0.025 mg/mL for EM. Both CEO and EM didn't exhibit potential to induce DNA damage. Taking all above mention into account, as strong antioxidants without genotoxicity and with weak cytotoxicity, CEO and EM could be considered as good candidates for further investigation of biological activities and potential for human use.

**KEYWORDS:** cinnamon essential oil, emulsion, cytotoxicity, genotoxicity, antioxidative activity





## IN SILICO TOKSIKOLOGIJA / IN SILICO TOXICOLOGY

### PREDVIĐANJE TOKSIČNOSTI SULFORAFANA I SULFORAFAN-GLUKOZINOLATA PRIMENOM *IN SILICO* US-EPA COMPTOX CHEMICALS DASHBOARD METODE

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In silico metode u toksikologiji podrazumevaju upotrebu kompjuterskih metoda koje omogućavaju analizu i predviđanje toksičnosti supstanci, pri čemu mogu usmeriti dalja eksperimentalna istraživanja. Sulforafan (SFN) je organosumporna supstanca iz grupe izotiocijanata, prisutna u povrću krstašica.

Dok se SFN-glukozinolat ekstrahuje iz brokolija i uključen je u brojne kliničke studije, SFN je hemijski sintetisan i koristi se samo u istraživačke svrhe. SFN je imunomodulator sa visokim potencijalom stimulacije imunskog sistema i ostvarivanja antitumorskog dejstva. Međutim, potencijal njegovih toksičnih efekata, a time i bezbednost njegove upotrebe nije dovoljno ispitana. Stoga, cilj rada je bio da se primenom in silico analize, istraži toksični potencijal SFN i SFN-glukozinolata.

Korišćen je onlajn server CompTox Chemicals Dashboard (<https://comptox.epa.gov/dashboard/predictions>) Agencije za zaštitu životne sredine Sjedinjenih Američkih Država (engl. United States Environmental Protection Agency, US EPA). Korišćeni su sledeći nazivi supstanci: SFN (DTKSSD8036732) i SFN-glukozinolat (DTKSSD90894071).



## IN SILICO TOKSIKOLOGIJA / IN SILICO TOXICOLOGY

Predviđena oralna srednja letalna doza (LD50) iznosila je 177,865 mg/kg za SFN, dok je oralni LD50 za SFN-glukozinolat iznosio 2443,698mg/L. Vrednost LD50 za SFN je 13,74 puta manja od letalne doze za SFN-glukozinolat.

Mutagenost je naznačena kao potencijalno svojstvo SFN, ali ne i SFN-glukozinolata. Utvrđeno je da se nijedna od ovih supstanci ne vezuje za estrogene receptore, dok je potencijalna toksičnost po rast i razvoj istaknuta za SFN, ali ne i za SFN-glukozinolat. Imajući u vidu dobijene rezultate in silico predikcije toksičnih efekata hemijski sintetisanog SFN, potrebna su dalja ispitivanja kako bi se utvrdio toksični potencijal i procenila bezbednost njegove primene.

**KLJUČNE REČI:** sulforafan, sulforafan-glukozinolat, toksičnost, in silico analiza, imunomodulatori

Srbija-Kina projekat: 451-03-1203/2021-09.



## SULFORAPHANE AND SULFORAPHANE - GLUCOSINOLATE TOXICITY PREDICTION USING THE *IN SILICO* US-EPA COMPTOX CHEMICALS DASHBOARD METHOD

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In silico methods in toxicology use computer methods to analyze and predict the toxicity of substances, which can serve as a basis for further experimental studies. Sulforaphane (SFN) is an organosulfur chemical from the isothiocyanate group, present in cruciferous vegetables. While SFN-glucosinolate is extracted from broccoli and is presently ongoing numerous clinical studies, SFN has been chemically synthesized, currently only for research purposes. SFN is recognized as immunomodulator with high immunostimulatory potential, e.g. antitumor activity. However, the potential for its toxic effects and thus the safety of its use has not been sufficiently investigated.

Therefore, by applying in silico analysis, the aim of this work was to investigate the toxic potential of SFN and SFN glucosinolates. For this purpose, prediction analyzer in the CompTox Chemicals Dashboard (<https://comptox.epa.gov/dashboard/predictions>) of United States Environmental Protection Agency (US EPA) was used. Chemical names were inserted into the search tab: SFN (DTXSD8036732) and SFN-glucosinolate (DTXSD90894071). The predicted oral rat 50% lethal dose (LD50) for SFN was 177.865 mg/kg, while the oral LD50 for SFN glucosinolate was 2443.698 mg/L. The LD50 value for SFN is 13.74 times lower than the lethal dose for SFN glucosinolate. Mutagenicity was reported as a potential property of SFN but not of SFN-glucosinolate. Neither compound was found to bind to estrogen receptors, while potential developmental toxicity was highlighted for SFN, but not SFN-glucosinolate. Given the in silico predicted toxic effects of chemically synthesized SFN, further studies are needed to determine the toxic potential and evaluate the safety of use.

**KEYWORDS:** sulforaphane, sulforaphane-glucosinolate, toxicity, in silico analysis, immunomodulators

Serbia -China project: 451-03-1203/2021-09.



## KOMPARATIVNA TOKSIKOGENOMSKA BAZA PODATAKA: NOVE MOGUĆNOSTI

IN SILICO  
TOXICOLOGY

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Poslednje dve decenije intenzivno se razvija toksikogenomika sa ciljem da prikupi, objedini i analizira podatke o uticaju toksičnih supstanci na nivou gena i da ih integriše u baze podataka, poput Komparativne toksikogenomske baze podataka (engl. Comparative Toxicogenomic Database, CTD; <http://ctdbase.org/>). Ova javno dostupna baza podataka omogućava razumevanje uticaja toksičnih supstanci iz životne sredine na razvoj bolesti, na osnovu podataka o uticaju supstanci na gene, kao i podacima koji geni su odgovorni za razvoj bolesti, pri čemu se pomoću nje mogu utvrditi relacije toksična supstanca-gen-bolest. Cilj rada je bio da prikaže nove mogućnosti ove baze nastale kroz šestogodišnje unapređenje. U proteklom periodu proširen je njen sadržaj, a prema najnovijim informacijama, CTD sadrži više od 50 miliona toksikogenomskih podataka.

Ažuriran je rečnik bolesti i uvedeni su sinonimi za supstance koji će olakšati njihovo prepoznavanje. Uveden je novi modul, Phenotypes, koji omogućava postavljanje hipoteza o molekularnom mehanizmu nastanka štetnih ishoda, koji nisu bolesti, a mogu nastati pod uticajem toksičnih supstanci. Takođe, uspostavljanjem relacije supstanca-gen-bolest-fenotip, moguće je izneti pretpostavke o mehanizmima razvoja bolesti unutar fenotipa. Objasnjavanje ovih mehanizama je dodatno olakšano uvođenjem novog alata, CTD tetramera, koji povezuje informacije o toksičnim supstancama, genima, bolestima i fenotipovima.

Uvedena je nova stranica, Anatomy, koja je povezana sa modulom Exposure, što omogućava klasifikaciju fenotipova na osnovu anatomskih pojmova i sagledavanje njihovih profila u slučaju izloženosti toksičnim supstancama. Ova i buduća ažuriranja baze pružiće korisnicima najnovije relevantne informacije potrebne za razumevanje mehanizama toksičnosti supstanci iz životne sredine i usmeravanje daljih in vitro i in vivo istraživanja.

**KLJUČNE REČI:** CTD; unapređenje; supstanca; in silico; relacija supstanca-gen-bolest



## THE COMPARATIVE TOXICOGENOMIC DATABASE: NEW POSSIBILITIES

IN SILICO  
TOXICOLOGY

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In the last two decades, toxicogenomics has been intensively developed with the aim of collecting, standardizing, and analyzing data on toxic substances that affect genes and integrating them into databases, such as the Comparative Toxicogenomic Database (CTD; <http://ctdbase.org/>). This publicly available database enables the understanding of the influence of environmental substances on disease development, determining the relationships between toxic substances, genes and diseases. This paper aimed to present the new possibilities of this database.

According to the latest information, during six years, its content expanded to more than 50 million toxicogenomic data. The disease lexicon has been updated and synonyms for substances have been introduced to facilitate their recognition. A new module, Phenotypes, has been introduced which allows hypothesizing the molecular mechanism of the occurrence of adverse outcomes which are not diseases, but might occur under the influence of substances. Moreover, by linking phenotypes and diseases via substances and genes important for both conditions, it is possible to infer the mechanism of disease development from phenotypes. Elucidation of these mechanisms has been further facilitated by the introduction of a new tool, CTD tetramer which connects chemicals, genes, diseases and phenotypes. A new Anatomy page was introduced, and linked to the Exposure module.

Hence, phenotypes can now be classified from an anatomical perspective and their chemical exposure profiles can be explored. This and other updates will provide users with the latest relevant information needed to understand the toxicity mechanisms of environmental chemicals and guide further in vitro and in vivo research.

**KEYWORDS:** CTD, update, substance, in silico, substance-gene-disease relationship



## MOGUĆNOSTI I IZAZOVI PRIMENE VEŠTAČKE INTELIGENCIJE U TOKSIKOLOGIJI

IN SILICO  
TOXICOLOGY

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Veštačka inteligencija predstavlja upotrebu mašina i softvera za konstrukciju modela koji oponašaju kognitivne sposobnosti ljudi u rešavanju problema iz realnog sveta. S obzirom na njenu sve veću primenu u različitim oblastima biomedicinskih nauka, cilj ovog rada je da se predstave mogućnosti i izazovi primene modela zasnovanih na veštačkoj inteligenciji u toksikologiji. Dosadašnja toksikološka istraživanja su generisala ogroman broj podataka i stoga je potreban automatizovan i pouzdan alat za njihovu analizu, odnosno modeli koji mogu tačno i brzo donositi odluke.

Primena ovih modela u toksikologiji je omogućila: procenu toksičnosti nove supstance na osnovu sličnosti strukture sa supstancama poznate toksičnosti (modeli kvantitativnog odnosa strukture i dejstva (QSAR modeli)) ili na osnovu toksikokinetičkih parametara dobijenih pomoću matematičkih jednačina (modeli toksikokinetike bazirani na fiziologiji (PBTK modeli)); procenu kancerogenosti novih supstanci (DeepCarc – skrining alat); određivanje da li supstanca dovodi do toksičnog efekta, kao i jačine i vremena ispoljavanja tog efekta (npr. Bajesov mrežni pristup) i mehanizama toksičnosti (npr. Komparativna toksikogenomska baza podataka), kao i određivanje prioritarnih supstanci za procenu rizika (npr. TOX21). Zahvaljujući veštačkoj inteligenciji razvijen je veliki broj softvera, baza podataka i alata koji omogućavaju dobijanje podataka od toksikološkog značaja, što će u budućnosti smanjiti broj eksperimentalnih istraživanja, ubrzati proces donošenja odluka i smanjiti neophodne resurse, a time i ukupne troškove ispitivanja toksičnosti. Postoji potreba za unapređenjem ovih modela kako bi bili lakši za upotrebu od strane korisnika, kao i dalji razvoj pouzdanih načina za upravljanje velikom količinom podataka.

**KLJUČNE REČI:** toksikologija, veštačka inteligencija, predviđanje toksičnosti, QSAR modeli, PBTK modeli



## POSSIBILITIES AND CHALLENGES OF APPLYING ARTIFICIAL INTELLIGENCE IN TOXICOLOGY

IN SILICO  
TOXICOLOGY

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Artificial intelligence involves the use of machines and software to create models that mimic the cognitive abilities of humans in solving real-world problems. Given their increasing application in various fields of biomedical sciences, this work aimed to present the possibilities of applying artificial intelligence-based models in toxicology. So far, toxicological research has produced a myriad of data. Hence, there is a need for automated and reliable tools for its analysis, i.e. models that can make accurate and fast decisions.

The application of these models in toxicology allows the evaluation of toxicity of a new substance based on similarity of the structure to substances of known toxicity (QSAR models) or based on toxicokinetic parameters determined using mathematical equations (PBTk models); evaluation of the carcinogenicity of new substances (DeepCarc – screening tool); determination of whether a substance is involved in the development of a toxic effect, magnitude and timing of the manifestation of the said effect (e.g. Bayesian network approach) and toxicity mechanisms (e.g. Comparative Toxicogenomics Database); and determining priority substances for risk assessment (e.g. TOX21). Artificial intelligence has been used to develop numerous software and databases, i.e. tools to generate data of toxicological importance, which will reduce the number of experimental studies in the future, speed up the decision-making process and reduce required resources and, consequently, the overall cost of toxicity testing. These models need to be improved to become more user-friendly, while more reliable methods for managing large amounts of data need to be developed.

**KEYWORDS:** toxicology, artificial intelligence, prediction of toxicity, QSAR models, PBTk models



## ISTRAŽIVANJE MOGUĆIH MEHANIZAMA POVREDE OKA IZAZVANE PRIMENOM JONIZUJUĆEG ZRAČENJA U INTERVENTNIM PROCEDURAMA: TOKSIKOGENOMSKA ANALIZA PODATAKA

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Usled ogromnog porasta primene jonizujućeg zračenja u interventnoj radiologiji na globalnom nivou, kao i složenosti i trajanja svake pojedinačne procedure, osoblje koje učestvuje u interventnim procedurama može biti izloženo značajnim količinama doze zračenja. Epidemiološke studije ukazuju na izraženu osetljivost očnog sočiva na jonizujuće zračenje koje može rezultovati oštećenjima i pri relativno niskoj ekspoziciji u dužem vremenskom periodu. Cilj ovog istraživanja bio je da se, toksikogenomskom analizom podataka, istraže molekularni mehanizmi oštećenja oka usled izlaganja jonizujućem zračenju kod osoblja uključenog u interventne procedure. Komparativna toksikogenomska baza podataka (engl. Comparative Toxicogenomic Database, CTD <http://ctdbase.org>), GeneMANIA (<https://genemania.org>) i ToppGene Suite (<https://toppgene.cchmc.org>) korišćeni su za in silico analizu. Fenotipovi povezani sa odgovorom na jonizujuće zračenje pretraživani su unutar CTD baze podataka. Izdvojeno je ukupno 5 gena povezanih sa jonizujućim zračenjem: ATM, CRIAB, SIRT1, IAP1 i TGFB1.

Analiza pomoću GeneMANIA alata pokazala je da je većina interakcija između ovih gena bila fizička (77,64%), praćena koekspresijom (8,01%). Najvažnijih 5 bioloških procesa u koje su uključeni ovi geni bili su povezani sa beta receptorom za transformišući faktor rasta (aktivnost i vezivanje za receptor), kao i sa aktivnošću transmembranskog receptora proteina serin/treonin kinaze, dok je najvažnijih 5 bioloških procesa bilo povezano sa hippo signalnim putem, uključenim u regulaciju ćelijske signalizacije i apoptoze, kao i formiranjem anatomske strukture u morfogenezi, cirkulatornom sistemu i razvoju krvnih sudova oka. Dobijeni rezultati o regulaciji ćelijske signalizacije i apoptoze ukazuju na moguće ćelijske mehanizme povezane sa povredom oka prouzrokovanom jonizujućim zračenjem i pružaju osnovu za dalja laboratorijska ispitivanja.

**KLJUČNE REČI:** X-zračenje, radijaciona povreda, očno sočivo,  
in silico analiza, molekularni mehanizmi





## INVESTIGATING POSSIBLE MECHANISMS OF EYE INJURY CAUSED BY THE APPLICATION OF IONIZING RADIATION IN INTERVENTIONAL PROCEDURES: TOXICOGENOMIC DATA MINING

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Due to the huge increase in the use of ionizing radiation in interventional radiology globally, as well as complexity and duration of each individual procedure, personnel participating in interventional procedures can be exposed to significant amounts of radiation dose. Epidemiological studies indicate a pronounced sensitivity of eye lens to ionizing radiation, which can, over a long period of time, result in damage even in the case of relatively low exposure. Aim of the current research was, by using toxicogenomic data-mining, to explore the molecular mechanisms of eye damage due to exposure to ionizing radiation in personnel involved in interventional procedures. Comparative Toxicogenomic Database (<http://ctdbase.org>), GeneMANIA (<https://genemania.org>) and ToppGene Suite (<https://toppgene.cchmc.org>) were applied for in silico analysis. Phenotypes were searched throughout CTD base for response to ionizing radiation.

Total of 5 genes were extracted for ionizing radiation: ATM, CRYAB, SIRT1, YAP1 and TGFB1. GeneMANIA analysis revealed that the majority of the interactions between these genes were physical (77.64%), followed by co-expression (8.01%). Top 5 biological processes connected with these genes were linked to transforming growth factor beta receptor (activity and binding), as well as transmembrane receptor protein serine/threonine kinase activity, while top 5 biological processes were connected to hippo signaling pathway, involved in cell proliferation and apoptosis, anatomical structure formation involved in morphogenesis, circulatory system and eye blood vessel development. The obtained results on the regulation of cellular signaling and apoptosis indicate possible cellular mechanisms associated with eye injury caused by ionizing radiation and provide a basis for further laboratory testing.

**KEYWORDS:** X-ray, radiation injury, eye lens, in silico analysis, molecular mechanisms



# IN SILICO TOKSIKOGENOMSKA STUDIJA SLUČAJA: ULOGA SMEŠE OLOVA I POLIHLOOROVANIH BIFENILA U REMEĆENJU FUNKCIJE ŽENSKOG REPRODUKTIVNOG SISTEMA

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Zagađujuće supstance iz životne sredine koje ometaju rad endokrino sistema mogu imati nepovoljan uticaj na zdravlje, utičući na različite organske sisteme. Ranije su toksikološke eksperimentalne studije bile fokusirane na testiranje potencijalnih štetnih efekata pojedinačnih hemikalija na zdravlje različitih bioloških sistema.

Međutim, u realnim okolnostima, mi smo istovremeno izloženi mnogim hemikalijama sa različitim putem unosa. In silico toksikogenomski pristup prikupljanju podataka je osmišljen kako bi se razjasnilo kako olovo (Pb) i polihlorovani-bifenili (PCB) mogu doprineti patofiziologiji poremećaja ženskog reproduktivnog sistema (FRSD). Komparativna toksikogenomska baza podataka, GeneMANIA и TopGene Suite su korišćeni za prikupljanje toksikogenomskih podataka. Naši rezultati se zasnivaju na uticaju Pb i PCB na 18 gena sa sličnom regulacijom koji se preklapaju.

Ženski reproduktivni sistem (ŽRS) je veoma osetljiv na endokrino ometanje. Ispitivana ekspresija gena, izmenjena koktelom ispitivanih zagađujućih supstanci, pokazala je potencijalnu inhibiciju ekspresije aromataze i moguće niže nivoe estrogena.



Efekti koktela Pb i PCB-a na ŽRS dovode do uzlazne regulacije CAT gena, verovatno zbog povećane proizvodnje ROS. Poremećaj antioksidantnih sistema u ŽRS može dovesti do patologije u sazrevanju jajnih ćelija, ovulaciji, oplodnji, implantaciji i razvoju embriona, što na kraju može uticati na ishod trudnoće.

Povećanje ekspresije gena PTGS2 (COX-2) može dovesti do povišene inflamacije u ŽRS. Predloženi pristup toksikonomskeg prikupljanja podataka može biti dragocen za jasniju sliku u vezi sa molekularnim mehanizmima koji leže u osnovi efekata koktela Pb i PCB-a koji su uključeni u remećenju rada ŽRS. Ipak, ovaj pristup treba smatrati preliminarnim, koji prethodi in vitro i in vivo testovima.

**KLJUČNE REČI:** olovo (Pb), polihlorovani bifenili (PCB), ženski reproduktivni sistem, oksidativni stres, aromataza

**ZAHVALNICE:** Ovo istraživanje je finansiralo Ministarstvo prosvete, nauke i tehnološkog razvoja Republike Srbije kroz Ugovor o dodeli bespovratnih sredstava sa Univerziteta u Beogradu-Farmaceutskog fakulteta broj: 451-108-202.



## IN SILICO TOXICOGENOMIC CASE STUDY: ROLE OF LEAD AND POLYCHLORINATED BIPHENYLS MIXTURE IN THE FEMALE REPRODUCTIVE SYSTEM DISRUPTION

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Endocrine disrupting pollutants from the environment can have unfavorable impact on health, influencing various organ systems. Previously, toxicological experimental studies have focused on testing potential adverse effects of single chemicals on the health of diverse biological systems. However, in real circumstances, we are exposed to many chemicals through multiple routes of exposure, most often simultaneously. In silico toxicogenomic data-mining approach was designed to clarify how lead (Pb) and polychlorinated-biphenyls (PCBs) may contribute to pathophysiology of female reproductive system disruption (FRSD).

Toxicogenomic data mining was done using Comparative Toxicogenomic Database, GeneMANIA, and ToppGene Suite. Our results were based on the 18 overlapped genes with similar gene regulation by Pb and PCBs. Female reproductive system (FRS) is highly sensitive to endocrine disruption. The researched gene expression, altered by an investigated pollutant cocktail, exhibited potential inhibition of aromatase expression and possibly to lower levels of estrogens. The cocktail effects of Pb and PCBs on FRS lead to up-regulation of CAT gene, probably due to elevated ROS production. Antioxidant systems disruption in FRS may lead to pathological outcomes in oocyte maturation, ovulation, fertilization, implantation, and embryo development, which can ultimately influence pregnancy outcomes. Upregulation of PTGS2 (COX-2) gene expression may lead to elevated inflammation in FRS.



Suggested toxicogenomic data-mining approach may be valuable for a clearer picture regarding molecular mechanisms underlying the cocktail effects of Pb and PCBs involved in FRS disruption. Still, this approach should be considered as preliminary which refers to further in vitro and in vivo tests.

**KEYWORDS:** lead (Pb), polychlorinated-biphenyls (PCB), female reproductive system, oxidative stress, aromatase



## IN SILICO ANALIZA TOKSIKOGENOMSKIH PODATAKA U CILJU KONSTRUISANJA AFLATOKSIN B1 REGULATORNI E MREŽE HUB GENA KOD HEPATOCELULARNOG KARCINOMA

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Aflatoksin B1 (AFB1) može dovesti do nastanka hepatocelularnog karcinoma (HCC) na taj način što izaziva mutacije u molekulu DNK. Pored toga, ovaj toksin može dovesti i do globalnih promena u ekspresiji gena u ćelijama jetre, međutim, mreža molekularnih puteva uključenih u HCC nakon izlaganja AFB1 nije poznata. U ovom radu smo koristili toksikogenomske podatke dobijene iz ćelija humane jetre koje su bile izložene AFB1 i in silico alate u cilju identifikacije mreže molekularnih puteva koji mogu biti uključeni u HCC nastao izlaganjem AFB1. In silico alati STRING, MCODE, cytoHubba, iRegulon, alat za predviđanje kinaza KEA3 i DAVID su korišćeni za identifikaciju mreža protein-protein interakcija, hub gena, transkripcionih faktora (TF), uzvodnih kinaza i bioloških procesa (BP). Pretpostavljeni molekularni događaji su potvrđeni preko eksternog skupa podataka, dok su hub geni u HCC validirani korišćenjem UALCAN baze podataka.

Rezultati ove studije su pokazali povezanost između AFB1 i hub gena uključenih u regulaciju ćelijskog ciklusa. Identifikovani su TF koji regulišu hub gene, a zatim su TF povezani sa uzvodnim kinazama kao što su kinaze zavisne od ciklina, protein kinaza aktivirana mitogenim stimulusima 1 i AKT. Ovakav pristup je omogućio konstrukciju regulatorne mreže posredovane AFB1 koja se sastoji od uzvodnih kinaza, TF, hub gena i BP, otkrivajući hijerarhiju signalizacije i protok informacija koji mogu doprineti nastanku ili razvoju HCC nakon izlaganja AFB1. Pristup opisan u ovom radu bi mogao biti korisno sredstvo u predviđanju molekularnih mehanizama uključenih u nastanak i razvoj bolesti izazvanih hemikalijama ukoliko postoje dostupni toksikogenomski podaci.

**KLJUČNE REČI:** aflatoksin B1, in silico, toksikogenomika, hub geni, hepatocelularni karcinom, signalni putevi

**ZAHVALNICA:** Istraživanje je podržano od strane Pokrajinskog sekretarijata za visoko obrazovanje i naučnoistraživačku delatnost Autonomne pokrajine Vojvodine (Br. 2695).



## IN SILICO TOXICOGENOMIC APPROACH IN CONSTRUCTING THE AFLATOXIN B1-MEDIATED REGULATORY NETWORK OF HUB GENES IN HEPATOCELLULAR CARCINOMA

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Aflatoxin B1 (AFB1) can cause hepatocellular carcinoma (HCC) through a mutagenic mode of action but can also lead to global changes in gene expression; however, the AFB1 network of molecular pathways involved in HCC is not known. Here, we used toxicogenomic data from human liver cells exposed to AFB1 to infer the network of AFB1-responsive molecular pathways involved in HCC. The following computational tools: STRING, MCODE, cytoHubba, iRegulon, kinase enrichment tool KEA3, and DAVID were used to identify protein-protein interaction network, hub genes, transcription factors (TFs), upstream kinases, and biological processes (BPs).

Predicted molecular events were validated with an external dataset, whereas the hub genes in HCC were validated using the UALCAN database. The results revealed an association between AFB1 and the hub genes involved in cell cycle. We identified TFs that regulate the hub genes and linked them with upstream kinases including cyclin-dependent kinases, mitogen-activated protein kinase 1, and AKT. This approach enabled the construction of the AFB1-mediated regulatory network consisting of upstream kinases, TFs, hub genes, and BPs, thus revealing the signaling hierarchy and information flow that may contribute to AFB1-induced HCC. This could be a useful tool in predicting the molecular mechanisms involved in chemical-induced diseases when available toxicogenomic data exist.

**KEYWORDS:** aflatoxin B1, in silico, hub genes, signaling pathways, hepatocellular carcinoma

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Poremećaj antioksidantnih sistema u ŽRS može dovesti do patologije u sazrevanju jajnih ćelija, ovulaciji, oplodnji, implantaciji i razvoju embriona, što na kraju može uticati na ishod trudnoće. Povećanje ekspresije gena PTGS2 (COX-2) može dovesti do povišene inflamacije u ŽRS.

Predloženi pristup toksikonomskeg prikupljanja podataka može biti dragocen za jasniju sliku u vezi sa molekularnim mehanizmima koji leže u osnovi efekata koktela Pb i PCB-a koji su uključeni u remećenju rada ŽRS. Ipak, ovaj pristup treba smatrati preliminarnim, koji prethodi in vitro i in vivo testovima.

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Aflatoksin B1 (AFB1) može dovesti do nastanka hepatocelularnog karcinoma (HCC) na taj način što izaziva mutacije u molekulu DNK. Pored toga, ovaj toksin može dovesti i do globalnih promena u ekspresiji gena u ćelijama jetre, međutim, mreža molekulskih puteva uključenih u HCC nakon izlaganja AFB1 nije poznata. U ovom radu smo koristili toksikogenomske podatke dobijene iz ćelija humane jetre koje su bile izložene AFB1 i in silico alate u cilju identifikacije mreže molekulskih puteva koji mogu biti uključeni u HCC nastao izlaganjem AFB1. In silico alati STRING, MCODE, cytoHubba, iRegulon, alat za predviđanje kinaza KEA3 i DAVID su korišćeni za identifikaciju mreža protein-protein interakcija, hub gena, transkripcionih faktora (TF), uzvodnih kinaza i bioloških procesa (BP). Pretpostavljeni molekulski događaji su potvrđeni preko eksternog skupa podataka, dok su hub geni u HCC validirani korišćenjem UALCAN baze podataka.

Rezultati ove studije su pokazali povezanost između AFB1 i hub gena uključenih u regulaciju ćelijskog ciklusa. Identifikovani su TF koji regulišu hub gene, a zatim su TF povezani sa uzvodnim kinazama kao što su kinaze zavisne od ciklina, protein kinaza aktivirana mitogenim stimulusima 1 i AKT. Ovakav pristup je omogućio konstrukciju regulatorne mreže posredovane AFB1 koja se sastoji od uzvodnih kinaza, TF, hub gena i BP, otkrivajući hijerarhiju signalizacije i protok informacija koji mogu doprineti nastanku ili razvoju HCC nakon izlaganja AFB1. Pristup opisan u ovom radu bi mogao biti korisno sredstvo u predviđanju molekulskih mehanizama uključenih u nastanak i razvoj bolesti izazvanih hemikalijama ukoliko postoje dostupni toksikogenomski podaci.

**KLJUČNE REČI:** aflatoksin B1, in silico, toksikogenomika, hub geni, hepatocelularni karcinom, signalni putevi

**ZAHVALNICA:** Istraživanje je podržano od strane Pokrajinskog sekretarijata za visoko obrazovanje i naučnoistraživačku delatnost Autonomne pokrajine Vojvodine (Br. 2695).



## AN *IN SILICO* TOXICOGENOMIC APPROACH IN CONSTRUCTING THE AFLATOXIN B1-MEDIATED REGULATORY NETWORK OF HUB GENES IN HEPATOCELLULAR CARCINOMA

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Aflatoxin B1 (AFB1) can cause hepatocellular carcinoma (HCC) through a mutagenic mode of action but can also lead to global changes in gene expression; however, the AFB1 network of molecular pathways involved in HCC is not known. Here, we used toxicogenomic data from human liver cells exposed to AFB1 to infer the network of AFB1-responsive molecular pathways involved in HCC. The following computational tools: STRING, MCODE, cytoHubba, iRegulon, kinase enrichment tool KEA3, and DAVID were used to identify protein-protein interaction network, hub genes, transcription factors (TFs), upstream kinases, and biological processes (BPs).

Predicted molecular events were validated with an external dataset, whereas the hub genes in HCC were validated using the UALCAN database. The results revealed an association between AFB1 and the hub genes involved in cell cycle. We identified TFs that regulate the hub genes and linked them with upstream kinases including cyclin-dependent kinases, mitogen-activated protein kinase 1, and AKT. This approach enabled the construction of the AFB1-mediated regulatory network consisting of upstream kinases, TFs, hub genes, and BPs, thus revealing the signaling hierarchy and information flow that may contribute to AFB1-induced HCC. This could be a useful tool in predicting the molecular mechanisms involved in chemical-induced diseases when available toxicogenomic data exist.

**KEYWORDS:** aflatoxin B1, in silico, hub genes, signaling pathways, hepatocellular carcinoma

**ACKNOWLEDGEMENT:** This work was supported by the Provincial Secretariat for Higher Education and Scientific Research of the Autonomous Province of Vojvodina (No. 2695).



## IN SILICO ANALIZA EFEKATA SMEŠE OLOVA I POLIHLOROVANIH BIFENILA NA MUŠKI REPRODUKTIVNI SISTEM

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Iako je izuzetno teško predvideti nezavisne efekte hemikalija koje ometaju endokrini sistem, one potencijalno mogu dovesti do toksičnijih efekata prilikom ekspozicije njihovoj smeši. Ova in silico toksikogenomska metoda analize podataka imala je za cilj da rasvetli efekte smeše olova (Pb) i polihlorovanih bifenila (PCB) na muški reproduktivni sistem muškaraca. Toksikogenomsko istraživanje podataka je sprovedeno korišćenjem komparativne toksikogenomske baze podataka, GeneMANIA i ToppGene Suite. Naši rezultati su zasnovani na 18 zajedničkih gena sa sličnom regulacijom gena od strane Pb i PCB-a.

Koekspresija je bila dominantna interakcija među ovim genima (47,11%). Najznačajnije izmenjene molekularne funkcije, prema studiji genske ontologije, bile su aktivnost oksidoreduktaze, vezivanje faktora transkripcije i vezivanje signalnih receptora. Ispitivana smeša promenom ekspresije gena može se povezati sa inhibicijom androgenih receptora, poremećajem nivoa testosterona i inhibicijom aromataze. Promena nivoa inflamatornih citokina i aktivacija topoizomeraze 2A mogu potencijalno smanjiti pokretljivost spermatozoida i izazvati defekte u glavama spermatozoida, redom. Rezultati analize su pokazali da primenjena in silico metodologija može biti efikasan pristup za bolje razumevanje molekularnih mehanizama koji leže u osnovi efekata koktela Pb i PCB-a uključenih u poremećaje endokrinog sistema. Ipak, rezultate ovog pristupa treba smatrati preliminarnim za dalje in vitro i in vivo testove.

**KLJUČNE REČI:** koktel efekat, IL-6, COX2, SOD2, HMOX1, TNF-alpha



## IN SILICO ANALYSIS OF LEAD AND POLYCHLORINATED BIPHENYLS MIXTURE EFFECTS ON THE MALE REPRODUCTIVE SYSTEM

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Even though it is exceedingly challenging to predict endocrine-disrupting chemicals' independent effects, they can potentially result in more severe toxicity when exposed together. This in silico toxicogenomic data-mining method aimed to shed light of the mixture's effects on male reproductive system, and how lead (Pb) and polychlorinated-biphenyls (PCBs) may contribute to endocrine system diseases (ESDs). Toxicogenomic data mining was conducted using the Comparative Toxicogenomic Database, GeneMANIA, and ToppGene Suite. Our results were based on the 18 overlapped genes with similar gene regulation by Pb and PCBs.

Co-expression was the dominant interaction among these genes (47.11%). The most significant altered molecular functions, according to a gene ontology study, were oxidoreductase activity, transcription factor binding, and signaling receptor binding. Investigated mixture by altering the expression of genes can be linked to inhibition of androgen receptors, disruption of testosterone levels, and inhibition of aromatase. The alteration in the level of inflammatory cytokines and activation of topoisomerase 2A can potentially decrease sperm motility and cause defects in sperm cell heads, respectively. These findings indicated that the proposed toxicogenomic data-mining approach may be effective for a better understanding of the molecular mechanisms underlying the cocktail effects of Pb and PCBs involved in ESDs. Nevertheless, this approach should be regarded as preliminary data for further in vitro and in vivo tests.

**KEYWORDS:** cocktail effect, IL-6, COX2, SOD2, HMOX1, TNF-alpha



## IN SILICO PREDVIĐANJE UTJECAJA RAZLIČITIH FUNKCIONALNIH SKUPINA N-ALKIL KVARTERNIH KINUKLIDINA NA NJIHOVA FIZIKALNO-KEMIJSKA SVOJSTVA I TOKSIČNOST

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Vodeni interesom za razvojem novih lijekova koji djeluju na kolinesteraze a imaju bolju biološku aktivnost i bioraspoloživost, sintetizirali smo 14 kvarternih kinuklidinskih spojeva s varijacijom u duljini N-alkilnog lanca i ugradnjom alkoholne ili oksimske glavne skupine na poziciju 3 kinuklidinskog prstena. Sučelje SwissADME korišteno je za određivanje osnovnih fizikalno-kemijskih svojstava i procjenu interakcije ispitivanih spojeva s odabranim enzimima sustava citokroma P450, što bi moglo utjecati na njihov metabolizam. Većina testiranih kinuklidina pokazala je odgovarajuća svojstva koja su definirana Lipinski pravilom 5, što ukazuje na svojstva dobrog lijeka. Uočene su iznimke za biskvarterne 3-hidroksi i 3-hidroksiimino spojeve s C8 i C10 alkilnim lancima koji premašuju broj rotirajućih veza i topološke polarne površine u usporedbi s preporučenim vrijednostima.

Nadalje, procijenjeno je da svi spojevi osim 3-hidroksiimino spoja s C16 alkilnim lancem imaju sposobnost prolaska kroz krvno-moždanu barijeru i povoljna svojstva za gastrointestinalnu apsorpciju. Međutim, svi ispitani kinuklidini imaju ili negativnu logP vrijednost ili ispod 1,5, što ukazuje na nisku lipofilnost i stoga nizak potencijal za pasivni transport preko bioloških barijera. Prema računalnim predviđanjima kinuklidini s dugim bočnim alkilnim lancem (C12 i C14) inhibiraju CYP2D6 što može biti jedan od uzroka farmakokinetičkih interakcija između lijekova koje dovode do toksičnih ili drugih neželjenih nuspojava. Razumijevanje načina na koji kombinacija različitih funkcionalnosti u molekuli utječe na njezina fizikalno-kemijska svojstva predstavlja dobro polazište za daljnja biološka istraživanja aktivnosti i toksičnosti.

**KLJUČNE RIJEČI:** novi kemijski entiteti, kinuklidin, predviđanje, profili slični lijekovima

**ZAHVALNICA:** Istraživanje je financirano iz projekta HrZZ- UIP-2017-05-7260 (M. Katalinić).



## IN SILICO PREDICTION HOW DIFFERENT FUNCTIONALITIES IN N-ALKYL QUATERNARY QUINUCLIDINES AFFECT THEIR PHYSICOCHEMICAL PROPERTIES AND TOXICITY

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Guided by an interest to develop new cholinesterase-acting drugs with better biological activity and bioavailability, we have synthesized 14 quaternary quinuclidine compounds with the variation in N-alkyl chain length and incorporation of alcohol or oxime headgroup at the position 3 of the quinuclidine ring. SwissADME interface was used to determine basic physicochemical properties and to evaluate whether tested compounds interact with selected enzymes of the cytochrome P450 system, which could affect their metabolism. Most of the tested quinuclidines complied with the Lipinski's rule-of-five ensuring their drug-likeness properties. Exceptions were noticed for bisquaternary 3-hydroxy and 3-hydroxyimino compounds with C8 and C10 alkyl chains which exceed the number of rotating bonds and topological polar surface area compared to the recommended values. Furthermore, it was estimated that all compounds except 3-hydroxyimino compound with C16 alkyl chain have an ability to cross the blood brain barrier and favorable properties for gastrointestinal absorption.

However, all tested quinuclidines have either negative logP value or below 1.5, indicating low lipophilicity and therefore low potential to be passively transported across biological barriers. According to computer prediction quinuclidines with a long side alkyl chain (C12 and C14) inhibit CYP2D6 that may be one of the causes of pharmacokinetics-related drug-drug interactions leading to toxic or other unwanted adverse effects. Understanding how combination of different functionalities in a molecule affects its physicochemical properties represents a good starting point for further biological research of activity and toxicity.

**KEYWORDS:** new chemical entities, quinuclidine, prediction, drug-like profiles

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## IN SILICO TOXICOGENOMIC CASE STUDY: ROLE OF LEAD AND POLYCHLORINATED BIPHENYLS MIXTURE IN THE ADRENAL SYSTEM DISRUPTION

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Human health risk associated with lifelong exposure to chemical mixtures, including endocrine disruptors (EDs), is one of toxicology's toughest concerns. Lead (Pb) and polychlorinated biphenyls (PCBs) have both been linked to the disruption of the adrenal glands (AG). An in silico toxicogenomic data-mining approach was created to give insight on Pb and PCB pathophysiology in adrenal gland disruption. The Comparative Toxicogenomic Database, GeneMANIA, and ToppGene Suite were used for toxicogenomic data mining. Our results were based on the 18 overlapped genes with similar gene regulation by Pb and PCBs. Adrenal glands affected by Pb exposure exhibited the potential to down-regulate the CCL-2 gene, which may disrupt macrophage infiltration, leading to changed tissue homeostasis, altered lipid metabolism, and reduced local aldosterone synthesis during stress.

The effect of Pb and PCBs on AG leads to up-regulation of the CAT gene and down-regulation of the SOD2 gene, possibly leading to interruptions in redox homeostasis within the AG and influencing steroidogenesis. In addition, the researched gene expression, altered by an investigated pollutant cocktail, exhibited potential inhibition of aromatase expression, which adds up to steroidogenesis disruption. Moreover, upregulation of TNF- $\alpha$  and IL-6, as important HPA-axis activators, could lead to increase in mineralocorticoids, glucocorticoids and androgens production. These findings revealed that the proposed toxicogenomic data mining method would be beneficial for gaining a deeper understanding of the molecular mechanisms underlying the combined effects of Pb and PCBs linked to diseases of the adrenal glands. This approach should be considered as preliminary information for further in vitro and in vivo studies.

**KEYWORDS:** Pb, PCB, adrenal glands, IL-6, CCL-2, TNF- $\alpha$



## IN VITRO TOKSIKOLOGIJA / IN VITRO TOXICOLOGY

# SULFORAFAN UTIČE NA MORFOLOŠKE PROMENE I VIJABILNOST ĆELIJA KOLOREKTALNOG KARCINOMA: EFEKAT ZAVISAN OD MUTACIJE P53

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Kolorektalni karcinom (CRC) je sve češći uzrok morbiditeta i mortaliteta širom sveta. Među biološki aktivnim jedinjenjima za koja se pokazalo da ispoljavaju citotoksične efekte na ćelije raka kod ljudi, uključujući CRC, je sulforafan (SFN), izotiocijanatno jedinjenje ekstrahovano iz povrća, posebno brokolija. Ova in vitro studija imala je za cilj da istraži efekat SFN-a na rast ćelija CRC-a kod ljudi i njegovu zavisnost od ekspresije p53. Dve ćelijske linije humanog CRC-a: HT-29 (p53 mutirana linija) i HT-116 (linija „divljeg“ tipa p53) tretirane su SFN-om u koncentracijama od 0, 4, 8, 16 do 32  $\mu\text{mol/L}$  tokom 72 sata kako bismo testirali njegov antikancerogeni efekat. Rezultati su pokazali da SFN izaziva morfološke promene ćelija i smanjuje ćelijsku vijabilnost. Tretiranje CRC ćelija SFN tokom 72 h je rezultiralo umerenom dozno-zavisnom citotoksičnošću, pri čemu su HCT-116 ćelije bile osetljivije ( $\text{IC}_{50} = 8,41 \mu\text{M}$ ) od HT-29 ćelija ( $\text{IC}_{50} = 24,83 \mu\text{M}$ ). Ovi rezultati ukazuju da SFN ostvaruje antikancerogeni efekat na CRC ćelijama koji je zavistan od p53 mutacije (projekat Srbija-Kina: 451-03-1203/2021-09).

**KLJUČNE REČI:** kolorektalni karcinom, sulforafan, vijabilnost ćelija, mutacija p53



## SULFORAPHANE AFFECTS MORPHOLOGICAL CHANGES AND CELL VIABILITY OF COLON CANCER CELLS: P53 MUTATION-DEPENDENT EFFECT

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Colorectal carcinoma (CRC) is an increasing cause of morbidity and mortality worldwide. Among biologically active compounds which have been shown to exert cytotoxic effects on human cancer cells, including CRC, is sulforaphane (SFN), an isothiocyanate compound extracted from cruciferous vegetables, especially broccoli. This in vitro study aimed to investigate the effect of SFN on the growth of human CRC cells and its dependency on the expression of p53. Two human CRC cell lines: HT-29 (a p53 mutated line) and HT-116 (a p53 wild-type line) were treated with SFN at concentrations of 0, 4, 8, 16, and 32  $\mu\text{mol/L}$  for 72 h to test its anticancer effect.

Results indicated that SFN induced cell morphological changes and decreased the total number of viable cells. Treatment of CRC cells with SFN for 72 h resulted in moderate dose-dependent cytotoxicity. HCT-116 cells, with a p53-wt, were more sensitive ( $\text{IC}_{50} = 8.41 \mu\text{M}$ ) than p53-mutated HT-29 cells ( $\text{IC}_{50} = 24.83 \mu\text{M}$ ). These results indicate that SFN exhibits the anticancer effect against CRC cells in p53 mutation-dependent manner (Serbia-China project: 451-03-1203/2021-09).

**KEY WORDS:** colorectal carcinoma, sulforaphane, cell viability, p53 mutation



## POTENCIJALNI PROTEKTIVNI UČINAK EKSTRAKTA PLODA JAGODE (*FRAGARIA X ANANASSA* DUCH.) NA DNA OŠTEĆENJA IZAZVANIH OKSIDACIJSKIM STRESOM

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Plod jagode je izrazito bogat bioaktivnim tvarima kao što su antocijani, fenolne kiseline i flavonoidi zbog čega njegova konzumacija ima pozitivan učinak na zdravlje. Prema kemijskom sastavu i biološkom potencijalu ovo voće se može smatrati funkcionalnom hranom. U ovom radu, pripremljen je vodeni ekstrakt liofiliziranog svježeg ploda jagode ultrazvučno induciranom ekstrakcijom u kojem je spektrofotometrijski određen polifenolni sastav. Citotoksični i antioksidativni učinak ekstrakta na ljudske stanične linije (CAL 27, AGS, Caco-2 i HepG2) određen je korištenjem Neutral Red i DCFH-DA metoda. Protektivni učinak na DNA ispitan je na modelu plazmida  $\Phi$ X174 RF1 DNA pomoću elektroforeze u agaroznom gelu. Prema dobivenim rezultatima, ekstrakt jagode sadrži najviše procijanidina ( $64,35 \pm 0,05$  mg/100 g), a najmanje flavonola ( $3,98 \pm 0,01$  mg/100 g).

Također, pokazalo se da je preporučena dnevna doza polifenola, preračunata na 5 L krvi i prosječnu težinu čovjeka, imala antiproliferativni učinak samo na staničnu liniju kolorektalnog adenokarcinoma (Caco-2), dok na ostale ispitivane stanične linije nije imala nikakav učinak te nema učinka na indukciju slobodnih radikala. Biološki aktivni spojevi u ekstraktu imaju dozno ovisan zaštitni učinak na DNA i sprječavaju stvaranje hidroksilnih radikala koji bi doveli do relaksacije superzavijene DNA. Citotoksični učinak jagode može se povezati s antiproliferativnim svojstvima hidroksicimetnih kiselina, koje su jedna od najzastupljenijih komponenti ekstrakta. Rezultati ovog rada potvrđuju potencijalnu ulogu polifenola jagode u borbi protiv oksidativnog stresa i predstavljaju dobru osnovu za daljnja istraživanja.

**KLJUČNE RIJEČI:** ekstrakt ploda jagode, DNA protektivni učinak, oksidativni stres, antiproliferativni učinak

**ZAHVALNICA:** Ovaj rad financiran je od strane Hrvatske zaklade za znanost (HRZZ-IP-2019-04-2105, "Tehnologija preprekama i 3D printanje za okolišno prihvatljivu proizvodnju funkcionalnih voćnih sokova"; voditeljica projekta: izv. prof. dr. sc. Danijela Bursać Kovačević).



## POTENTIAL DNA PROTECTIVE EFFECT OF AN EXTRACT FROM STRAWBERRY FRUIT (*FRAGARIA X ANANASSA* DUCH.) AGAINST OXIDATIVE STRESS DAMAGE

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Strawberry fruit is rich in various bioactive compounds such as anthocyanins, phenolic acids and flavonoids, and has many health-promoting properties. Due to its chemical composition and biological potential, this fruit can be considered as a functional food. In this study, the aqueous extract was prepared by ultrasonic extraction from a freeze-dried fresh strawberry fruit. The content of the polyphenolic compounds was measured spectrophotometrically. The cytotoxic and antioxidant effects of the extract on human cell lines (CAL 27, AGS, Caco-2, and HepG2) were performed using the Neutral Red and DCFH-DA methods, respectively. The DNA protective effect was tested on a plasmid model  $\Phi$ X174 RF1 DNA using agarose gel electrophoresis.

According to the results, strawberry extract contains the most procyanidins ( $64.35 \pm 0.05$  mg/100 g) and the least flavonols ( $3.98 \pm 0.01$  mg/100 g). It was shown that the recommended daily dose of polyphenols, calculated on 5 L of blood and the average weight of a human, had an antiproliferative effect only on the colorectal adenocarcinoma cell line (Caco-2), while it had no effect on the other cell lines tested, and no effect on the induction of free radicals. Moreover, the biologically active compounds in the extract have a dose-dependent protective effect on DNA and prevent the formation of hydroxyl radicals that would lead to the relaxing of supercoiled DNA. The cytotoxic effect of strawberry may be related to the antiproliferative properties of hydroxycinnamic acids, which are one of the most abundant components of the extract. The results of this work confirm the potential role of strawberry polyphenols against oxidative stress and represent a good basis for further research.

**KEYWORDS:** strawberry fruit extract, DNA protective effect, oxidative stress, antiproliferative effect

**ACKNOWLEDGEMENT:** This work was supported by the Croatian Science Foundation (project identifier: HRZZ-IP-2019-04-2105 "Hurdle technology and 3D printing for sustainable fruit juice processing and preservation"; project manager: PhD Danijela Bursać Kovačević, Associate Professor).



## LOGANSKA KISELINA INDUKUJE APOPTOZU U MONONUKLEARNIM ĆELIJAMA PERIFERNE KRVI ČOVEKA

IN VITRO  
TOXICOLOGY

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Loganska kiselina (La) je sekundarni metabolit biljnog reda Gentianales. Uticaj ovog iridoidnog jedinjenja je označen kao osteoprotektivan, anti-inflamatoran i antioksidativan, detektovan u eksperimentalnim okruženjima gde su ćelije bile paralelno izložene toksičnim agensima. Budući da je u humanim primarnim mononuklearnim ćelijama periferne krvi (PBMC), istovremeno izloženim agensima koji oštećuju DNK i La, La imao citoprotektivni efekat, naš cilj je bio da analiziramo uticaj koncentracije samog tretmana La na vijabilnost, kao i tip izazvane ćelijske smrti odabranom citotoksičnom koncentracijom. Vijabilnost ćelija posle 48 h tretmana sa 20, 50, 100 i 130  $\mu\text{M}$  La procenjena je testom isključenja tripan plave boje. La je pokazao citotoksični potencijal u PBMC kulturama, što je dovelo do smanjenja broja ćelija nakon 48 h tretmana sa koncentracijama od 50  $\mu\text{M}$  i više.

Nakon tretmana sa 50  $\mu\text{M}$  La, izvršeni su kolorimetrijski test fragmentacije DNK i imunoblot analiza markera proteina apoptoze, kaspaze-3 i PARP1 za procenu tipa ćelijske smrti. Nivo fragmentacije DNK u PBMC kulturama nakon tretmana sa 50  $\mu\text{M}$  La bio je 3 puta veći u poređenju sa kontrolnim uzorcima. Smrt ćelija usled aktivacije apoptoze potvrđena je imunoblot analizom, koja je pokazala povišenje nivoa fragmenata kaspaze-3 i PARP1 89 kDa. Naši nalazi su pokazali da tretman La može smanjiti vijabilnost ćelija usled aktivacije apoptotske ćelijske smrti. Potencijalna zaštitna svojstva La bi mogla opravdati La tretman, ali se mora izvoditi sa oprezom.

**KLJUČNE REČI:** loganska kiselina, vijabilnost, fragmentacija DNK, apoptoza

**PRIZNANJE:** Ovaj rad je finansijski podržalo Ministarstvo prosvete, nauke i tehnološkog razvoja Republike Srbije, grant broj 451-03-68/2022-14/200017



## LOGANIC ACID INDUCES APOPTOSIS IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Loganic acid (La) is a secondary metabolite of the plant order Gentianales. These iridoid compound properties are reported as osteoprotective, anti-inflammatory and antioxidative, detected in experimental settings where cells were exposed to toxic agents in parallel. Since in primary human peripheral blood mononuclear cells (PBMCs) exposed to DNA damaging agents and La, La had a cytoprotective effect, our goal was to analyze the concentration impact of La treatment alone, on viability and the type of cell death induced by selected cytotoxic concentration. Cell viability after 48 h of treatment with 20, 50, 100 and 130  $\mu\text{M}$  La was assessed by the trypan blue dye exclusion test. La displayed cytotoxic potential in PBMC cultures, which led to a decrease in cell number upon 48 h treatment with concentrations of 50  $\mu\text{M}$  and higher. Following 50  $\mu\text{M}$  La treatment, a colorimetric DNA fragmentation assay and immunoblot analysis of the apoptosis protein markers, caspase-3 and PARP1, were performed for the assessment of cell death type. The level of DNA fragmentation in PBMC cultures upon 50  $\mu\text{M}$  La treatment was 3 times higher compared to control samples. The cell death due to apoptosis activation was confirmed by immunoblot analysis, which revealed the elevation of cleaved caspase-3 and PARP1 89 kDa fragments. Our findings showed that La treatment may decrease cell viability due to the activation of apoptotic cell death. Potential protective properties of La could justify La's treatment but it must be performed with caution.

**KEYWORDS:** Loganic acid, viability, DNA fragmentation, apoptosis

**ACKNOWLEDGMENT:** This work was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-68/2022-14/200017



## PRELIMINARNO ISPITIVANJE TOKSIČNOSTI GRAFENSKIH KVANTNIH TAČKICA (GKT) *IN VITRO*

IN VITRO  
TOXICOLOGY

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Grafenske kvantne tačkice (GKT) su najmlađe superzvezde porodice ugljika. Njihova fizičko-hemijska svojstva čine ih atraktivnim nanomaterijalom za biomedicinske primene. Jedan od ključnih izazova u njihovoj primeni je toksičnost, stoga je cilj istraživanja procena in vitro biološkog uticaja tri GKT materijala pripremljena sonikacijom u tri različita medija: čista voda (GKT – H<sub>2</sub>O), 1 M vodeni rastvor Na<sub>2</sub>SO<sub>4</sub> (GKT – Na<sub>2</sub>SO<sub>4</sub>) i 1,8 M vodeni rastvor H<sub>2</sub>SO<sub>4</sub> (GKT – H<sub>2</sub>SO<sub>4</sub>). Sve suspenzije su dijalizovane nakon ultrazvučnog tretmana, da bi se uklonili rastvoreni neorganski joni, centrifugirane, i na kraju supernatanti su sakupljeni za ispitivanje toksičnosti i karakterizaciju. Humane embrionalne ćelije bubrega (HEK 293T) korišćene su za procenu citotoksičnosti kolorimetrijskim (MTT) testom nakon 24 h izlaganja, dok je procena genotoksičnosti alkalnim komet testom izvršena nakon 3 h izlaganja. GKT su dodate u ćelijske kulture u tri različite koncentracije.

Rezultati su testirani analizom varijanse sa nivoom značajnosti postavljenim na  $p < 0,05$ . Za sva tri materijala, MTT testom uočena je najniža citotoksičnost u najnižoj testiranoj koncentraciji. Značajno niža citotoksičnost uočena je za GKT – H<sub>2</sub>O, u najnižoj koncentraciji u poređenju sa druga dva tretmana. U komet testu, najveća koncentracija GKT – Na<sub>2</sub>SO<sub>4</sub> i GKT – H<sub>2</sub>SO<sub>4</sub> rezultirala je povećanjem oštećenja DNK u poređenju sa negativnom kontrolom (netretirane ćelije). Međutim, za materijal GKT – H<sub>2</sub>O, oštećenje DNK nije se značajno razlikovalo između tretmana. Rezultati trenutne studije mogu biti od pomoći za buduća poboljšanja, proizvodnju i primenu GKT-a. Međutim, potrebno je više studija da bi se dobili podaci za visokokvalitetne GKT za biomedicinske aplikacije.

**KLJUČNE REČI:** grafenske kvantne tačkice, MTT esej, komet test, DNK oštećenje, in vitro procena toksičnosti

**ZAHVALNICA:** Istraživanje podržano od strane Ministarstva za nauku, visoko obrazovanje i mlade Kantona Sarajevo (grant broj: 27-02-11-41250-2/21).





## PRELIMINARY INVESTIGATION OF GRAPHENE QUANTUM DOTS (GQDS) TOXICITY *IN VITRO*

IN VITRO  
TOXICOLOGY

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Graphene quantum dots (GQDs) are the youngest superstars of the nanocarbon family for biomedical applications. Toxicity is one of the key challenges in their applications, therefore, we aimed to evaluate in vitro biological influence of three GQDs materials prepared by sonication in three different media: pure water (GQDs – H<sub>2</sub>O), 1 M aqueous Na<sub>2</sub>SO<sub>4</sub> (GQDs – Na<sub>2</sub>SO<sub>4</sub>), and 1.8 M aqueous H<sub>2</sub>SO<sub>4</sub> (GQDs – H<sub>2</sub>SO<sub>4</sub>). All suspensions were dialyzed after ultrasonic treatment in order to remove dissolved inorganic ions and centrifuged to obtain supernatants for toxicity testing and characterization.

Human embryonic kidney cells (HEK 293T) were used for cytotoxicity evaluation by colorimetric (MTT) assay after 24 h of exposure, while genotoxicity assessment by alkaline comet assay was performed after 3h of exposure. GQDs were added into cell cultures in three different concentrations.

Results were tested by analysis of variance with significance level set at  $p < 0.05$ . For all three materials, MTT assay revealed the lowest cytotoxicity in the lowest concentration tested. A significantly lower cytotoxicity of GQDs – H<sub>2</sub>O, in the lowest concentration, was revealed compared to the other two treatments. In comet assay, the highest concentration of GQDs – Na<sub>2</sub>SO<sub>4</sub> and GQDs – H<sub>2</sub>SO<sub>4</sub> resulted in the increase in DNA damage when compared with negative control (untreated cells). However, for the GQDs – H<sub>2</sub>O material, DNA damage did not significantly differ between treatments. The results of the current study can be helpful for future improvements, production and applications of GQDs. However, more studies are needed to gain data for high-quality GQDs for biomedical applications.

**KEYWORDS:** graphene quantum dots, MTT assay, comet assay, DNA damage, in vitro toxicity assessment

**ACKNOWLEDGEMENT:** This work has been supported by the Ministry of Science, Higher Education, and Youth of Sarajevo Canton (grant No: 27-02-11-41250-2/21).



# PROCENA TOKSIČNOSTI MEZOPOROZNIH NANOČESTICA SILICIJUM DIOKSIDA PRI RAZLIČITIM POSTUPCIMA EKSTRAKCIJE

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Mezoporozne nanočestice silicijum dioksida (MSNP) su opsežno proučavane poslednjih godina kao agens sa potencijalnom primenom u biomedicini. Njihov veliki odnos površine prema zapremini, velika poroznost i stabilnost čine ih pogodnim terapijskim nosačima. S obzirom na njihovu potencijalnu primenu, procena toksičnosti je od velikog značaja. Citotoksičnost i indukcija oksidativnog stresa u zavisnosti od oblika, koncentracije i sastava medijuma ćelijske kulture su objavljeni u literaturi za ispitivane MSNP. Cilj ovog rada je bio testiranje efekata novosintetisanog SBA-15 MSNP sa visokom specifičnom površinom (641 m<sup>2</sup> g<sup>-1</sup>) i porama između 1,9 i 25 nm na vijabilnost HEK-293 ćelija (XTT esej) i prooksidativni/antioksidativni balans (PAB esej). Da bi se procenili efekti sastava medijuma, DMEM sa 10% serumom (DMEM-ser) i medijum bez seruma (DMEM-free) korišćeni su za ekstrakciju SBA-15 (20 mg/mL) tokom 24 sata na 37°C. Ćelije su tretirane dobijenim ekstraktima u finalnim koncentracijama od 100, 250 i 500 µg/mL tokom 72 sata.

Rezultati su pokazali da i DMEM-ser i DMEM-free ekstrakt značajno smanjuju vijabilnost ćelija, u zavisnosti od koncentracije i izraženije nakon DMEM-ser tretmana ( $p < 0,001$ ). S druge strane, nivoi PAB-a su se smanjili nakon najveće koncentracije DMEM-free tretmana ( $p < 0,05$ ), dok je tretman najvišom koncentracijom DMEM-ser povećao PAB vrednosti ( $p < 0,01$ ), što ukazuje na moguću interakciju SBA-15 sa komponentama seruma. Ovi rezultati sugerišu da novosintetisani SBA-15 deluje citotoksično u zavisnosti od koncentracije, dok proces ekstrakcije utiče na nivo toksičnosti. Potrebna je dalja optimizacija sinteze da bi se dobile bezbedne čestice za ljudsku upotrebu.

**KLJUČNE REČI:** mezoporozne nanočestice silicijum dioksida, vijabilnost ćelija, prooksidativni/antioksidativni balans, SBA-15

**PRIZNANJE:** Ovaj rad je finansijski podržalo Ministarstvo prosvete, nauke i tehnološkog razvoja Republike Srbije, grant broj 451-03-68/2022-14/200017



# TOXICITY ASSESSMENT OF MESOPOROUS SILICA NANOPARTICLES UNDER DIFFERENT EXTRACTION PROCEDURES

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Mesoporous silica nanoparticles (MSNP) have been extensively studied in recent years as a promising agent in biomedicine. Their high surface area-to-volume ratio, great porosity, and stability make them robust platforms as therapeutic carriers. Considering their potential applications, toxicity assessment is of great importance. Cytotoxicity and oxidative stress induction dependent on the shape, concentration, and cell culture medium composition have been reported for investigated MSNPs. Our aim was to test the effects of newly synthesized SBA-15 MSNP with a high specific surface area (641 m<sup>2</sup> g<sup>-1</sup>) and pores between 1.9 and 25 nm on the viability of HEK-293 cells (XTT assay) and pro-oxidant/anti-oxidant balance (PAB assay).

To assess the effects of media composition, DMEM supplemented with 10% serum (DMEM-ser) and serum-free media (DMEM-free) were used for SBA-15 extraction (20 mg/mL) for 24 hours at 37°C. Cells were treated with obtained extracts at final concentrations of 100, 250 i 500 µg/mL for 72 hours. The results showed that both DMEM-ser and DMEM-free extracts significantly reduced cell viability, concentration dependently and more pronounced after DMEM-ser treatment (p<0.001). On the other hand, PAB levels decreased after DMEM-free treatment at the highest concentration (p<0.05), while DMEM-ser treatment increased PAB levels at the highest concentration (p<0.01), indicating possible SBA-15 interaction with serum components. These results suggest that newly synthesized SBA-15 display cytotoxic effect in a concentration-dependent manner, whereas extraction process influences level of toxicity. Further synthesis optimization is required to obtain safe particles for human use.

**KEY WORDS:** Mesoporous silica nanoparticles, cell viability, pro-oxidant/anti-oxidant balance, SBA-15

**ACKNOWLEDGMENT:** This work was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-68/2022-14/200017



## WELLS-DAWSON POLIOKSOVOLFRAMATI SUPSTITUISANI HAFNIJUMOM KAO POTENCIJALNA KONTRASTNA SREDSTVA: UTICAJ NA DNK *IN VITRO*

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**UVOD:** Istraživanja pokazuju da su metalne nanočestice pogodni kandidati za kontrastna sredstva (CESA-contrast-enhancing staining agents) nove generacije za kompjuterizovanu tomografiju (CT) zahvaljujući velikoj gustini i sposobnosti da apsorbuju X-zrake.

**CILJ:** Studije toksičnosti in vivo ukazuju na štetno dejstvo polioksometalata, što bi mogla biti glavna prepreka u kliničkoj primeni ovih bioaktivnih kompleksnih jedinjenja. Stoga je cilj ovog istraživanja da se ispituju genotoksične osobine polioksovolframata Wells-Dawson tipa supstituisanih sa hafnijumom (Hf-WD 1:2), K16[Hf(α2-P2W17O61)2]·19H2O, koji je pokazao dobre osobine kao kontrastno sredstvo za vizuelizaciju tkiva bubrega i dugih kostiju miša.

**METODOLOGIJA:** Hf-WD 1:2 je sintetisan prema metodi opisanoj u literaturi. Uzorci humane pune krvi su uzeti od zdravih donora i izloženi različitim koncentracijama (10<sup>-6</sup>-10<sup>-4</sup> mol/L) Hf-WD 1:2 tokom 4 i 24 sata, na 37 °C. Standardna metoda za alkalni komet test je korišćena za praćenje genotoksičnih efekata Hf-WD 1:2.

**REZULTATI:** Stepen oštećenja DNK humane periferne krvi nakon izlaganja Hf-WD 1:2 je izražen kao relativno povećanje repne DNK u odnosu na kontrolu. Nije uočena statistički značajna razlika u količini prekida DNK lanca u poređenju sa odgovarajućom kontrolom, za oba vremena izlaganja (4 i 24 sata) i za sve ispitivane koncentracije polioksovolframata.

**ZAKLJUČAK:** In vitro ispitivanje genotoksičnosti pokazalo je da Hf-WD 1:2 ne menja značajno strukturu DNK zdravih humanih ćelija pune krvi i stoga se može smatrati bezbednim u smislu genotoksičnosti u dodatnim studijama kao potencijalnog kontrastnog sredstva za CT.

**KLJUČNE REČI:** hafnijum(IV)-supstituisani Wells-Dawson polioksovolframati, kontrastno sredstvo, alkalni komet esej, genotoksičnost in vitro



## HAFNIUM(IV)-SUBSTITUTED WELLS-DAWSON BASED CONTRAST-ENHANCING STAINING AGENT: EFFECT ON DNA *IN VITRO*

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**INTRODUCTION:** Metallic nanoparticles have been reported as promising candidates for the development of new-generation contrast-enhancing staining agents (CESAs) for computed tomography (CT) due to their high X-ray attenuation and density.

**AIM:** In vivo toxicity studies revealed side effects of polyoxometalate clusters, which could be a key obstacle in the potential clinical application of these bioactive compounds. Therefore, the aim of this investigation was to perform the genotoxicity evaluation of 1:2 hafnium(IV)-substituted Wells-Dawson polyoxotungstate (Hf-WD 1:2), K<sub>16</sub>[Hf(α<sub>2</sub>-P<sub>2</sub>W<sub>17</sub>O<sub>61</sub>)<sub>2</sub>]-19H<sub>2</sub>O that was reported as a promising CESA candidate to visualize murine long bones and kidneys.

**METHODOLOGY:** Hf-WD 1:2 was prepared using the method described in the literature. Human whole blood samples were taken from healthy donors and exposed to different Hf-WD 1:2 concentrations (within the range of 10<sup>-6</sup>-10<sup>-4</sup> mol/L) for 4 and 24 h, at 37 °C. The standard procedure for alkaline comet assay was carried out for monitoring the genotoxic effects of Hf-WD 1:2.

**RESULTS:** The degree of DNA damage in human peripheral blood cells after exposure to Hf-WD 1:2 was expressed as a relative increase of tail DNA related to the control. No statistically significant difference in the amount of DNA strand breaks in comparison with the corresponding control was observed for both 4 and 24 h, regardless of the Hf-WD 1:2 concentrations tested.

**CONCLUSION:** In vitro genotoxicity assessment indicated that Hf-WD 1:2 does not affect DNA structure in healthy human blood cells, accordingly might be regarded as genotoxicity safe in further research as a potential CESA for CT.

**KEY WORDS:** hafnium(IV)-substituted Wells-Dawson polyoxotungstate, contrast-enhancing staining agent, alkaline comet assay, genotoxicity in vitro



## EVALUACIJA GENOTOKSIČNOSTI NANOKLASTERA POLIOKSOVOLFRAMATA KAO POTENCIJALNOG KONTRASTNOG AGENSA

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**UVOD:** Poliooksovolframati su negativno naelektrisana neorganska kompleksna jedinjenja koja se dve decenije intenzivno proučavaju kao moćni bioaktivni agensi. Skorašnje studije na dugim kostima i bubrezima miša su pokazale da poliooksovolframati mogu da se koriste kao agensi za bojenje za poboljšanje kontrasta (CESAs) za kompjuterizovanu tomografiju (CT).

**CILJ:** S obzirom da razvoj novih medicinskih agenasa zahteva procenu njihove bezbednosti, cilj ove studije je da se evaluiraju genotoksične osobine in vitro poliooksovolframata Wells-Dawson tipa (parent WD),  $\alpha_2$ -K6P2W18O62.14H2O, koji je u prethodnim istraživanjima pokazao bolje osobine u odnosu na joheksol, standardni kontrastni agens za CT.

**METODOLOGIJA:** Parent WD je sintetisan sledeći objavljenu proceduru. Uzorci humane krvi dobijeni od zdravih donora su tretirani ispitivanim poliooksovolframatom, a zatim inkubirani na 37 °C tokom 4 i 24 sata. Za procenu genotoksičnosti parent WD korišćen je alkalni komet test kako je opisano u literaturi.

**REZULTATI:** Uzorci pune krvi su tretirani parent WD poliooksovolframatom u opsegu koncentracija 1-100  $\mu\text{mol/L}$ . Oštećenje DNK, koje se koristi kao pokazatelj genotoksičnosti, je izraženo kao % repne DNK. Dobijeni rezultati su pokazali da sve ispitivane koncentracije parent WD nisu značajno uticale na oštećenje DNK u odnosu na odgovarajuću kontrolu, posle 4 i 24 sata tretmana.

**ZAKLJUČAK:** Parent WD nanoklaster nije izazvao genotoksični efekat na zdravim ćelijama periferne humane krvi u svim ispitivanim koncentracijama. U skladu sa tim, ispitivani potencijalni CESA kandidat za CT bi se u daljim istraživanjima mogao smatrati bezbednim sa stanovišta genotoksičnosti.

**KLJUČNE REČI:** Wells-Dawson poliooksovolframati, kontrastni agens, DNK oštećenje, genotoksičnost in vitro



## GENOTOXICITY EVALUATION OF A POLYOXOTUNGSTATE NANOCLUSTER AS A PROMISING CONTRAST AGENT

IN VITRO  
TOXICOLOGY

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**INTRODUCTION:** Polyoxotungstates are negatively charged inorganic cage complexes that were studied as potent bioactive agents for the last two decades. They were recently reported as promising contrast-enhancing staining agents (CESAs) for computed tomography (CT) of murine long bones and kidney tissues.

**AIM:** Taking into account the fact that the development of novel medications requires an evaluation of their safety, the purpose of this study was to assess the genotoxic properties in vitro of parent Wells-Dawson polyoxotungstate (parent WD),  $\alpha_2$ -K6P2W18O62.14H<sub>2</sub>O that showed superior imaging capabilities compared to the standard CT contrast agent iohexol.

**METHODOLOGY:** Parent WD was synthesized according to the published procedure. Human whole blood samples obtained from healthy donors were treated with parent WD, and then incubated at 37 °C for 4 and 24 h. For the genotoxicity assessment of the studied nanocluster alkaline comet assay was performed as described in the literature.

**RESULTS:** Whole blood samples were treated with parent WD within the concentration range of 1-100  $\mu\text{mol/L}$ . DNA damage was expressed as % of tail DNA and used as the indicator of genotoxicity. The obtained results demonstrated that parent WD at all investigated concentrations did not significantly affect DNA damage with respect to the corresponding control, for both 4 and 24 h.

**CONCLUSION:** Parent WD nanocluster did not induce a genotoxic effect on normal non-target human peripheral blood cells at all studied concentrations. Thus, this promising CESA candidate for CT could be considered in further research as safe from a genotoxicity point of view.

**KEY WORDS:** Wells-Dawson polyoxotungstate, contrast agent, DNA damage, genotoxicity in vitro



## CITOTOKSIČNI I GENOTOKSIČNI POTENCIJAL KOMPOZITA NA BAZI NANOČESTICA RESVERATROLA I SELENA, I NJEGOVA SPOSOBNOST INDUKCIJE SUPEROKSIDNIH ANJONA

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Kontinuirano interesovanje za nanočestice različitih materijala i njihove kompozite stvorilo je brojna pitanja u vezi njihove potencijalne toksičnosti. Polifenol resveratrol i jedinjenja sa selenom su među agensima čijoj je upotrebi nanotehnologija omogućila nov pristup. U formi nanočestica, oni pokazuju poboljšanu biokompatibilnost i biološke efekte, kao što je antimikrobna aktivnost.

Na osnovu podataka iz literature, nanočestice selena su najčešće manje toksične nego jedinjenja sa selenom. U našem istraživanju, najpre smo pripremili kompozitni materijal na bazi selena i resveratrola.

Nakon toga, kompozit i njegove komponente bili su testirani pomoću MTT tetrazolijumske boje za utvrđivanje citotoksičnosti, komet testom na genotoksičnost i nitroplavo-tetrazolijumskim testom za procenu nivoa superoksidnih anjona. U svim eksperimentima je korišćena zdrava ćelijska linija fibroblasta čoveka (MRC-5). Rezultati su pokazali da, iako čestice resveratrola ispoljavaju citotoksičnost u koncentracijama preko 12 µg/ml, one nisu genotoksične i ne indukuju stvaranje superoksidnih anjona. Sa druge strane, nanočestice selena nisu bile citotoksične ispod 150 µg/ml, ali su njima tretirane ćelije pokazale znake oštećenja DNK i povećanje produkcije superoksidnih anjona, čak i na 18 µg/ml. Kompozit je pokazao drugačije efekte, indukujući višu citotoksičnost i oksidativni stres nego ekvivalentne koncentracije komponenti, ali istovremeno nije bio genotoksičan. Moguće je da su interakcije nanočestica selena sa nanočesticama resveratrola dovele do pojave jedinstvenih osobina kompozita, potvrđujući izuzetnu važnost testiranja nanokompozitnih materijala uporedo sa njihovim komponentama.

**KLJUČNE REČI:** nanočestice selena, resveratrol, kompozitni materijali, citotoksičnost, komet test, antioksidansi





## CYTOTOXIC AND GENOTOXIC POTENTIAL OF COMPOSITE BASED ON RESVERATROL AND SELENIUM NANOPARTICLES, AND IT'S POTENTIAL FOR SUPEROXIDE ANION INDUCTION

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The continuous interest in engineered nanoparticles of various materials, and their composites, has raised numerous questions regarding their toxicological profiles. Polyphenol resveratrol and selenium compounds are among the agents to which nanotechnology has given all new approach. Their particulate forms possess improved bioavailability and biological effects, such as antibacterial activity. Furthermore, selenium nanoparticles usually have significantly lower toxicity compared to selenium compounds. In this study, we prepared composite material consisting of selenium and resveratrol nanoparticles.

Then, composite material and its components were assessed by using tetrazolium MTT dye for determining cytotoxicity, comet assay for genotoxicity study, and nitroblue tetrazolium assay (NBT) assay to estimate levels of superoxide anions. The normal human fibroblast cells (MRC-5) were used in all the experiments. Results showed that, although the resveratrol component exhibited cytotoxicity at 12 µg/ml, it was not genotoxic, and did not induce superoxide anion production. Selenium nanoparticles, on the other hand, were not cytotoxic below 150 µg/ml, but treated cells showed signs of DNA damage and elevation in superoxide anion production even at 18 µg/ml. Composite material behaved differently, inducing higher cytotoxicity and oxidative stress than comparable concentrations of components, but also showed lack of genotoxicity. Possible interactions of selenium nanoparticles with resveratrol nanoparticles could have created unique composite properties, confirming the great importance of toxicity testing of nanocomposite materials along with their components.

**KEYWORDS:** selenium nanoparticles, resveratrol, composite materials, cytotoxicity testing, comet assay, antioxidants



## CYTOTOXIC AND ESTROGENIC ACTIVITIES OF RESMETHRIN IN MCF-7 CELLS

IN VITRO  
TOXICOLOGY

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Resmethrin is a commonly used pyrethroid insecticide. Therefore, it is being used more nowadays and may cause potential hazards to human health, making it necessary to evaluate their safety for humans at exposure doses. The goal of this study is to investigate the potential cytotoxic activity using cytotoxicity assays and estrogenic activity of resmethrin using the E-Screen assay in human breast cancer cells (MCF-7).

The cytotoxicity was evaluated using MTT, lactate dehydrogenase release, and trypan blue exclusion assays, and its estrogenic activity using a cell proliferation assay. Resmethrin caused cell death at high concentrations. Resmethrin at 0.34 mg/L promoted cell proliferation, with a relative proliferative effect ratio of 75%, relative to  $\beta$ -estradiol (at  $10^{-9}$  mol/L). These findings provide insights into the underlying mechanisms of cytotoxic and estrogenic activity of resmethrin and should also be considered when assessing the safety of resmethrin.

**KEYWORDS:** resmethrin, in vitro, endocrine disrupting, pyrethroids, human breast cells



## KLINIČKA TOKSIKOLOGIJA / CLINICAL TOXICOLOGY

### TROPONIN I VISOKE OSETLJIVOSTI I DUŽINA BORAVKA U BOLNICI KOD AKUTNO OTROVANIH PACIJENATA SA RABDOMIOLIZOM

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Srčani troponini (cTn) su regulatorni proteini koji igraju ključnu ulogu u interakciji između aktina i miozina, kontrolišući kontrakciju i opuštanje skeletnog i srčanog mišića. Visokoosetljivi srčani troponin I nije u potpunosti specifičan za povredu miokarda. Cilj rada je utvrditi ulogu hs-Troponin I na dužinu boravka u bolnici kod pacijenata sa rabdomiolizom akutno otrovanih psihotropnim i hemijskim supstancama. U klinički kontrolisanoj prospektivnoj studiji, 140 pacijenata sa rabdomiolizom podeljeno je u dve grupe u zavisnosti od opojne supstance (psihotropne ili hemijske). Rabdomioliza je definisana kao kreatin kinaza (CK)>250 U/L prema skor u ozbiljnosti trovanja (PSS).

Analiziramo hs-Troponin I u obe grupe po prijemu. Kod pacijenata sa rabdomiolizom u grupama psihotropnih intoksikacija, nivo hs-Troponin I pri prijemu je imao značajan uticaj na dužinu hospitalizacije od 17,7% ( $R^2 = 0,177$ ). Povećanje vrednosti nivoa hs-Troponin I po jedinici pri prijemu je povećalo dužinu boravka u bolnici u proseku za 0,016 dana. Kod pacijenata sa rabdomiolizom u grupama sa hemijskom intoksikacijom, nivo hs-Troponin I pri prijemu nema uticaja na dužinu boravka u bolnici ( $R^2 = 0,000$ ). Povećani nivo hs-Troponin I prvog dana nije uticao na varijabilnost dužine boravka u bolnici. Kod pacijenata sa rabdomiolizom koji su akutno otrovani psihotropnim supstancama, hs-Troponin I se može koristiti za predviđanje dužine boravka u bolnici.

**KLJUČNE REČI:** rabdomioliza, visokoosetljivi troponin I, intoksikacija, hospitalizacija



## HIGH-SENSITIVITY TROPONIN I AND HOSPITAL LENGTH OF STAY IN PATIENTS ACUTELY INTOXICATED WITH RHABDOMYOLYSIS

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Cardiac troponins (cTn) are regulatory proteins that play a pivotal role in the interaction between actin and myosin, controlling the contraction and relaxation of skeletal and cardiac muscle. The high-sensitivity cardiac troponin I assay is not entirely specific for myocardial injury. The aim is to determine the role of hs-TroponinI on the hospital length of stay in patients with rhabdomyolysis acutely intoxicated with psychotropic and chemical substances. In a clinically controlled prospective study, 140 patients with rhabdomyolysis were divided into two groups depending on the intoxicating substance (psychotropic or chemical). Rhabdomyolysis was defined as a creatine kinase (CK) >250 U/L according to the poisoning severity score (PSS).

We analyze hs-TroponinI in both groups upon admission. In patients with rhabdomyolysis in the group of psychotropic intoxications, the level of hs-TnI on admission significantly affected the length of hospitalization in 17.7% ( $R^2 = 0.177$ ). Increasing the value of the hs-TnI level per unit on admission increased the length of hospital stay by an average of 0.016 days. In patients with rhabdomyolysis in the chemical intoxication group, the hs-TnI level on admission has no influence on the hospital length of stay ( $R^2 = 0.000$ ). The increased hs-TnI level on the first day did not affect the hospital length of stay variability. In patients with rhabdomyolysis acutely intoxicated with psychotropic substances, hs-TnI can be used to predict the length of hospital stay.

**KEYWORDS:** rhabdomyolysis, high-sensitivity troponin I, intoxication, hospitalization



## POSTOJI LI VEZA IZMEĐU HEMATOLOŠKE TOKSIČNOSTI I ODGOVORA NA NEOADJUVANTNU HEMORADIOTERAPIJU KOD PACIJENATA SA LOKALNO UZNAPREDOVALIM KARCINOMOM REKTUMA?

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Standardni tretman lokalno uznapredovalog karcinoma rektuma (LARC) je neoadjuvantna hemioradioterapija (nCRT), nakon čega sledi operacija. Cilj ove studije bio je da se proceni povezanost između hematološke toksičnosti i odgovora tumora na terapiju (TR). U periodu od juna 2020. do januara 2022. godine, u studije je prospektivno uključeno 75 pacijenata sa LARC-om koji su lečeni dugotrajnim režimom CRT-a. RT je isporučena korišćenjem volumnog rotacionog planiranja zračne terapije i simultanog integrisanog boost-a. Istovremena hemioterapija (5-FU, leukovorin) je primenjivana tokom prve i pete nedelje RT. TR je procenjen u 8. nedelji, nakon završetka nCRT. Za pacijente sa potpunim kliničkim odgovorom (cCR) nije predložena neposredna radikalna operacija.



Grupa pacijenata koja je dobro odgovorila na sprovedeno lečenje (eng. responders, R) je definisana sa cCR i TRG1 i TRG2 postoperativnim kategorijama, prema Mandardovoj klasifikaciji. Grupa koja je lošije odgovorila (eng. non-responders, NR) definisana je kao TRG3-5. Akutna toksičnost je procenjena nedeljno u skladu sa zajedničkim terminološkim kriterijumima za neželjene događaje (CTCAE) v.5.0. R su obuhvatali 46,6% pacijenata.

Tokom nCRT, javili su se ukupni hematološki neželjeni efekti: anemija 40,0%, leukopenija 32,0% i neutropenija 21,3%. Anemija 3. stepena, leukopenija i neutropenija su se javile kod 6,7%, 2,7% i 4% pacijenata, respektivno. Nije prijavljena toksičnost 4. stepena. Limfopenija je primećena kod 94,7% pacijenata; 50,7% njih imalo je stepen toksičnosti 3/4. Značajna povezanost između hematološke toksičnosti i TR nije dokazana. Negativan trend je primećen kod anemije; pacijenti bez anemije tokom nCRT bolje su reagovali na lečenje ( $p=0,05$ ). Ova povezanost može biti dovedena u vezu sa hipoksijom tumora i posledičnom radiorezistentnošću. Limfopenija izazvana zračenjem i njena potencijalna povezanost sa TR biće ispitana u nastavku ove studije. Dalje analize će biti usmerene na ispitivanje subpopulacija zahvaćenih limfocita korišćenjem CyTOF metodologije masene citometrije.

**KLJUČNE REČI:** lokalno uznapredovali karcinom rektuma, neoadjuvantna hemioradioterapija, hematološka toksičnost.

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## IS THERE A RELATION BETWEEN HEMATOLOGICAL TOXICITY AND RESPONSE TO NEOADJUVANT CHEMORADIOTHERAPY IN PATIENTS WITH LOCALLY ADVANCED RECTAL CANCER?

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The standard treatment for locally advanced rectal cancer (LARC) is neoadjuvant chemoradiotherapy (nCRT) followed by surgery. The aim of this study was to evaluate the association between different hematological toxicity and tumor response (TR). We prospectively included 75 LARC patients treated with long-course CRT between June 2020 and January 2022. RT was delivered using volumetric modulated arc therapy-simultaneous integrated boost. Concomitant chemotherapy (5FU, Leucovorine) was given during the first and fifth week of RT. TR was assessed in week 8, after nCRT completion. No immediate radical surgery was suggested for patients with complete clinical response (cCR). Responders were defined as patients with cCR and TRG1 and TRG2 postoperative categories, according to the classification by Mandart. Non-responders were classified as TRG3-5. Acute toxicity was evaluated weekly according to common terminology criteria for adverse events (CTCAE) v.5.0.



Responders group comprised 46.6% of patients. During nCRT, all-grade hematological adverse effects occurred: anemia 40.0%, leukopenia 32% and neutropenia 21.3%. Grade 3 anemia, leukopenia and neutropenia occurred in 6.7%, 2.7% and 4% of patients, respectively. No grade 4 toxicity was reported. Lymphopenia was observed in 94.7% of patients; 50.7% of them had grade 3/4 toxicity. No significant association was found between hematological toxicities and TR.

A negative trend was observed for anemia; patients without anemia during nCRT responded better to treatment ( $p=0.05$ ). This association might be connected to tumor hypoxia and consequent radioresistance. Radiation-induced lymphopenia and its potential association with TR remain to be understood. Further analyses will be directed to the investigation of the affected lymphocytes subpopulations using a CyTOF mass cytometry methodology.

**KEY WORDS:** locally advanced rectal cancer, neoadjuvant chemoradiotherapy, hematological toxicities.

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## USPEŠNA PRIMENA ALBUMINSKE DIJALIZE U LEČENJU AMANITINSKE INTOKSIKACIJE

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Trovanje Amanita phalloides-om uzrokuje teško oštećenje jetre sa visokim stepenom mortaliteta. Terapija najtežih formi amanitinskog sindroma osim primene N-acetilcisteina (NAC), hepatoprotektiva i suportivnih mera zahteva primenu ekstrakorporalnih procedura uključujući i albuminsku dijalizu (SPAD). Cilj rada je prikazati uspešnu primenu SPAD kod pacijenta koji je razvio akutnu insuficijenciju jetre sa encefalopatijom i akutnom bubrežnom insuficijencijom. Ovo je bio prvi slučaj primene ove ekstrakorporalne procedure u Vojnomedicinskoj akademiji. Pacijent muškog pola, starosti 48 g., premešten je iz lokalnog zdravstvenog centra (ZC) u Nacionalni centar za kontrolu trovanja (NCKT) zbog intoksikacije pečurkama skupljenih u prirodi.

Tri sata nakon ingestije razvija gastrointestinalne (GI) tegobe zbog kojih se narednog dana javlja u matični ZC u teškom stanju sa parametrima lezije jetre (ALT 775 U/L, AST 1066 U/L, Bil 34  $\mu$ mol/L, INR 2,5) i bubrega (urea 15 mmol/L, kreatinin 306  $\mu$ mol/L) zbog čega je odmah upućen u NCKT (24h od ingestije) gde je neposredno po prijemu otpočeto je sa intenziviranom parenteralnom suportivnom terapijom uključujući primenu NAC po 21h protokolu. Uprkos navedenim merama pacijent fulminantno razvija dodatno pogoršanje hepatične insuficijencije (Bil 186  $\mu$ mol/L, ALT 1793 U/L, AST 1507 U/L, ggT 132 U/L, alb 29 g/L, INR 4,9, apTT 78 sec) uz GI hemoragiju i encefalopatiju (amonijak 136  $\mu$ mol/L). Počev od 2. dana otpočelo se sa ekstrakorporalnim procedurama (hemoperfuzija i plazmaferezna procedura koja je ponovljena i 3. dana), a potom naredna 2 dana SPAD nakon kojih razvija značajno kliničko poboljšanje uz postepeni oporavak jetrene i bubrežne funkcije. Rana primena SPAD (sa ili bez terapijskih izmena plazme) kod pacijenata sa hepatičnom i bubrežnom insuficijencijom te encefalopatijom u sklopu amanitinske intoksikacije ima višestruko koristan terapijski efekat. Blagovremena primena iste zahteva promptnu i tesnu multidisciplinarnu saradnju.

**KLJUČNE REČI:** albuminska dijaliza, akutna hepatična insuficijencija, ekstrakorporalna procedura



## SUCCESSFUL USE OF ALBUMIN DIALYSIS IN THE MANAGEMENT OF *AMANITA PHALLOIDES* POISONING

CLINICAL  
TOXICOLOGY

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*Amanita phalloides* poisoning causes severe liver damage with high mortality. Therapy of the most severe forms of amanita syndrome, apart from the use of N-acetylcysteine (NAC), hepatoprotective agents and supportive measures requires the use of extracorporeal procedures, including single pass albumin dialysis (SPAD). Objective of this study was to demonstrate the successful application of SPAD in a patient who developed acute liver failure with encephalopathy and acute renal failure. This was the first case of use of this extracorporeal procedure at the Military Medical Academy. A male patient, aged 48, was transferred from the local medical center to National Poison Control Center (NPCC) due to intoxication with mushrooms collected in nature.

Three hours after the ingestion, he developed gastrointestinal (GI) complaints, due to which he appeared in the local medical center in the following day in a serious condition with parameters of liver lesions (SGPT 775 U/L, SGOT 1066 U/L, Bil 34  $\mu\text{mol/L}$ , INR 2,5) and kidneys (urea 15 mmol/L, creatinine 306  $\mu\text{mol/L}$ ) when he was immediately referred to NPCC (24h after ingestion) where, immediately after admission he was treated with intensified parenteral supportive therapy including the administration of NAC according to the 21hours protocol. Despite the mentioned measures, the patient fulminantly developed additional worsening of hepatic insufficiency (Bil 186  $\mu\text{mol/L}$ , ALT 1793 U/L, AST 1507 U/L, ggT 132 U/L, alb 29 g/L, INR 4,9, apTT 78 sec) with GI hemorrhage and encephalopathy (ammonia 136  $\mu\text{mol/L}$ ). Starting from the 2nd day, extracorporeal procedures were started (hemoperfusion and plasmapheresis procedure, which was repeated on the 3rd day), followed by SPAD for the next 2 days, after which he developed a significant clinical improvement with a gradual recovery of liver and kidney function. Early application of SPAD (with or without therapeutic plasma changes) in patients with hepatic and renal insufficiency and encephalopathy as part of amanita intoxication, can have a multiple beneficial therapeutic effect. Its timely application requires prompt and close multidisciplinary cooperation.

**KEY WORDS:** Albumin dialysis, acute liver failure, extracorporeal procedure



## AKCIDENTALNA DUBOKA HIPOTERMIJA U TEŠKOM TROVANJU KARBAMAZEPINOM

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Hipotermija je snižavanje telesne temperature ispod 35°C koja može biti posledica izlaganja hladnoći ili nekog drugog stanja uključujući povredu i trovanje. Sa snižavanjem telesne temperature, u slučaju blage hipotermije (35-32°C), osoba počinje da kompenzuje proizvodnjom temperature drhtanjem i centralizacijom krvotoka. U dubokoj hipotermiji (<28°C) većina pacijenata gubi svest i cerebrovaskularnu regulaciju. Pritom je rizik od ventrikularne fibrilacije i asistolije visok, uz smanjenu potrebu mozga za kiseonikom. Ksenobiotici (etanol, opioidi, sedativo-hipnotici, anestetici itd.) mogu da pogoršaju ovo stanje remećenjem fiziološke termoregulacije.

Dvadesetčetvorogodišnja pacijentkinja primljena je u NCKT zbog polimedikamentozne intoksikacije, dominantno karbamazepinom, nakon tri dana od ingestije lekova. Primljena je u komi, sa reakcijom na grube draži, midrijatičnih nereaktivnih zenica, respiratno insuficijentna (saturacije O<sub>2</sub> 86%) sa pukotima pri auskultaciji i nemerljivom telesnom temperaturom. EKG je pokazao nodalni ritam, SF 50/min, proširen QRS (114ms), izražen „J-Ozbornov“ talas, produženi QT (627ms) i QTc (572ms). Odmah po prijemu u Kliniku za urgentnu i kliničku toksikologiju NCKT dolazi od srčanog i respiratornog zastoja. Pacijentkinja je priključena na mehaničku ventilaciju i date su 16 ampula adrenalina i 6 ampula atropina praćene stimulacijom dopaminom. Nadoknada tečnosti zagrejanim kristaloidima, kao i zagrevanje električnim čebetom su nastavljani uz profilaktički niskomolekularni heparin, hiperproteinsku i visokoenergetsku dijetu itd.. Šestog dana je ekstubirana i 14. dana, potpuno oporavljena upućena je psihijatru na dalji tretman. Intoksikacija lekovima može pospešiti nastanak hipotermije, što znači da kardiopulmonalna reanimacija treba trajati duže kod ovih pacijenata nego kod onih sa drugim stanjima.

**KLJUČNE REČI:** hipotermija, karbamazepin, kardiopulmonalna reanimacija



## ACCIDENTAL PROFOUND HYPOTHERMIA IN SEVERE CARBAMAZEPINE INTOXICATION

CLINICAL  
TOXICOLOGY

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Accidental hypothermia is a body core temperature below 35.0°C which may be caused by exposure to a cold environment or triggered by other conditions, including trauma and poisoning. As the body temperature lowers, in the case of mild hypothermia (35-32°C), a person starts compensating with heat generation by shivering and centralizing the blood flow. In deep hypothermia (<28°C) most patients lose consciousness and cerebrovascular regulation. Thus, the risk of ventricular fibrillation and asystole is high, with brain having a reduced need for oxygen. Xenobiotics (ethanol, opioids, sedative-hypnotics, anesthetics etc.) can worsen this condition by impairing the physiological thermoregulation. A 24-year old female was admitted to the NPCC for drug poisoning, predominantly by carbamazepine, three days after drug ingestion. At the admission she was in coma, with reaction to painful stimuli, mydriatic, unreactive pupils, respiratory insufficiency (Sat O<sub>2</sub> 86%) and rales on auscultation, cold extremities and unmeasurable body temperature.

ECG showed a nodal rhythm, heart rate 50/min, widened QRS (114 ms), pronounced “J-Osborn wave”, prolonged QT (627 ms) and QTc (572 ms). Immediately after being admitted to the Clinic of Emergency and Clinical Toxicology NPCC cardiac and respiratory arrest occurred. She was placed on mechanical ventilation, 16 ampoules of Adrenaline and 5 ampoules of Atropine were administered, followed by Dopamine stimulation. Fluid recoupment using heated crystalloids as well as heating by an electric blanket were continued along with prophylactic low-molecular heparin, hyperprotein and high-energy diet, etc.). On Day 6 she was extubated, and on Day 14, fully recovered she was referred to a psychiatrist for further treatment. Drug intoxication can facilitate the onset of hypothermia which means that cardiopulmonary reanimation should last longer in these patients than in those with other conditions.

**KEYWORDS:** hypothermia, carbamazepine, cardiopulmonary reanimation



## AKUTNO TROVANJE GAMA-HIDROKSIBUTIRATOM (GHB) PRIKAZ SLUČAJA

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Gama-hidroksibutirat (GHB), takozvana „klupska droga“ ili „droga za silovanje“ postao je čest uzrok trovanja u hitnim službama, zbog zablude mladih da je relativno bezbedan. Koristi se zbog povećanog seksualnog nagona, euforičnih i sedativnih efekata. GHB se vezuje za GABA-B i GHB-specifične receptore, blokirajući oslobađanje dopamina u sinapsama što dovodi do povećanja neuronskog dopamina. Teško je proceniti nefatalne prijeme u bolnicu sa GHB jer se analiza ne obavlja rutinski, s obzirom da je to supstanca koja je prirodno prisutna u telu. Klasična prezentacija GHB toksičnosti je povraćanje, iznenadni gubitak svesti, respiratorna depresija, konvulzije, bradikardija, hipotenzija, halucinacije i hipotermija. Muškarac star 36 godina stigao je kolima hitne pomoći u Kliniku za urgentnu i kliničku toksikologiju NCKT intubiran, stanja svesti nivoa kome, nemerljivog arterijskog pritiska sa respiratornom insuficijencijom i cijanozom.

Prema podacima njegovog prijatelja, on je uzeo bočicu GHB, uznemirio se, imao samoubilačke misli i istrčao na ulicu gde je kasnije pronađen u koma. Njegov početni Glazgov koma skor (GCS) bio je 3, frekvencija disanja 8/min, puls 40/min, SatO<sub>2</sub> 55%. GHB je u krvi potvrđen LC-MS metodom u koncentraciji >10 mg/L, uz etanol 0,28%. Primljen je u Odeljenje intenzivne nege NCKT, gde je uspostavljen venski pristup, primenjena infuziona terapija i stavljen na mehaničku ventilaciju (SIMV modalitet). Ekstubiran je 5 sati kasnije, ali je i dalje imao periode pospanosti i blage uznemirenosti. Opušten je potpuno oporavljen trećeg dana. Ovaj slučaj pokazuje da lekari hitne pomoći treba da razmišljaju o GHB, njegovoj tipičnoj prezentaciji, kao i o diferencijalnoj dijagnozi koma nepoznate etiologije.

**KLJUČNE REČI:** GHB, koma, respiratorna insuficijencija



## ACUTE GAMMA-HYDROXYBUTYRATE (GHB) POISONING: CASE REPORT

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Gamma-hydroxybutyrate (GHB), so-called „club drug“ or „date rape drug“ has become a more frequent cause of poisoning in emergency departments, due to misconceptions of young adults that it is relatively safe. It is used for its increased sex drive, euphoric and sedative effects. GHB binds to GABA-B and GHB-specific receptors, blocking dopamine release at synapses which produces an increase in neuronal dopamine. It is difficult to assess non-fatal hospital admissions with GHB as analysis is not routinely performed, and it is a substance naturally present in the body. The classic presentation of GHB toxicity is vomiting, a sudden loss of consciousness, respiratory depression, convulsions, bradycardia, hypotension, hallucinations, and hypothermia. A 36-year old male arrived by ambulance at the Clinic of Emergency and Clinical Toxicology NPCC intubated, with coma, immeasurable arterial pressure, respiratory insufficiency and cyanosis.

According to the data from his friend, he had taken a bottle of GHB, became agitated, with suicidal thoughts and ran out to the street where he was later found in coma. His initial Glasgow Coma Score (GCS) was 3, respiratory rate 8/min, heart rate 40/min, SatO<sub>2</sub> 55%. GHB was confirmed in blood by LC-MS in concentration of >10 mg/L, alongside with ethanol 0,28‰. He was admitted to the Intensive Care Unit of the NPCC, where venous access was established, infusion therapy administered, and he was placed on mechanical ventilation (SIMV modality). He was extubated 5 hours later, however continued to have alternating period of somnolence and mild agitation. He was discharged fully recovered on Day 3. This case highlights that emergency physicians should be alerted to GHB, its typical presentation, but also to consider it when a patient presents to the ER with undifferentiated coma.

**KEYWORDS:** GHB, coma, respiratory insufficiency



## EVALUACIJA TROVANJA PREGABALINOM NA JUGOISTOKU SRBIJE U PETOGODIŠNJEM PERIODU

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Pregabalin je depresor CNS-a, koji je prvobitno registrovan za lečenje neuropatskog bola i parcijalnih epileptičnih napada. Širom sveta je primećen porast u propisivanju i zloupotrebi, razvoj zavisnosti i fatalnih intoksikacija, usled trovanja pregabalinom, pre svega kod opioidnih zavisnika. Cilj rada je utvrđivanje učestalosti i karakteristika trovanja pregabalinom na jugoistoku Srbije. Studija preseka je sprovedena u Nišu, u jugoistočnoj Srbiji, za period od januara 2018, do decembra 2022. godine. Korišćeni su podaci Zavoda za sudsku medicinu u Nišu, a obrađeni su programom Excel 2013. Pregledano je 4710 postmortem i 1215 antemortem slučajeva. Zabeleženo je 24 (0,51%) postmortem i 64 (5,27%) antemortem slučajeva trovanja pregabalinom.

Procenat postmortem slučajeva je porastao od 0,09% 2018. do 1,06% 2021. godine, dok je primećen porast antemortem slučajeva od 1,93% 2018. do 9,44% 2021. godine. Vrednosti 2022. godine su ostale približne vrednostima u 2021. Statistički značajniji broj trovanja muške populacije je zabeležen u postmortem slučajevima (70,83%), dok je kod antemortem slučajeva bio veći procenat trovanja u ženskoj populaciji (53,13%). Samo 3 slučaja trovanja isključivo pregabalinom je zabeleženo. Pregabalin je najčešće kombinovan sa sedativima (36,86%; 32%) i opioidima i opioidnim supstituentima (14,14%; 30,66%) u antemortem i postmortem slučajevima, redom. Izuzetno je značajno povećati svest lekara o potencijalu zloupotrebe pregabalina. Primena pregabalina sa drugim depresorima CNS-a je opasna, zbog interakcija i potencijalnog letalnog ishoda. Lekari i toksikolozi moraju pratiti porast propisivanja i zloupotrebe novoregistrovanih lekova.

**KLJUČNE REČI:** trovanje lekovima, pregabalin, zloupotreba



## PREGABALIN INTOXICATION PATTERN IN SOUTHEAST SERBIA DURING FIVE YEARS PERIOD

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Pregabalin is a CNS depressant initially registered for neuropathic pain and partial-onset seizures. An increase in prescription, abuse, dependence and fatal intoxication, especially in opioid addicts, was noticed worldwide. The aim of the study was to identify patterns of pregabalin intoxication in southeast Serbia. The cross-sectional study was conducted in Nis, southeast Serbia, from January 2018 to December 2022. The data were extracted from the Institute of Forensic Medicine records and statistically evaluated using Excel 2013. 4710 postmortem and 1215 antemortem cases were analysed. 24 (0.51%) postmortem cases and 64 (5.27%) antemortem cases of pregabalin intoxication are recorded. The percentage of postmortem cases raised from 0.09% in 2018 to 1.06% in 2021 and from 1.93% in 2018 to 9.44% in 2021 of antemortem cases.

In 2022 the numbers remained stable. Male statistically significant predominance was observed in postmortem cases (70.83%), while there were 53.13% of antemortem cases, in the female population. Only 3 cases of pregabalin itself intoxication were recorded. Pregabalin was most commonly combined with sedatives (36.86%; 32%) and opioids and opioid substituents (14.14%; 30.66%) in antemortem and postmortem cases respectively. Raising awareness of drug prescribers about the pregabalin abuse potential is imperative. Prescribing pregabalin with CNS depressants should be carefully considered, as they can interact and cause lethal intoxication. Clinicians and toxicologists must be up to date with newly registered drugs and carefully monitor the rise in prescription and abuse of drugs.

**KEYWORDS:** drug intoxication, pregabalin, abuse





## SAMOTROVANJA ANTIDEPRESIVIMA NOVIJE GENERACIJE – KOLIKO SMO BEZBEDNI?

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Selektivni inhibitori ponovnog preuzimanja serotonina (SSRI) su lekovi sa širokim terapijskim indeksom i širokim indikacionim područjem što ih čini čestim agensom u samotrovanjima. Cilj rada je prikazati osnovne karakteristike trovanja i predoziranja SSRI, distribuciju u odnosu na pol i životno doba, učestalost pojedinih agenasa, procena težine trovanja, te uticaj koingestiranih supstanci. Retrospektivna analiza istorija bolesti 71 bolesnika hospitalizovanih zbog akutne intoksikacije sa SSRI u četvorogodišnjem periodu kod kojih je ingestirana doza leka bila veća od petostruke preporučene dnevne doze i/ili dokazane toksične koncentracija leka.

Prosečna starost bolesnika je bila 42±16 godine i većinu (60,71%) su činile žene. Trovanje je bilo recidivantno u 52% slučajeva. Najzastupljeniji agens je bio sertralina (39), potom citalopram/escitalopram (25%), fluoksetin (18%) i paroksetin (17%). Više od 90% bolesnika imalo je koingestirane druge supstance (benzodiazepini, neuroleptici, antiepileptici i alkohol). Trovanje lakog stepena (PSS 1) imalo 62%, srednje teškog 17%, teškog stepena (PSS 3) 18% bolesnika, a dva trovanja (3%) su imala letalni ishod (PSS 4). Svi bolesnici sa težim trovanjima, kao i letalnim ishodom su imali koingestirane druge supstance. Ispitivanjem odnosa koncentracija pojedinog SSRI agensa u krvi i dužine QTc intervala nije utvrđena statistička značajnost ni za jedan agens. Konvulzije su registrovane kod jednog pacijenta (citalopram). Kriterijume serotoniniskog sindroma nije imao ni jedan pacijent. Klinička slika akutnih trovanja SSRI, koja su često recidivantna, uglavnom je lakog stepena. Visoke ingestirane doze i često prisustvo drugih koingestiranih lekova i supstanci te njihove međusobne interakcije značajno utiču na težinu ispoljene kliničke slike.

**KLJUČNE REČI:** selektivni inhibitori ponovnog preuzimanja serotonina, akutno samotrovanje



## DELIBERATE SELF-POISONINGS WITH NEW ANTIDEPRESSANTS – HOW SAFE ARE WE?

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Selective serotonin reuptake inhibitors (SSRIs), widely prescribed medications with a high therapeutic to toxicity ratio, are agents often involved in deliberate self-poisonings. The aim was to analyze the main clinical manifestations of acute SSRI poisoning, age, gender and agents distributions, overdose severity according Poisoning Severity Score (PSS), type of concomitants and its influence on clinical features. Data from medical records of 71 patients hospitalized in 4 years period with ingested doses five times higher than daily therapeutic dose and/or confirmed toxic concentration of drugs in blood were retrospectively analysed. Average age of patients was 42±16 years; most (60,71%) were female. In 52% of cases deliberate self-poisoning was repeated. The most common agent was sertraline (39%), then citalopram/escitalopram (25%), fluoxetine (18%) and paroxetine (17%).

In more than 90% of patients concomitants (benzodiazepines, neuroleptics, antiepileptics and ethanol) were presented. The PSS was 1 in 62%, 2 in 17% and 3 in 18% of patients. There were two (3%) lethal outcomes. All cases with severe clinical features and lethal outcome had concomitants ingested. Correlation between agents blood concentrations and length of QTc interval was not found for any type of agent. Seizures were registered in one patient (citalopram poisoning). Serotonin syndrome was not present in any patient. Symptoms observed following acute overdose of SSRI are typically mild. Large ingested doses, frequent presence of concomitants and their interactions contribute to the severity of clinical manifestations.

**KEYWORDS:** selective serotonin reuptake inhibitors, deliberate self-poisonings



## FAKTORI KOJI UTIČU NA RAZVOJ RABDOMIOLIZE TEŠKOG STEPENA U AKUTNIM TROVANJIMA

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Rabdomioliza je jedna od najčešćih komplikacija akutnih intoksikacija. Cilj rada je analiza faktora koji utiču na razvoj rabdomiolize teškog stepena u akutnim trovanjima. Retrospektivna analiza istorija bolesti pacijenata lečenih zbog akutne intoksikacije u petogodišnjem periodu sa vrednostima kreatin kinaze (CK) >1500 U/l. Analizirani su dob, pol, agens; arterijski pritisak (TA), saturacija kiseonika (SaO<sub>2</sub>) i lokalni nalaz na prijemu; vreme proteklo od ekspozicije; maksimalni CK; parametri bubrežne funkcije, hemodijaliza i ishod. Od ukupno 269 pacijenata (muškarci 58%, žene 42%, prosečne dobi 42g, 21% (56) je imalo CK >10000U/l. Prosečna vrednost CK kod pacijenata sa teškom rabdomiolizom je bila 50598.09 ± 49744.78U/l (CKmax 212,000U/l), većina su bili muškarci (73%), a dominantni agens sredstva zloupotrebe uključujući alkohol (50%; uglavnom opiodi).

Skoro 70% pacijenata sa CK>10000U/l primljeno je >12h nakon ekspozicije; vrednosti SaO<sub>2</sub> i TA na prijemu bile su značajno niže u odnosu na pacijente sa CK<10000U/l (83% vs. 91%, p<0.001; 103/66mmHg vs. 120/69mmHg, p<0.001). Hemodijaliza je sprovedena kod 10 pacijenata. Neurološke sekvele (postnokslična encefalopatija i oštećenje perifernih nerava) zabeležene su kod 29% bolesnika. Svi pacijenti sa CK>10000U/l kod kojih je došlo do smrtnog ishoda (10;18%) bili su muškog pola; opiodi su bili primarni agens u 70% slučajeva; 90% je primljeno u komi, >12h od ekspozicije; 70% je imalo lokalni nalaz (otok, crvenilo); srednje vrednosti SaO<sub>2</sub> i TA na prijemu su bile niže u odnosu na druge pacijente sa teškom rabdomiolizom. Glavni faktori rizika za razvoj rabdomiolize teškog stepena u akutnim trovanjima su muški pol, opiodi, produženo vreme od ekspozicije, hipotenzija, niska saturacija kiseonika i lokalni nalaz na prijemu.

**KLJUČNE REČI:** rabdomioliza teškog stepena, akutna trovanja



## CONTRIBUTING FACTORS FOR THE ONSET OF SEVERE RHABDOMYOLYSIS IN ACUTE POISONINGS

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Rhabdomyolysis is one of the most often complications in acute poisonings. The aim was to analyze factors that influence the onset of severe rhabdomyolysis in acute poisonings. Retrospectively were analysed data from medical records of acutely poisoned patients with creatine kinase (CK)>1500 U/l treated in five years period. Data were reviewed for age, gender, agents; blood pressure (BP), oxygen saturation (SaO<sub>2</sub>) and local findings on admission; the time elapsed since exposure; maximal CK; renal function parameters, hemodialysis and outcome. Out of 269 patients (male 58%, female 42%, average age 42y), 21% (56) had CK>10000U/l. The average value of CK in patients with severe rhabdomyolysis was 50598.09 ± 49744.78U/l (CKmax 212,000 U/l), the majority were male (73%), dominant agents were drugs of abuse including ethanol (50%; mostly opioids).

The time from exposure to admission was >12h in almost 70% of patients with CK>10000U/l; SaO<sub>2</sub> and BP on admission were significantly lower compared to patients with CK<10000U/l (83% vs. 91%, p<0.001; 103/56mmHg vs. 120/69mmHg, p<0.001). Hemodialysis was performed in 10 patients. Neurological sequelae (postanoxic encephalopathy, peripheral nerve injury) were noted in 29% patients. All patients with CK>10000U/l who died (10;18%) were male; opioids were agents in 70% cases; 90% were admitted >12h since exposure; 70% had local findings (redness, swelling); average SaO<sub>2</sub> and BP on admission were lower compared to other patients with severe rhabdomyolysis. Male gender, opioids, prolonged time elapsed since exposure, hypotension, low SaO<sub>2</sub> and local findings were main risk factors for severe rhabdomyolysis in acute poisonings.

**KEYWORDS:** severe rhabdomyolysis, acute poisoning



## TROVANJA KOJA VIDIMO SVE ČEŠĆE: KVETIAPIN

CLINICAL  
TOXICOLOGY

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Trovanje sa tvarima iz skupine psihijatrijskih lijekova sve su češća pojava u hitnom prijemu. Iako se je do sada dominantno radilo o namjernim ili nenamjernim izlaganjima benzodiazepinima, u zadnje vrijeme se sve češće vidaju i trovanja sa drugim, puno opasnijim lijekovima. U hitni prijem dovežena je 50-godišnja pacijentica poremećenog stanja svijesti, koja je, prema navodima izvanbolničke hitne medicinske pomoći, u pokušaju samoubojstva popila 50 tableta od 100mg kvetiapina (sveukupna doza 5g). Pri dolasku je pacijentica dubokog poremećaja stanja svijesti, sa GCS 3, hipotenzivna i tahikardna, te bez znakova promjena u EKG-u.

Pacijentica je intubirana u brzom slijedu u hitnom prijemu, te je hospitalizirana u jedinici intenzivnog liječenja, gdje je par sati nakon prijema razvila i QT prolongaciju koja je uspješno liječena sa magnezijevim sulfatom. Pacijentica je ekstubirana nakon 28 sati od dolaska u hitni prijem, premještena na odjel psihijatrije gdje je dalje nastavljeno njeno liječenje. U ovom radu prezentirati ćemo ovaj slučaj, a kroz pregled literature i načine kako brzo prepoznati i liječiti ovo sve češće, i ponekad po život opasno trovanje.

**KLJUČNE REČI:** hitni prijem, toksikologija, kvetiapin



## ON THE RISE: QUETIAPINE TOXICITY

CLINICAL  
TOXICOLOGY

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Poisoning with substances from the group of psychiatric drugs is an increasingly common occurrence in the emergency department. Although until now it was mainly about intentional or unintentional exposure to benzodiazepines, poisonings with other, much more dangerous drugs have been seen more and more recently. A 50-year-old female patient with decreased consciousness was brought to the emergency department. According to the out-of-hospital emergency medical services, she ingested 50 tablets of 100 mg quetiapine (total dose 5 g) in an attempt to commit suicide. On arrival, the patient had a profoundly decreased state of consciousness, with GCS 3, she was hypotensive and tachycardic, and without signs of changes in the ECG.

The patient was intubated in rapid sequence in the emergency department and was admitted to the intensive care unit, where a few hours after admission she developed QT prolongation, which was successfully treated with magnesium sulfate. The patient was extubated 28 hours after arriving at the emergency department and transferred to the psychiatric ward, where her treatment continued. In this paper, we will present this case, and through a review of the literature, present ways to quickly recognize and treat this increasingly frequent, and sometimes life-threatening poisoning.

**KEYWORDS:** emergency medicine, toxicology, quetiapine



## ANTIDOTI U HITNOM PRIJEMU: TREBAMO IH, ALI IH NEMAMO UVIJEK!

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U Hrvatskoj ne postoji razvijena subspecijalizacija iz kliničke toksikologije, a samim time niti razvijena strategija niti mreža toksikoloških centara. Činjenica je da se u svakodnevnom radu susrećemo sa trovanjima, a postoji nejednaki prisup antidotima u hitnim prijemima ili centralnim bolničkim ljekarnama. Svrha ovog predavanja je pregled literature o temi dostupnosti antidota u akutnim bolnicama, vremenske i količinske, ali i vlastitih iskustva o izgradnji takve prakse u OHBP-u KBC-a Zagreb, a sa namdom da će u budućnosti postojati strategija koja će dati preporuke o tome koje antidote moramo imati uvijek u hitnom prijemu, kada ih dajemo, u kojim dozama, i kakve zalihe bolnice moraju uvijek imati u centralnim ljekarnama.

**KLJUČNE REČI:** hitna medicina, toksikologija, antidoti



## ANTIDOTES IN THE EMERGENCY DEPARTMENT: NEED THEM, BUT DON'T HAVE THEM!

CLINICAL  
TOXICOLOGY

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In Croatia, there is no sub-specialization in clinical toxicology, and therefore neither a developed strategy nor a network of toxicology centers. We encounter poisoning in our daily work, and there is an unequal supply of antidotes in emergency departments or central hospital pharmacies. The purpose of this lecture is to review the literature on the topic of the availability of antidotes in acute hospitals, in terms of time and quantity, as well as our own experiences on the construction of such a practice in the OHBP of KBC Zagreb, with the hope that in the future there will be a strategy that will give recommendations about this which antidotes must we always have in the emergency room, when do we give them, in what doses, and what supplies must hospitals always have in central pharmacies.

**KEYWORDS:** emergency medicine, toxicology, antidotes





## SISTEMSKA INTOKSIKACIJA KOBALTOM I HROMOM NAKON TOTALNE ARTROPLASTIKE KUKA

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Poznato je da kod nosioca endoproteza može doći do oslobađanja jona kobalta i hroma poreklom iz metalnih delova proteze, posebno u slučaju preloma proteze kada postoji rizik od razvoja metaloze. Predstavljamo pacijenta kod koga je nakon totalne artroplastike kuka došlo do razvoja sistemske intoksikacije kobaltom i hromom sa ozbiljnim kliničkim manifestacijama trovanja. Muškarcu starom 50 godina je urađena totalna artroplastika kuka zbog avaskularne nekroze levog kuka. U narednih pet godina, usled dva preloma keramičkih komponenti kuka, urađene su delimične revizije operacije kuka.

Pacijent je počeo da se žali na bol i nelagodnost u ingvinalnoj i glutealnoj regiji sa epizodama ataksije, progresivnim gubitkom vida i sluha i stalnim osećanjem umora. U početku, simptomi nisu prepoznati kao moguće trovanje metalnim jonima. Nakon opsežnih dijagnostičkih procedura, na kraju su urađene analize Co i Cr u krvi i izmereni su povišeni nivoi jona (133,9 µg/L, odnosno 40,3 µg/L). Nakon potpunog uklanjanja endoproteze, nivoi Co i Cr u serumu su značajno opali (1,5 µg/L i 4,2 µg/L, respektivno), ali gubitak sluha i vida se nije oporavio. Nivoi jona Co i Cr u serumu mogu se povećati nakon zamene kuka što može dovesti do sistemskih manifestacija trovanja. Rutinski testovi iz krvi se obično ne rade, iako predstavljaju pogodan način praćenja funkcionalnosti proteze i potencijalnog razvoja metaloze. Pored toga, to bi moglo skratiti opsežnu dijagnozu što bi dovelo do uspešnijeg lečenja.

**KLJUČNE REČI:** intoksikacija kobaltom i hromom, zamena kuka, metaloza



## SYSTEMIC COBALT AND CHROMIUM INTOXICATION AFTER TOTAL HIP REPLACEMENT

CLINICAL  
TOXICOLOGY

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**Introduction** It is well known that endoprosthesis wearers can experience the release of cobalt (Co) and chromium (Cr) ions from the metal parts of the endoprosthesis, especially in the case of prosthesis breakage, when the risk of metallosis arises. We present a patient who underwent a total hip replacement and developed systemic Co and Cr intoxication followed by serious clinical manifestations. A 50-year-old man had a total hip replacement due to avascular necrosis of the left hip. In the following five years, partial revision surgeries had to be performed due to two breakages of ceramic components. The patient began to complain of pain and discomfort in the inguinal and gluteal region with ataxic episodes, progressive loss of vision and hearing, and a constant feeling of fatigue. At first, the symptoms were not recognized as possible metal ion poisoning.

After extensive diagnostic procedures, blood Co and Cr tests were performed eventually and elevated ion levels were measured (133.9 µg/L and 40.3 µg/L, respectively). After the complete removal of an endoprosthesis, serum Co and Cr levels dropped significantly (1.5 µg/L and 4.2 µg/L, respectively), but his hearing and sight loss did not recover. Levels of serum Co and Cr ions may increase after hip replacement which could lead to systemic body manifestations. Routine blood tests are usually not performed, although it represents a convenient way to monitor the functionality of the prosthesis and the potential development of metallosis. Additionally, it could shorten the extensive diagnosis leading to more successful treatment.

**KEYWORDS:** cobalt and chromium intoxication, hip replacement, metallosis



## FATAL ACUTE SELF-POISONING WITH ONE PHOSTOXIN TABLET – A CASE REPORT

CLINICAL  
TOXICOLOGY

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Aluminum phosphide (AIP) is well known as an effective pesticide for grain preservation, but also as a suicidal agent due to its high human toxicity. Phosphine gas (PH<sub>3</sub>), which is released in contact with stomach acid, is responsible for systemic toxicity and high mortality. We report the first case of severe intentional AIP poisoning treated in our clinic with a fatal outcome and review the therapeutic modalities. A 35-year-old woman with a history of epilepsy was admitted to the University Clinic for Toxicology two hours after the intentional ingestion of a phostoxin (AIP) tablet. She presented with vomiting, abdominal pain, leukocytosis, ECG changes (prolonged PT interval, inverted T waves in D3, AVF, and left precordial leads), hypotension (80/40 mmHg, heart rate 120/min) and respiratory failure, after which she was transferred to the ICU.

Signs of hepatic lesion, rhabdomyolysis, renal failure and metabolic acidosis (pO<sub>2</sub> 9.6 kPa, pCO<sub>2</sub> 4.14 kPa, pH 7.15, bicarbonate 11 mmol/L, BE -15) were noted. Despite the application of mechanical ventilation, fluid supplementation and inotropic support, hemodynamic instability worsened. Cardiopulmonary resuscitation was performed three times, but unfortunately the patient had a fatal outcome on the fourth day of hospitalization. Freshly opened phostoxin tablets (3 g) are very toxic, and the dose of AIP they contain is sufficient to cause progressive life-threatening symptoms and a fatal outcome. In the absence of antidote, consensus on treatment and elucidated mechanisms of toxicity, the key to treatment is rapid decontamination and initiation of resuscitation measures.

**KEYWORDS:** aluminum phosphide, phosphine, toxicity, treatment protocols, outcome



## AKUTNO TROVANJE ZOLPIDEMOM I MOGUĆNOSTI ANALITIČKE POTVRDE

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Z-lekovi (zolpidem, zaleplon) su hipnotici koji se koriste u terapiji nesanice. U novije vreme učestalost akutnih trovanja zolpidemom, pojedinačna ili u kombinaciji sa alkoholom i benzodiazepinima, raste. Određivanje koncentracije zolpidema u serumu pacijenata sa sumnjom na akutno trovanje ovim lekom može se uspešno izvršiti metodom tačne hromatografije sa UV skenirajućim detektorom (HPLC/PDA). U radu je opisano akutno trovanje zolpidemom, pacijentkinje starosti 27 godina, koja je u cilju suicida popila 10 tableta Sanval® (zolpidem) od 10 mg u kombinaciji sa alkoholom, nakon čega je hospitalizovana u Centru za kontrolu trovanja VMA.

Pacijentkinja je na prijemu bila somnolentna, dizartična, eupnoična, normalno prebojene kože i sluzokože. Određivanje koncentracije zolpidema izvršeno je metodom tačne hromatografije sa UV skenirajućim detektorom (HPLC/PDA), a određivanje etanola metodom gasne hromatografije sa plameno-jonizacionim detektorom i "head space" injektorom. Toksikološko-hemijske analize krvi potvrdile su prisustvo zolpidema u koncentraciji od 1,26 mg/l i etanola u koncentraciji 1,06 mg/ml. Nakon primene suportivne terapije i stabilizacije opšteg stanja, pacijentkinja je otpuštena kući dva dana od prijema.

Detekcija i određivanje koncentracije zolpidema u serumu akutno otrovanih bolesnika je važna u dijagnostici, lečenju i praćenju toksičnih efekata. Metoda HPLC/PDA je specifična, osetljiva i relativno brza metoda za određivanje koncentracije zolpidema u serumu.

**KLJUČNE REČI:** zolpidem, akutno trovanje, HPLC/PDA



## ACUTE POISONING WITH ZOLPIDEM AND POSSIBILITIES OF ANALYTICAL CONFIRMATION

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Z-drugs (zolpidem, zaleplon) are hypnotics used in treatment of insomnia. Recently, frequency of acute zolpidem poisoning, either alone or in combination with alcohol and benzodiazepines, is increasing. Determination of zolpidem concentration in serum of patients with suspected acute poisoning with zolpidem can be successfully performed using liquid chromatography with ultraviolet scanning detector (HPLC/PDA). The paper describes the acute poisoning of a 27-year-old patient, with 10 tablets of Sanval® (zolpidem) of 10 mg in combination with alcohol in order to commit suicide, after which she was hospitalized in the Poison Control Center of the Military Medical Academy.

On admission, the patient was somnolent, dysarthric, eupnoic, with normally discolored skin and mucous membranes. Zolpidem concentration was determined using the method (HPLC/PDA), while determination of ethanol was performed using method of gas chromatography with a flame-ionization detector and "head space" injector. Toxicological and chemical blood analyzes confirmed the presence of zolpidem in a concentration of 1.26 mg/l and ethanol in a concentration of 1.06 mg/ml. After the application of supportive therapy and stabilization of the general condition, the patient was discharged home two days after admission. Detection and determination of zolpidem concentration in serum of acutely poisoned patients is important in diagnosis, treatment and monitoring of toxic effects. The HPLC/PDA method is a specific, sensitive and relatively fast method for determining the concentration of zolpidem in serum.

**KEYWORDS:** zolpidem, acute poisoning, HPLC/PDA



## RAZVOJ IMUNOTERAPIJE POREMEĆAJA UZROKOVANIH ZLOUPOTREBOM PSIHOAKTIVNIH SUPSTANCI

CLINICAL  
TOXICOLOGY

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Visoke stope mortaliteta i po život opasni poremećaji uzrokovani zloupotrebom psihoaktivnih supstanci (PAS) predstavljaju veliki teret za javno zdravlje. Primera radi, presek zloupotrebe PAS-i za 2021. godinu, među Evropljanima i globalnom populacijom od 15-64 godine, iznosi 31,3 odnosno 345 miliona; kao i da je 11,8 miliona smrtnih slučajeva godišnje udruženih sa zloupotrebom PAS-i, više od svih karcinoma zajedno. Ograničenja konvencionalne farmakoterapije PAS-udruženih poremećaja zdravlja podstiču uvođenje novih terapijskih pristupa, uključujući imunoterapiju [biološki lekovi, kao što su vakcine, monoklonska antitela (mAbs) i kompleksi haptena-nosača].

Osnovni mehanizam ove terapije je stimulacija B-limfocita vakcinisanih individua da proizvode Abs protiv PAS-antitela ili kompleksa PAS-haptena-nosača, sprečavajući prolazak kroz krvno-moždanu barijeru i efekte na centralni nervni sistem i druga tkiva. Ukupno registrovanih prekliničkih/kliničkih studija imunoterapije PAS-udruženih poremećaja zdravlja kod američkog Instituta za zdravlje (NIH) ima: opiodi [oksidon-1/1, heroin-1/0, morfijum-2/0, fentanil-2/0]; nikotin-4/6; kokain-5/4; metamfetamin-2/1; katinon-1/0; sintetički kanabinoidi-1/0; i ketamin-0/1. Samo su dve tekuće studije, planirane od decembra 2025, odnosno 2023: faza 1, za kokainsku dAd5GNE vakcinu (kokain haptena (GNE) konjugovan je sa proteinima kapsida adenovirusa (Ad) tipa 5); faza 1/2, za oksidon Oki(Gli)4-sKLH vakcinu (modifikovan oksidon je kovalentno vezan za protein-nosač hemocijanina (KLH)).

Uprkos mnogim obećavajućim rezultatima prekliničkim ispitivanjima, potrebni klinički zahtevi nisu dosegnuti. Stoga, nijedan biološki lek za PAS-udružene poremećaje nije odobren od strane američke agencije Administracije za hranu i lekove (FDA). Najsavremenije formulacije vakcina, novi biomarkeri (korisni za procenu kvaliteta, efikasnosti i bezbednosti bioloških lekova) i duboko razumevanje ove naučne oblasti su prerogativi za naprednu imunoterapiju PAS-udruženih poremećaja zdravlja, koja se uskoro očekuje.

**KLJUČNE REČI:** imunoterapija, psihoaktivne supstance, prekliničke/kliničke studije



## EMERGING IMMUNOTHERAPY OF PSYCHOACTIVE SUBSTANCES ABUSE ASSOCIATED DISORDERS

CLINICAL  
TOXICOLOGY

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High mortality rate and life-threatening psychoactive substance-associated disorders (PASADs) are a prodigious burden for public health. Thus, a cross-section on psychoactive substance (PAS) abuse for 2021, among Europeans and the global population aged 15-64, is 31.3 and 345 million, respectively; 11.8 million/year deaths are PASADs associated, more than all cancers combined.

The conventional PASADs pharmacotherapy limitations urge the introduction of novel therapeutic approaches, including immunotherapy [biological drugs, such as vaccines, monoclonal antibodies (mAbs) and hapten-carrier complexes]. The underlying mechanism compels B- lymphocytes of the vaccinated individuals to produce Abs against a PAS-antibody or PAS-hapten-carrier complex, preventing passage through the blood-brain barrier and the effects on the central nervous system and other tissues.

Totals of the pre-clinical/clinical trials registered by National Institute of Health, USA, respectively are: opioids [oxycodone-1/1, heroin-1/0, morphine-2/0, fentanyl-2/0]; nicotine-4/6; cocaine-5/4; methamphetamine-2/1; cathinone-1/0; synthetic cannabinoids-1/0; and ketamine-0/1. Underway are just two studies, planned to be completed by December 2025 and 2023, respectively: phase 1 for cocaine dAd5GNE vaccine (cocaine hapten (GNE) conjugated to adenovirus (Ad) type 5 capsid proteins); phase 1/2 for oxycodone Oxy(Gly)4-sKLH vaccine (comprised of the modifying oxycodone covalently bond to the keyhole limpet hemocyanin (KLH) carrier protein).

Despite many promising pre-clinical trial results, desired clinical endpoints have not encountered. In addition, none of PASAD biologics is approved by the Food and Drug Administration. State-of-the-art vaccine formulations, novel biomarkers (useful to evaluate the quality, effectiveness and safety of the biologics) and the profound understandings are prerogatives for advanced PASADs immunotherapy, awaited soon.

**KEYWORDS:** immunotherapy, psychoactive substances, preclinical/clinical studies



## FAKTORI KOJI UTIČU NA ELIMINACIJU, KLINIČKU SLIKU I ISHOD AKUTNE INTOKSIKACIJE BENZODIAZEPINIMA KOD PACIJENATA STARIJE ŽIVOTNE DOBI

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Benzodiazepini su najčešće korišćeni lekovi u slučaju akutnih samotrovanja. Cilj rada je analiza faktora koji utiču na proces eliminacije benzodiazepina, težinu kliničke slike, razvoj komplikacija i ishod akutne intoksikacije kod osoba starije životne dobi. Sprovedena je klinička opservaciona kohortna studija. Ukupno je analizirano 95 bolesnika hospitalno lečenih u Klinici za toksikologiju Vojnomedicinske akademije. Podeljeni su u tri grupe u odnosu na životnu dob (18-40 godina, 41-65 godina, stariji od 65) i praćeni su u skladu sa kliničkom slikom i detektovanim toksičnim koncentracijama minimalno 48 sati.

Najčešće su intoksikacije bromazepamom, a poređenjem srednjih koncentracija bromazepama na prijemu među različitim dobnim grupama, u grupi starijih od 65 godina one su bile više, bez statističke značajnosti u odnosu na mlađe dobne grupe. Eliminacija benzodiazepina je analizirana poređenjem koncentracija lekova na prijemu i određivanjem procentulanog smanjenja koncentracije posle 24 i 48 sati.

Dominantan klinički znak u akutnim intoksikacijama benzodiazepinima je poremećaj stanja svesti različitog stepena procenjivan pomoću Glasgow Coma Scale (GCS). Procena težine trovanja vršena je u skladu sa Skalom težine trovanja (engl. Poisoning Severity Score, PSS). Pokazano je da je kod osoba starije životne dobi u poređenju sa drugim starosnim grupama metabolički kapacitet manji, a renalna eliminacija sporija. Kod starijih od 65 godina uočena je i češća hipoalbuminemija što doprinosi dužem održavanju toksičnih koncentracija benzodiazepina. Kod bolesnika starijih od 65 godina zabeležena je teža klinička slika (dugotrajniji poremećaji svesti, češći kardiovaskularni poremećaji), duža hospitalizacija, veća potreba za primenom antidota, češće komplikacije (aspiraciona pneumonija i rabdomioliza), oporavak sporiji, a letalitet veći.

**KLJUČNE REČI:** intoksikacije, benzodiazepini, stare osobe





## FACTORS AFFECTING BENZODIAZEPINES ELIMINATION, CLINICAL FEATURES AND OUTCOME IN ELDERLY PEOPLE ACUTELY POISONED WITH BENZODIAZEPINES

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Benzodiazepines are the most frequent drugs used in cases of deliberate self-poisoning. The aim is to analyze the factors affecting the elimination process of benzodiazepines, severity of clinical features, complications and acute poisoning outcome in elderly patients. Clinical observation cohort study was made including 95 patients hospitalized in the Department of Clinical Toxicology in Belgrade. Patients were divided into three groups according to their age (18-40; 41-65; ≥65 years old). Patients were monitored in accordance with the clinical features as well as the detected toxic concentrations of benzodiazepines in 48-hour period.

The most common ingested benzodiazepine was bromazepam. Comparing the mean concentration of bromazepam among different age groups, drug concentration is slightly higher in group of elderly people, but not statistically important compared to younger age groups. Drug elimination was analyzed by comparing its concentration after 24 and 48 hours. Dominant clinical characteristic of an acute benzodiazepine intoxication is different level of consciousness disorder estimated by Glasgow Coma Scale (GCS). Evaluation of the poisoning was performed in accordance with the Poisoning Severity Score (PSS). The study shows that metabolic capacity decreases and renal elimination is slower in elderly people compared to other age groups. More common hypoalbuminemia is also observed in group of elderly people, contributing to extended maintenance of toxic concentrations of benzodiazepines. Severe clinical features is observed in elderly population- deeper and prolonged disorders of consciousness, more common cardiovascular disorder, longer hospitalization, increased need for antidotes, frequent complications (aspiration bronchopneumonia and rhabdomyolysis), slower recovery and higher lethality.

**KEYWORDS:** self-poisoning, benzodiazepines, elderly



## UPOTREBA KANABISA I AMFETAMINA KOD PACIJENATA NA PROGRAMU ODRŽAVANJA BUPRENORFINOM

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Zloupotreba više droga je poznat problem među pacijentima na održavanju opioidne terapije. Cilj rada je proceniti profil pacijenata na programu održavanja buprenorfinom koji paralelno koriste kanabis, amfetamin ili kanabis i amfetamin. Ova studija je sprovedena 2021. godine na Univerzitetskoj klinici za toksikologiju u Skoplju i ima prospektivan dizajn. Studija je analizirala uzorak od 112 pacijenata sa zavisnošću od opioida na programu održavanja buprenorfinom. Podaci su prikupljeni u skladu sa prethodno dizajniranim upitnikom koji je prilagođen prema "treatment demand indicator". Analizirane su sledeće varijable: pol, starost, trajanje upotrebe opioida, način primene, trajanje lečenja buprenorfinom, doza buprenorfina, upotreba kanabisa i amfetamina.

Većina pacijenata bili su pripadnici muškog pola (88,4%). Prosečna starost pacijenata bila je 38,7±4,3 godine. Prosečno trajanje upotrebe opioida bilo je 16,8±6,8 godina; trajanje terapije buprenorfinom je bilo 6,3±3,3 godina; doza buprenorfina je bila 12,5±4,9 mg. Paralelnu upotrebu buprenorfina sa kanabisom izjavilo je 43,7% pacijenata i bili su značajno mlađi u odnosu na ostale ( $p=0,014$ ). Kombinaciju buprenorfina sa amfetaminom koristilo je 14,3%, značajno mlađe starosti u odnosu na ostale ( $p=0,003$ ). Buprenorfin sa kanabisom i amfetaminom praktikovalo je 15,2% pacijenata. Nije pronađena značajna korelacija između ostalih varijabli ispitivane grupe i paralelne upotrebe kanabisa i amfetamina.

Skoro polovina pacijenata na supstitucionoj terapiji buprenorfinom prijavila je paralelnu upotrebu kanabisa. Pacijenti koji su se izjasnili o paralelnoj upotrebi kanabisa i amfetamina bili su mlađi u odnosu na druge.

**KLJUČNE REČI:** buprenorfin, kanabis, amfetamin



## CANNABIS AND AMPHETAMINE USE IN PATIENTS ON BUPRENORPHINE MAINTENANCE TREATMENT

CLINICAL  
TOXICOLOGY

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Polydrug abuse is a known problem among patients on opioid maintenance treatment. Objective is to assess the profile of patients on buprenorphine substitution treatment who use cannabis, amphetamine or cannabis and amphetamine in parallel. This study was conducted in 2021 year at University Clinic for toxicology, Skopje and has a prospective cohort design that included 112 patients with opioid use disorder on buprenorphine maintenance treatment.

The data were collected according to a previously designed self-administered questionnaire adapted according to the “treatment demand indicator”. The following variables were analyzed: gender, age, duration of opioid use disorder, route of administration, duration of buprenorphine treatment, buprenorphine dose, cannabis and amphetamine use. The majority of patients were male (88.4%). The mean age of patients was 38.7±4.3 years. The average duration of opioid use disorder was 16.8±6.8 years; the duration of buprenorphine treatment was 6.3±3.3 years; buprenorphine dose was 12.5±4.9mg. Parallel use of buprenorphine with cannabis was declared in 43.7% of the patients and they were significantly younger compared to others ( $p=0.014$ ). A combination of buprenorphine with amphetamine was used in 14.3% with significantly younger ages compared to others ( $p=0.003$ ). Buprenorphine with both cannabis and amphetamine was practiced in 15.2% of the patients. No significant correlation was found between other variables of the studied group and the parallel use of cannabis and amphetamine. Almost half of the patients on buprenorphine substitution treatment reported parallel use of cannabis. The patients who declared parallel use of cannabis and amphetamines were of younger age compared to others.

**KEYWORDS:** buprenorphine, cannabis, amphetamine



## KONTROLA VAZDUŠNIH PUTEVA I VENTILACIJE KOD BOLESNIKA SA AKUTNIM TROVANJEM

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Mali broj studija analizira ventilatornu podršku u akutnim trovanjima. Cilj ovog rada je: analiza indikacija za respiratornu podršku, najprikladniji modaliteti i sedacija, intubacija i dalje sprovođenje mehaničke ventilacije (MV) u akutnim trovanjima. Sprovedena je jednogodišnja retrospektivna analiza istorija bolesti akutno otrovanih pacijenata. MV je bila neophodna 10.5% (80 od 760) bolesnika. Iako su intubacija i MV indikovane kod GCS<8, kod pacijenata otrovanih psihoaktivnim lekovima, pogotovu benzodiazepinima, u 80% slučajeva nije bilo potrebe za intubacijom. Slabost respiratorne muskulature je najčešće bila razlog za intubaciju i višednevnu MV u trovanjima organofosfornim jedinjenjima. ARDS i akutno oštećenje pluća koje je zahtevalo MV, najčešće prilikom predoziranja opijatima je bilo kratko (1-2 dana). Hemodinamska slabost je, nezavisno od vrste agensa, takođe bila česta indikacija za primenu MV.

Parametri za monitoring MV kod pacijenata sa akutnim trovanjem su isti kao u drugim stanjima. Najčešće inicijalno upotrebljavan modalitet je kontrolisana MV sa početnim FiO<sub>2</sub> 0,5 i Tidal volumenom 7,5 mg/kg, potom asistirana kontrolisana ventilacija. Potreba za sedacijom prilikom intubacije pacijenata sa akutnim trovanjem u komi je relativna, zavisi od rizika od aspiracije. Potreba za održavanjem sedacije nije jasno definisana, pogotovo ako se radi o komi prouzrokovanoj psihoaktivnim lekovima. Može biti neophodna da olakša adaptaciju na ventilator, kod nepotpuno budnih ili agitiranih pacijenata. Kontrola vazdušnih puteva je jedna od glavnih mera urgentne reanimacije u lečenju akutnih trovanja. Iako je neophodno pridržavati se opštih principa sprovođenja intubacije i MV, treba imati u vidu i specifičnosti intoksikacija, prvenstveno kada se radi o poremećaju svesti kao indikaciji, te potrebi za dodatnom sedacijom ovih bolesnika.

**KLJUČNE REČI:** akutna trovanja, intubacija, mehanička ventilacija



## NEEDS OF ANALYSIS FOR INTUBATION AND MECHANICAL VENTILATION IN PATIENTS WITH ACUTE POISONING

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A small number of studies are focused on ventilator support in acute poisonings. The aim of this paper was: analyzing the indications for respiratory support, the most appropriate modalities and sedation, intubation and further implementation of MV in acute poisonings. Retrospectively were analyzed data from medical records of acutely poisoned patients in one year period. MV was necessary in 10.5% (80/760) patients. Although intubation and MV are indicated in patients with GCS <8, in patients poisoned with psychotropic drugs, particularly benzodiazepines, in most of cases there was no need for intubation. The weakness of the respiratory muscles is usually a reason for intubation and several daily MV of organophosphate poisoning.

Adult respiratory distress syndrome and acute lung injury due to which it was necessary MV are most frequent during opiate overdose, and duration of MV was short. Hemodynamic weakness is, independent from the type of the agent, also a frequent indication for usage of MV. Parameters for monitoring of MV in patients with acute poisoning are the same as in other conditions. The most commonly initiated usage mode is controlled MV FIO<sub>2</sub> 0.5 and Tidal volume of 7.5 mg / kg, followed by a controlled-assisted ventilation. The need for sedation during intubation of patients with acute poisoning in a coma is relative, depending on the risk of aspiration.

The need for maintenance of sedation is not clearly defined, especially in the case of coma caused by psychoactive medication. It may be necessary to facilitate the adaptation of the ventilator, particularly in incomplete awake or agitated patients. Securing of airways is one of the main measures of urgent resuscitation in the treatment of acute poisoning. Although it is necessary to follow the general principles of the intubation and MV, we should bear in mind the specifics of intoxication, especially when it is about consciousness disorder as an indication, as well as the need for additional sedation in these patients.

**KEYWORDS:** acute poisoning, intubation, mechanical ventilation



## PSYCHOTROPIC DRUGS MODULATE THE RETINOL TO RBP4 RATIO AND PARAOXONASE 1 ACTIVITIES IN SERUM OF PARTICIPANTS OF THE DR. ESQUERDO COHORT

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Psychotropic drugs (PsyD), as like antidepressants and mood-stabilizers, are prescribed in severe mental illness (e.g. schizophrenia or major depression) producing metabolic impairment and increasing the risk of diseases. The retinoid system (RS) is involved in the regulation of the energy homeostasis. The paraoxonase 1 (PON1), an antioxidant enzyme, have been shown to be compromised in cases suffering cardiometabolic diseases.

The aims of this study were 1) to measure retinol and its transporter (RBP4) along with four PON1 activities in serum of patients of the Dr. Esquerdo Psychiatric Hospital in Spain and 2) to assess the associations between the mentioned variables with PsyD doses by pharmacological group. Serum samples were extracted from 80 volunteers (34 women). Clinical, pharmacological, and socio-demographic data were acquired from the clinical histories. Retinol levels were quantified by HPLC and those of RBP4 by ELISA. Dihydrocoumarin, 4-(chloromethyl)phenyl acetate (CMPA) and phenyl acetate (PA) in low and high salt conditions were used as substrates to determinate the following PON1 activities: lactonase, CMPase, PALSase and PAHSase spectrophotometrically. PAHSase and CMPase were used to infer the Q192R functional genotype of PON1. Mood-stabilizers dose were positive related with the RBP4/ROH ratio ( $p=0.031$ ). The most frequent Q192R genotype was QQ (46%). CMPase ( $p=0.021$ ) and PAHSase ( $p=0.023$ ) were positive associated with antidepressant dose. The treatment with PsyD could affect both the RS and PON1 activities, which might be involved in the mechanism of the metabolic adverse effects.

**KEYWORDS:** mental illness, psychotropic drugs, metabolic disease, paraoxonase 1, retinoid system



## IMMOBILIZED ENZYMES AS A COST-EFFECTIVE AND SUSTAINABLE APPROACH IN THE STUDY OF COMPOUND TOXICITY

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The vast majority (~95%) of molecular targets of currently known xenobiotics (e.g. toxins or drugs) are proteins (e.g. enzymes). It is therefore essential to test the influence of these compounds on target protein function. The main challenge when working with proteins is their cost, limited stability, possible interference with analytical methods, and inability to recycle. In this project, human recombinant forms of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), monoamine oxidase A (MAO-A), and monoamine oxidase B (MAO-B) were immobilized on the surface of magnetic microparticles in order to overcome these limitations.

The recombinant form of human cholinesterases and monoamine oxidases were immobilized on the surface of Dynabeads™ His-Tag magnetic non-porous microparticles by a non-covalent bond using IMAC technology. The resulting biocatalysts were analysed and compared with free enzymes in terms of preservation of enzyme activity, stability, kinetic parameters, and interaction with known inhibitors (reversible, quasi-irreversible, and irreversible). For this purpose, the Ellman assay and kynuramine assay were adapted for a 96-well plate. In conclusion, by attaching these enzymes to magnetic solid support, a powerful, cost-effective, stable, recyclable, and easy-to-use tool for studying enzyme kinetics, inhibition mechanism and reactivation of OP-inhibited ChE has emerged.

**KEYWORDS:** immobilization, magnetic microparticles, IMAC, cholinesterases, monoamine oxidases

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## NEUTROPENIJA KOD AKUTNE INTOKSIKACIJE OLANZAPINOM

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Prikazali smo slučaj neutropenije kod akutne intoksikacije olanzapinom. Pacijent u dobi od 18 godina, primljen u Kliniku za toksikologiju zbog samotrovanja olanzapinom, nekoliko sati nakon ingestije oko 300 mg olanzapina. Pacijent nije ranije lečen psihijatrijski. Na prijemu komatozan, afebrilan, tahipnoičan, tahikardičan, frekvence do 170/min, TA: 110/50 mmHg, sO<sub>2</sub> 87%, zenice miotične, prisutna hipersalivacija. Odmah na prijemu intubiran, spontanog disanja.

U EKG se detektuje sinusna tahikardija frekvence 150/min. U analizama WBC  $4,2 \times 10^9$ , CK 4252 U/l, uz normalne parametre bubrežne funkcije i uredne elektrolite. Koncentracija olanzapina na prijemu je 1,10 mg/l (toksične preko 0,15 mg/l). Radiografija pluća ukazuje na postojanje konsolidacije donjeg i srednjeg plućnog polja levo. U terapiji pacijent dobijao infuzione rastvore, niskomolekularni heparin, benzo-diazepine, bikarbonate i antibiotsku terapiju (ceftriakson, metronidazol). Drugog dana hospitalizacije, se javlja febrilnost uz leukopeniju, WBC  $1,39 \times 10^9$ , neutrofili  $0,86 \times 10^9$ . Ordiniran granulostimulisujući factor rasta (G-CSF), korigovana antibiotska terapija, uvedeni vankomicin i meropenem.

Leukociti se normalizuju narednog dana (WBC  $9,60 \times 10^9$ , neutrofili  $8,64 \times 10^9$ ). Održavaju se visoke vrednosti olanzapina, drugog dana 0,82 mg/l, trećeg 0,76 mg/l. Pacijent u potpunosti oporavlja stanje svesti te je ekstubiran trećeg dana. Takođe, dolazi do regresije inflamatornih promena na plućima pa se pacijent petog dana hospitalizacije otpušta na lični zahtev i upućuje na dalje psihijatrijsko lečenje. Neutropenija indukovana olanzapinom je redak, potencijalno opasan neželjeni efekat u terapiji olanzapinom, retko opisan kod akutne intoksikacije. U našem slučaju, ovaj efekat je imao verovatno dozno zavisnu komponentu i naglašava važnost praćenja leukocita kod akutnog trovanja. Povišena vrednost CK ukazuje na miotoksičnost olanzapina.

**KLJUČNE REČI:** olanzapin, neutropenija, prikaz slučaja





## OLANZAPINE OVERDOSE INDUCED NEUTROPENIA

CLINICAL  
TOXICOLOGY

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We report a case of neutropenia in acute olanzapine overdose. A 18-years-old male, was admitted to the Clinic for self poisoning with olanzapine, a few hours after ingesting about 300mg of olanzapine. The patient has not been previously treated psychiatrically. On admission, comatose, afebrile, tachypneic, tachycardic, heart rate up to 170/min, BP: 110/50 mmHg, sO<sub>2</sub> 87%, pupils miotic, present hypersalivation. Immediately on admission, intubated. In ECG detected sinus tachycardia with a frequency of 150/min. In the blood analyses, WBC  $4.2 \times 10^9$ , CK 4252 U/l, with normal parameters of renal function and electrolytes.

The concentration of olanzapine on admission is 1.10 mg/l. Radiography of the lungs indicates the existence of consolidation of the lower and middle lung fields on the left. Therapy included fluids, LMWH, benzodiazepines, bicarbonates and antibiotic therapy (ceftriaxone, metronidazole).

Second day of hospitalization, he was febrile with leukopenia, WBC  $1.39 \times 10^9$ , neutrophils  $0.86 \times 10^9$ . Granulocyte colony-stimulating factor (G-CSF) was administered and corrected antibiotic therapy (vankomycin, meropenem). Leukocytes are normalized the next day (WBC  $9.60 \times 10^9$ , neutrophils  $8.64 \times 10^9$ ). High values of olanzapine are maintained, on the second day 0.82 mg/l, on the third 0.76 mg/l. The patient fully recovers of consciousness and was extubated on the third day. Also, there is a regression of inflammatory changes in the lungs, so the patient is discharged on the fifth day of hospitalization at his own request and referred to further psychiatric treatment. Olanzapine-induced neutropenia is a rare, potentially dangerous side effect in olanzapine therapy, rarely described in acute intoxication. In our case, this effect had a probably dose-dependent component and highlights the importance of leukocyte monitoring in acute poisoning. An elevated CK value indicates myotoxicity of olanzapine.

**KEYWORDS:** olanzapine, neutropenia, case report



## UČESTALOST SAOBRAĆAJNIH NESREĆA POD DEJSTVOM PSIHOAKTIVNIH SUPSTANCI NA TERITORIJI BEOGRADA

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Korišćenje psihoaktivnih supstanci (PAS) koje ispoljavaju efekte na centralni nervni sistem utiče na sposobnost vozača da upravlja vozilom i dovodi do povećanja rizika od saobraćajnih nezgoda (SN). Pored testiranja učesnika SN na prisustvo alkohola u krvi, u Nacionalnom centru za kontrolu trovanja (NCKT) se vrši testiranje učesnika SN i na prisustvo PAS (heroin, kokain, jedinjenja amfetaminske strukture, kanabinoidi). Cilj rada je prikazati učestalost zloupotrebe PAS kod učesnika SN registrovanih u NCKT u 2021 i 2022. godini. U istraživanju su korišćeni podaci NCKT dobijeni analizom urina na prisustvo PAS, nakon intervencije saobraćajne policije. Detekcija PAS vršena je primenom imunohromatografskih test traka, a potvrda prisustva primenom tačne hromatografije sa UV skenirajućim i maseno-spektrometrijskim detektorom. U 2021 i 2022. godini, skrining na PAS bio je zahtevan za 103 učesnika SN (55 u 2021. godini i 48 u 2022. godini). Oko 60% analiziranih uzoraka bilo je negativno na prisustvo PAS.

Psihostimulansi, kokain i jedinjenja amfetaminske strukture, potvrđeni su u 18% analiza. Posmatrano pojedinačno, najčešće korišćena PAS bila je marihuana u 19% slučajeva i kokain u 12% slučajeva, Registrovan je po jedan slučaj korišćenja MDMA, metadona i buprenorfina. Upotreba samo jedne PAS bila je zabeležena kod 31 ispitanika, dok je kod 4 ispitanika zabeležena kombinacija kanabinoida sa kokainom (3 slučaja) i amfetaminom (1 slučaj). Testiranje učesnika SN na prisustvo PAS od strane saobraćajne policije se vrši kada postoji sumnja na njihovo korišćenje. Prikazani podaci ukazuju na usku povezanost vožnje pod dejstvom PAS i saobraćajnih nezgoda.

**KLJUČNE REČI:** psihoaktivne supstance, saobraćajne nezgode, vožnja pod dejstvom PAS



## FREQUENCY OF TRAFFIC ACCIDENTS UNDER THE INFLUENCE OF PSYCHOACTIVE SUBSTANCES IN THE TERRITORY OF BELGRADE

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Use of psychoactive substances (PAS) that have effects on central nervous system and affect the driver's ability to drive and leads to risk increase of traffic accidents (TA). Besides testing TA participants for alcohol presence in their blood, National Poison Control Center (NPCC) also tests TA participants for presence of PAS (heroin, cocaine, compounds of amphetamine structure, cannabinoids). Objective was to show frequency of PAS abuse in traffic accidents (TA) participants, registered in NPCC in 2021 and 2022. This research used NPCC data obtained after urine analysis for PAS presence, after traffic police intervention.

Detection of PAS presence was performed using immuno-chromatographic test strips, and confirmation was performed using a liquid chromatography with UV scanning and mass spectrometric detector. In 2021 and 2022, PAS screening was required at 103 TA participants (55 in 2021 and 48 in 2022). About 60% of analyzing samples were negative on PAS. Psychostimulants, cocaine and amphetamines confirmed in 18% of samples. Considered individually, most frequently used PAS was marijuana in 19% of cases, followed by cocaine in 12% of cases. One case of using MDMA, methadone and buprenorphine was registered. The use of only one PAS was recorded in 31 respondents, while in four respondents, combination of cannabinoids and cocaine (three cases) and amphetamine (one case) was recorded. Testing of TA participants for the presence of PAS by the traffic police is carried out when there is suspicion of their use. The presented data indicate a close connection between driving under influence of PAS and road traffic accidents

**KEY WORDS:** psychoactive substances, traffic accidents, driving under the influence of PAS



## NEOBIČAN TOK TROVANJA KOKAINOM SA SMRTNOM ISHODOM: PRIKAZ SLUČAJA

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Od Evropskog monitoring centra za droga i zavisnosti od droga dobijaju se podaci o nekoliko hiljada hitnih slučajeva akutnog trovanja kokainom godišnje, što predstavlja značajno opterećenje za zdravstveni sistem. Najčešću kliničku sliku akutne intoksikacije kokainom karakteriše prekomerna adrenergička aktivnost uključujući tahidisritmiju, hipertenziju, koronarni vazospazam, ekstremnu dijaforezu i hipertermiju koje dovode do akutnog koronarnog sindroma, moždanog udara i smrti. Epileptični napadi se mogu javiti u roku od nekoliko sekundi, minuta ili sati nakon uzimanja kokaina, usled sniženog praga nadražaja neurona.

Osobe starije životne dobi i one sa postojećim srčanim oboljenjima su im obično sklonije, ali se mogu javiti čak i kod nekoga ko nije imao ličnu anamnezu pozitivnu za epilepsiju ili prethodne probleme sa srcem. Mladić star 29 godina doveden je kolima hitne pomoći u Kliniku za urgentnu i kliničku toksikologiju NCKT potpuno svestan, komunikativan, eupnoičan, sa midrijazom. Sam je dao podatak da je uzeo kokain ušmrkavanjem, posle kojeg je usledilo kratkotrajno paranoično ponašanje. Po dolasku GKS je bio 15, arterijski krvni pritisak 135/75 mmHg, respiratorna frekvencija 12/min, srčana frekvencija 95/min, SatO<sub>2</sub> 97%. Kokain, benzoilekgonin i levamisol su potvrđeni u krvi pomoću LC-MS. Grand mal napad, koji se desio deset minuta po dolasku u prijemnu ambulantu, uspešno je kupiran diazepamom. Nekoliko minuta kasnije dolazi do razvoja epileptičnog statusa, posle čega je usledio kardiorespiratorni zastoj. Pacijent je intubiran, reanimiran tokom 40 min, ali reanimacija nije bila uspešna. Kardiovaskularni i cerebrovaskularni poremećaji, kao i neurološka oštećenja su najčešći neželjeni efekti povezani sa intoksikacijom kokainom. Prisustvo kokaina i njegovog metabolita u biološkom materijalu sugerise da je pacijent koristio kokain. Čak i kada nema znakova prekomerne adrenergičke aktivnosti, lekari hitne pomoći treba da budu svesni mogućih zdravstvenih posledica povezanih sa kokainom.

**KLJUČNE REČI:** kokain, konvulzije, epileptični status, smrtnost



## UNUSUAL COURSE OF COCAINE POISONING WITH FATAL OUTCOME: A CASE REPORT

CLINICAL  
TOXICOLOGY

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The European Monitoring System for Drugs and Drug Addiction reports several thousands of cocaine-related emergencies per year, representing a significant burden on services. The most common presentation of acute toxicity is characterized by adrenergic overactivity including tachydysrhythmia, hypertension, coronary vasospasm, extreme diaphoresis and hyperthermia leading to acute coronary syndrome, stroke and death. Seizures can occur within seconds, minutes or hours after taking cocaine, due to a lowered threshold of neuronal stimulation. Usually, older adults and those with pre-conditioned heart problems are more prone to it, however they can occur even in someone with no history of seizures or previous heart problems.

A 29-year old male arrived by ambulance at the Clinic of Emergency and Clinical Toxicology NPCC fully conscious, communicative, euphoric, with mydriasis. He self-reported cocaine snorting, followed by short-term paranoid thinking. His initial Glasgow Coma Score (GCS) was 15, arterial blood pressure 135/75 mmHg, respiratory rate 12/min, heart rate 95/min, SatO<sub>2</sub> 97%. Cocaine, benzoylecgonine and levamisole were confirmed in blood by LC-MS. Grand mal seizure, occurring ten minutes after arrival to the emergency room, was successfully treated by diazepam. A couple of minutes later, a series of convulsions developed, followed by cardiorespiratory arrest. The patient was intubated, reanimated for 40 min, but resuscitation was unsuccessful. Cardiovascular and cerebrovascular disorders and neurologic impairments are the most common adverse effects associated with cocaine intoxication. The presence of cocaine and its metabolite in biological samples suggested that the man was a cocaine user. Even when no signs of adrenergic overactivity are present, emergency physicians should be aware of possible cocaine-related health consequences.

**KEYWORDS:** cocaine, seizures, status epilepticus, mortality



## SVEST I STAVOVI BUDUĆIH ZDRAVSTVENIH RADNIKA U SRBIJI O NOVIM PSIHOAKTIVNIM SUPSTANCAMA

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Znanje zdravstvenih radnika (ZR) o novim psihoaktivnim supstancama (NPAS) predstavlja kamen temeljac za inicijative kliničkih toksikologa u suočavanju sa ovim novim svetskim fenomenom. Rezultati nedavno objavljenih studija ukazuju na neadekvatno osnovno znanje i veštine ZR neophodnih za uspešno lečenje pacijenata koji koriste NPAS. Istraživanje je sprovedeno kako bi se procenila svest (znanje) i stavovi budućih ZR o NPAS u kontekstu njihove nadolazeće uloge u prevenciji i lečenju predoziranja i zavisnosti od NPAS. Takođe, analizirani su i svi parametri koji stupaju u korelaciju sa percepcijom i korišćenjem NPAS kod budućih ZR. U studiji preseka, koja je sprovedena 2017. godine, učestvovalo je 490 studenata medicine, farmacije i stomatologije Medicinskog fakulteta Univerziteta u Novom Sadu. Svest o NPAS je bila bolja kod studenata farmacije (IRR: 1,926, CI: 1,173-3,163, p=0,010) nego kod studenata medicine – studenti farmacije su prepoznali čak 92,6% više NPAS imena od svojih vršnjaka koji studiraju medicinu.

Studentkinje su znale 36,5% manje NPAS imena od svojih muških kolega (IRR: 0,635, CI: 0,399-1,013, p=0,049). Broj NPAS imena koje su učesnici studije znali rastao je za 15,9% sa svakom godinom uzrasta – što je starost studenata bila veća, znali su više o NPAS (IRR: 1,159, CI: 1,025-1,310, p=0,018). Studenti koji su koristili marihuana znali su 52,6% više NPAS imena od onih koji nikada nisu imali iskustva sa kanabisom (IRR: 1,526, CI: 0,953-2,445, p=0,049). Iako je veliki broj budućih ZR tvrdio da zna šta su NPAS, uočene su brojne zablude. Neophodni su dalji obrazovni naponi kako bi se poboljšalo njihovo znanje i stavovi u vezi sa NPAS.

**KLJUČNE REČI:** nove psihoaktivne supstance, svest, stavovi, studenti, zdravstveni radnici



## AWARENESS AND ATTITUDES OF FUTURE HEALTH CARE PROFESSIONALS IN SERBIA TOWARDS NEW PSYCHOACTIVE SUBSTANCES

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Health care provider awareness is recognized as a headstone of clinical toxicology initiatives in taking up the upcoming threat presented by new psychoactive substances (NPS). Recently published studies have underlined the inadequacy in essential knowledge and skills required by health care professionals (HCPs) to successfully treat patients overdosed by NPS. The study was conducted in order to evaluate prospective HCPs awareness and attitudes regarding NPS in the context of their future role in prevention and treatment of NPS overdose and addiction. Correlates of NPS perception and use were also examined.

This cross-sectional survey was performed on 490 students of the Faculty of Medicine, Novi Sad, Serbia, during 2017. NPS awareness was better in pharmacy students (IRR: 1.926, CI: 1.173-3.163,  $p=0.010$ ) than in medicine students – pharmacy students recognized 92.6% more NPS names than their peers studying medicine. Female students knew 36.5% less NPS names than their male colleagues (IRR: 0.635, CI: 0.399-1.013,  $p=0.049$ ). Number of NPS names students know was rising 15.9% with each age year—the higher the age, the larger the number of NPS they were aware of (IRR: 1.159, CI: 1.025-1.310,  $p=0.018$ ). Students who had used marijuana knew 52.6% more NPS names than those who had never had experience with cannabis (IRR: 1.526, CI: 0.953-2.445,  $p=0.049$ ). Although a high number of future HCPs claimed to know what NPS are, numerous misconceptions were noticed. Further educational efforts are necessary to improve their awareness and attitudes regarding NPS.

**KEYWORDS:** new psychoactive substances, awareness, attitudes, students, health care professionals



## SUPSTANCE ZLOUPOTREBE – PREZENTACIJE U TOKSIKOLOŠKOJ AMBULANTI NACIONALNOG CENTRA ZA KONTROLU TROVANJA

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Bolnički podaci o hitnim slučajevima pružaju jedinstven uvid u akutne zdravstvene štete povezane sa zloupotrebom droga. Od 2021. godine Nacionalni centar za kontrolu trovanja je deo mreže EURO-DEN Plus koja prikuplja podatke o prezentacijama vezanim za toksičnost supstanci zloupotrebe u toksikološkoj ambulanti. Podaci su izvučeni iz baze podataka Nacionalnog centra za kontrolu trovanja za sve prezentacije zloupotrebe droga od 1. januara do 31. decembra 2021. Za svaku supstancu zloupotrebe registrovana je distribucija po polu, životnoj dobi i težini trovanja prema Poisoning Severity Score (PSS). Od 4324 prezentacije u urgentnoj službi NCKT, 339 se odnosilo na zloupotrebu droga. Heroin je registrovan kod 34,2% pacijenata, zatim amfetamini (18,2%), kanabis (16,5%), metadon (12,1%), kokain (8,8%), GHB (2,6%), buprenorfin (5,3%) i MDMA (1,2%).

Prezentacije u vezi sa zloupotrebom droga bile su najčešće u dobnim grupama 30-65 (60,2%) i 25-29 (16,5%), a zatim kod mladih pacijenata (20-24 godine) (13,3%). Međutim, 7,6% je bilo mlađe od 18 godina. Preovladavali su muškarci (81,8% prema 18,2%). Umerena i teška trovanja registrovana su kod 41,9% pacijenata, ali nije bilo smrtnih slučajeva. Iako je samo manjina pacijenata primljena u bolnicu (5,6%), oni su zahtevali lečenje u Odeljenju za intenzivno lečenje NCKT. Predoziranja supstancama zloupotrebe predstavljaju značajno opterećenje za hitne zdravstvene službe. Najzastupljeniji su heroin i sintetički stimulanasi, a tri četvrtine su bili muškarci. Iako su prezentacije bile najčešće među onima starosti 35-60 godina, pacijenti mlađi od 24 godine su i dalje u značajnom broju (20,9%). Predoziranje supstancama zloupotrebe zahteva brzu dijagnostiku i često specifične terapijske mere i lečenje u jedinici intenzivne nege, posebno u slučaju predoziranja opioidima.

**KLJUČNE REČI:** droge, predoziranje, hitna služba





## DRUGS OF ABUSE - RELATED PRESENTATIONS TO THE EMERGENCY DEPARTMENT OF THE NATIONAL POISON CONTROL CENTRE

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Hospital emergency data provide unique insight into acute health harms related to drugs of abuse. Since 2021 the National Poison Control Centre is a part of the EURO-DEN Plus network which collects data on emergency presentations with drugs of abuse toxicity. Data were extracted from the National Poison Control Centre database for all drugs of abuse presentations from 1 January to 31 December 2021. For each drug of abuse, the distribution of sex, age group, severity of poisoning according to the Poisoning Severity Score (PSS) was assessed. Out of 4324 NPCC emergency department presentations, 339 were related to drugs of abuse.

Heroin was registered in 34.2% of patients, followed by amphetamines (18.2%), cannabis (16.5%), methadone (12.1%), cocaine (8.8%), GHB (2.6%), buprenorphine (5.3%), and MDMA (1.2%). Drugs of abuse - related presentations were the most frequent in age groups 30-65 (60.2%) and 25-29 (16.5%), followed by younger patients 20-24 (13.3%). However, 7.6% were younger than 18 years. Males were predominant (81.8 versus 18.2%). Moderate and severe poisonings were registered in 41,9% of patients, but there were no fatalities. Although only a minority of patients were admitted to hospital (5,6%), they required treatment at the NPCC Intensive Care Unit. Presentations with drugs of abuse constitute a significant burden on emergency health services. Heroin and synthetic stimulants are the most common, and three quarters were male. Although presentations were most common among those aged 35-60, patients younger than 24 are still prominent (20.9%). Drugs of abuse overdose requires prompt diagnostics and often specific therapeutic measures and treatment in intensive care unit, especially for opioid overdose.

**KEYWORDS:** drugs, overdose, emergency



## THE TREND OF CORROSIVE POISONINGS IN NORTH MACEDONIA – IS THERE A CHANGE IN THE PATTERN OF CORROSIVE POISONINGS?

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Corrosive poisonings (CP) has remained highly presented in developing countries as opposite to the developed ones where they are declining. Objective was comparing the epidemiological characteristics of CP in the last two years to the predicted values (PV) calculated according previous 10 year-period trend analysis. There were 1668 acute CP from 2010 to 2021. CP decreased during the last 12 years period ( $y = -6.5 + 181.4 \cdot R^2 = 0.56$ ), increased by 1.5% during 2020 due to COVID-19 curfews, and decreased by 25.5% in 2021, compared to PV. Females increased during 2020 by 7.7% and then continued to decrease during 2021 by 27.5%, while males declined during 2020 and 2021 by 16.6% and 32.4% respectively, compared to PV, becoming almost equally represented in 2021.

During 2020 and 2021 CP increased in adolescents (by 12.8% and 80.0%) and older than 75 years (by 2.5% and 6.2%), but not in adults who rose during 2020 by 3.7% and declined in 2021 by 17.9% compared to PV. The suicidal CP presented in proportion slightly rose ( $y = 0.01x + 0.56$ ,  $R^2 = 0.07$ ) during 12 years period, decreased in 2020 by 4.6% and increased by 9.9% in 2021 compared to PV. The CP declined during the last 12 years in North Macedonia with an increase in suicidal CP and more significant reduction in females compared to males. The age group patterns changed by increasing poisonings in adolescent and older the 75 years during the last two years, becoming target vulnerable groups that require increased medico-social attention.

**KEYWORDS:** corrosive poisoning, trend, suicide



## EPIDEMIJA TROVANJA DIVLJIM PEČURKAMA TOKOM JESENI 2022. U HRVATSKOJ

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U Hrvatskoj se u jesen 2022. godine dogodila epidemija trovanja pečurkama. Objavljeni podaci o trovanju ljudi pečurkama u Hrvatskoj i okolnim zemljama su oskudni. Izvršen je retrospektivni pregled sumnjivih trovanja pečurkama tokom jeseni 2022. godine, koji je prijavljen telefonskim konsultacijama sa Hrvatskim centrom za kontrolu trovanja (CKO) sa ciljem da se identifikuju najčešće otrovne pečurke i stekne bolje razumevanje karakteristika slučajeva konzumiranja pečuraka. Primili smo ukupno 54 poziva: 3,7% dece (medijana uzrasta 11,5 godina), 55,7% ženskog pola. U jesen 2021. bilo je samo 5 slučajeva konzumiranja pečuraka prijavljenih CKO-u.

Većina izloženosti (81,4%) dogodila se u oktobru 2022. Slučajevi su zabeleženi uglavnom u Zagrebu i okolini (51,8%), Primorsko-goranskoj i istarskoj županiji (26%). Najčešće vrste pečuraka bile su Amanita pantherina (14,8%), Macrolepiota venenata (14,8%), Amanita phalloides (7,4%), Boletus satanas (5,5%), Amanita caesarea (5,5%), sa 22,2% neidentifikovanih pečuraka. Zabrinjavajuće je što su se teški simptomi razvili kod 24% pacijenata. Najteži simptomi bili su posle unošenja Amanite pantherine (vrtoglavica, ataksija, teška konfuzija) i Amanite phalloides (akutno otkazivanje jetre i bubrega, trombocitopenija). U preostalim slučajevima dominirali su uglavnom gastrointestinalni simptomi (mučnina, povraćanje i dijareja). Unošenje je bilo nenamerno, sa samo jednim pokušajem samoubistva (sa neidentifikovanim pečurkom). Nagoveštena je potreba za edukacijom i upozoravanjem stanovništva na opasnost branja i konzumiranja pečuraka sa ciljem sprečavanja trovanja pečurkama.

**KLJUČNE REČI:** Amanita phalloides, Amanita pantherina, otrovne pečurke, centar za kontrolu trovanja



## OUTBREAK OF WILD MUSHROOM POISONING DURING AUTUMN 2022 IN CROATIA

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An outbreak of mushroom poisonings occurred in Croatia in the autumn of 2022. Published data on human mushroom poisonings in Croatia and surrounding countries are scarce. A retrospective review of suspected mushroom poisonings during autumn of 2022 reported by telephone consultations with the Croatian Poison Control Centre (CPCC) was performed with the aim of identifying the most common poisonous mushrooms and gaining a better understand of the characteristics of mushroom ingestion cases. We received a total of 54 calls: 3.7% children (median age 11.5 years), 55.7% female. In autumn of 2021, there were only 5 cases of mushroom ingestions reported to the CPCC. The majority of exposures (81.4%) occurred in October 2022.

Cases were reported mostly in Zagreb and the surrounding area (51.8%), Primorsko-Goranska and Istria counties (26%). The most common mushroom species were *Amanita pantherina* (14.8%), *Macrolepiota venenata* (14.8%), *Amanita phalloides* (7.4%), *Boletus satanas* (5.5%), *Amanita caesarea* (5.5%), with 22.2% of unidentified mushrooms. It is concerning that severe symptoms developed in 24% of patients. The most severe symptoms were following ingestion of *Amanita pantherina* (vertigo, ataxia, severe confusion) and *Amanita phalloides* (acute liver and kidney failure, thrombocytopenia). In the remaining cases, mostly gastrointestinal symptoms (nausea, vomiting and diarrhea) dominated. Ingestions were unintentional, with only one suicide attempt (with an unidentified mushroom). The need for educating and warning the population about the dangers of picking and consuming mushrooms with the aim of preventing mushroom poisonings is indicated.

**KEYWORDS:** *Amanita phalloides*, *Amanita pantherina*, poisonous mushrooms, poison control centre



## NEUROTOKSIKOLOGIJA / NEUROTOXICOLOGY

# UTICAJ SUBAKUTNE IZLOŽENOSTI SMEŠI NISKIH DOZA OLOVA, KADMIJUMA, ŽIVE I ARSENA ZNAČAJNIH ZA IZLOŽENOST IZ ŽIVOTNE SREDINE NA AKTIVNOST ACETILHOLINESTERAZE KOD PACOVA: BENCHMARK PRISTUP

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Brojne studije ukazuju na povezanost između izloženosti toksičnim metalima/metaloidima i pojave štetnih efekata na nivou centralnog nervnog sistema. Cilj rada je bio ispitati povezanost između izloženosti smeši olova, kadmijuma, žive i arsena i promene aktivnosti acetilholinesteraze (AChE) u moždanom tkivu pri subakutnoj oralnoj izloženosti pacova primenom Benchmark metodologije.

Sprovedena je studija na eksperimentalnim životinjama: 30 pacova je podeljeno u 6 grupa: kontrolnu, 5 grupa peroralno tretiranih 28 dana rastućim dozama smeše metala u opsegu koncentracija kojima su ljudi izloženi u životnoj sredini: MIX1- MIX5 (mg/kg t.m./dan) (Pb: 0,003, 0,01, 0,1, 0,3, 1; Cd: 0,01, 0,03, 0,3, 0,9, 3; Hg: 0,0002, 0,0006, 0,006, 0,018, 0,06; As: 0,002, 0,006, 0,06, 0,18, 0,6). U tkivu mozga pacova spektrofotometrijski je ispitana aktivnost AChE. Odnos doza-odgovor proračunat je korišćenjem PROASTveb 70.1 softvera, a rezultati predstavljeni kao Benchmark interval (BMDI), pri čemu je Benchmark odgovor (BMR) podešen na 5%. Tri najviša dozna opsega primenjene smeše metala dovela su do značajnog smanjenja aktivnosti AChE.



## NEUROTOKSIKOLOGIJA / NEUROTOXICOLOGY

Odnos doza-odgovor je potvrđen za sve ispitivane metale prisutne u smeši i aktivnost AchE (BM-DI:  $1.54e-06 - 0.165 \text{ mg Pb/kg t.m./dan}$ ;  $1.83e-06 - 0.503 \text{ mg Cd/kg t.m./dan}$ ;  $1.06e-06 - 0.0101 \text{ mg Hg/kg t.m./dan}$ ;  $1.59e-06 - 0.101 \text{ mg As/kg t.m./dan}$ ).

Ovim rezultatima je pokazano da subakutna izloženost niskim doznim nivoima smeše ispitivanih metala/metaloida može prouzrokovati dozno-zavisno smanjenje aktivnosti AChE u tkivu mozga pacova, ukazujući na to da bi čak i vrednosti Pb, Cd, Hg i As (prisutnih u smeši) više od  $1.54e-06$ ,  $1.83e-06$ ,  $1.06e-06$  i  $1.59e-06$ , redom, mogle dovesti do sniženja aktivnosti AChE za 5%.

**KLJUČNE REČI:** toksični metali, acetilholinesteraza, smeše, relevantne doze za izloženost iz životne sredine, benchmark pristup



# INFLUENCE OF LEAD, CADMIUM, MERCURY AND ARSENIC LOW DOSED MIXTURES RELEVANT TO ENVIRONMENTAL EXPOSURE ON ACETYLCHOLINESTERASE ACTIVITY IN SUBACUTE EXPOSURE IN RATS: A BENCHMARK APPROACH

NEUROTOXICOLOGY

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Numerous studies indicate a connection between increased toxic metal(oid)s level in the blood and the CNS harmful effects, affecting the acetylcholinesterase (AChE) activity. The aim of the work was to examine the relationship between exposure to mixtures of lead, cadmium, mercury and arsenic and changes in AChE activity in brain tissue during subacute oral exposure of rats using the Benchmark methodology. Experimental animals: 30 rats were divided into 6 groups: control, 5 groups orally treated (28 days) with increasing, environmentally relevant doses of metal mixture: MIX1- MIX5 (mg/kg bw/day) (Pb: 0.003, 0.01, 0.1, 0.3, 1; Cd: 0.01, 0.03, 0.3, 0.9, 3; Hg: 0.0002, 0.0006, 0.006, 0.018, 0.06; As: 0.002, 0.006, 0.06, 0.18, 0.6).

Rat brain AChE activity was examined spectrophotometrically. The dose-response relationship results (PROASTweb 70.1 software) presented as the Benchmark Interval (BMDI, 5% Benchmark Response). The three highest doses of the applied metal mixture led to a significant decrease in AChE activity. The dose-response relationship was confirmed for all investigated metals and AChE activity (BMDI: 1.54e-06 – 0.165 mg Pb/kg b.w./day; 1.83e-06 – 0.503 mg Cd/kg b.w./day; 1.06e-06 – 0.0101 mg Hg/kg b.w./day; 1.59e-06 – 0.101 mg As/kg b.w./day). These results showed that subacute exposure to low dose levels of the investigated metal(oid)s mixture can cause a dose-dependent decrease in AChE activity in rat brain tissue, indicating that even the values of Pb, Cd, Hg and As (present in the mixture) more than 1.54e-06, 1.83e-06, 1.06e-06 and 1.59e-06, respectively, could lead to a 5% decrease in AChE activity.

**KEYWORDS:** toxic metals, acetylcholinesterase, mixtures, environmentally relevant doses, benchmark approach



## OKSIM K-870 BOLJE OD OBIDOKSIMA ŠTITI PROTIV TROVANJA PARAOKSONOM U PACOVA

NEUROTOXICOLOGY

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Paraokson (POX) je organofosfatni ireverzibilni inhibitor acetilholinesteraze (AChE). Cilj ovog istraživanja bio je da se utvrdi antidotski potencijal novog oksima K-870 u poređenju sa obidoksimumom, kao monoterapija i u kombinaciji sa atropinom. Studija je sprovedena na pacovima Wistar. Određeni su zaštitni indeks (PR) antidota protiv POX – PR od: obidoksima (22 mg/kg), oksima K-870 (35 mg/kg), kao monoterapija ili sa atropinom (10 mg/kg).





Doze oksima su izabrane kao 20% njihovih LD50 vrednosti. U biohemijskom delu eksperimenata primenjen je POX 0,25 mg/kg sc (0,75% LD50), a minut kasnije K-870 ili obidoksim im. Životinje su žrtvovane: 5, 15, 30, 45, 60, 120, 240, 480, 960 i 1440 min nakon POX. Vrednosti AChE određivane su spektrofotometrijski iz eritrocita, velikog mozga, malog mozga, moždanog stabla i dijafragme. Aktivnosti karboksilesteraze određivane su titrimetrijski iz plazme i jetre.

**REZULTATI.** PR atropina, obidoksima i oksima K-870 bio je 2,76, 1,79 i 2,27, redom. PR oksima K-870 plus atropin (125,00) bio je značajno veći od PR obidoksima plus atropina (68,79). Oba oksima su uspešno reaktivirala AChE u mozgu (velikom mozgu, malom mozgu i moždanom stablu), dijafragmi i eritrocitima, a oksim K-870 je bio bolji od obidoksima. Oba oksima reaktiviraju karboksilesterazu, obidoksim bolje u plazmi, a K-870 bolje u jetri. Oksim K-870, kada se primenjuje zajedno sa atropinom, je efikasniji antidot od obidoksima i atropina kod trovanja POX kod pacova. Osim toga, oksim K-870 generalno pokazuje viši stepen reaktivacije esteraza inhibiranih POX-om.

**KLJUČNE REČI:** oksimi, oksim K-870, obidoksim, paraokson, antidote, inhibitori acetilholinesteraze



## OXIME K-870 BETTER THAN OBIDOXIME PROTECTS AGAINST PARAOXON POISONING IN RATS

NEUROTOXICOLOGY

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Paraoxon (POX) is an organophosphorus irreversible acetylcholinesterase (AChE) inhibitor. The aim of this study was to determine the antidotal potential of the new oxime K-870 compared to obidoxime, as monotherapy and in combination with atropine. The study was conducted in Wistar rats. Protective ratios (PRs) of antidotes against POX were determined – PRs of: obidoxime (22 mg/kg), oxime K-870 (35 mg/kg), as a monotherapy or with atropine (10 mg/kg).



Doses of oximes were chosen as 20% of their LD50 values. In the biochemical part of the experiments, POX 0.25 mg/kg sc (0.75% LD50) was administered, followed one minute later by K-870 or obidoxime im. Animals were sacrificed: 5, 15, 30, 45, 60, 120, 240, 480, 960 and 1440 min after POX. AChE values were determined spectrophotometrically from erythrocytes, cerebrum, cerebellum, brainstem and diaphragm.

Carboxylesterase activities were determined titrimetrically from plasma and liver. The PRs of atropine, obidoxime and oxime K-870 were 2.76, 1.79 and 2.27, respectively. The PR of oxime K-870 plus atropine (125.00) was significantly higher than the PR of obidoxime plus atropine (68.79). Both oximes successfully reactivated AChE in the brain (cerebrum, cerebellum and brainstem), diaphragm and erythrocytes, and oxime K-870 performed better than obidoxime. Both oximes reactivated carboxylesterase, obidoxime better in plasma, and K-870 better in the liver. Oxime K-870, when co-administered with atropine, is a more effective antidote than obidoxime and atropine in POX poisoning in rats. Besides, oxime K-870 generally shows a higher degree of reactivation of esterases inhibited by POX.

**KEYWORDS:** oximes, oxime K-870, obidoxime, paraoxon, antidotes, acetylcholinesterase inhibitor



## ORGAN SPECIFIČNA TOKSIKOLOGIJA / ORGAN SPECIFIC TOXICOLOGY

### ISPITIVANJE TOKSIČNOSTI D,L-SULFORAFANA NA MODELU ZEBRICE

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Sulforafan (SFN) spada u grupu izotiocijanata i prisutan je kod mnogih biljaka krstašica, naročito brokolija. Pozitivna svojstva ovog bioaktivnog molekula u obliku ekstrakata su pokazana u brojnim istraživanjima i uključuju antibakterijsko, kardioprotektivno i neuroprotektivno dejstvo, antioksidativni i imunomodulatorni efekat, kao i pozitivno dejstvo kod različitih karcinoma. Međutim, potencijal ispoljavanja štetnih efekata SFN nije dovoljno ispitan, posebno za hemijski dobijen D,L- sulforafan. Cilj ovog rada je bio da se ispita toksičnost D,L-sulforafana na modelu zebrice (Danio rerio). Korišćeni su embrioni divljeg (AB) soja i transgenih linija zebrice sa fluorescentno obeleženim ćelijama jetre (Tg(fab-p:EGFP)) i endotelnim ćelijama krvnih sudova (Tg(fli1:EGFP)), tretirani različitim koncentracijama SFN u opsegu od 1 do 20 µg/mL.

Tokom pet dana praćeno je preživljavanje embriona, toksičnost na rast i razvoj, funkciju jetre i kardiovaskularnog sistema. Koncentracija od 20 µg/mL D,L-sulforafana izazvala je smrt svih embriona, dok je dobijeno da srednja smrtna koncentracija (LC50) iznosi 14,2 µg/mL. D,L-sulforafan ispoljava toksična dejstva u koncentracijama višim od 3 µg/mL i to prvenstveno na razvoj mehura za plivanje (4 µg/mL), rast i razvoj embriona (4,58 µg/mL), dok su štetni efektni na jetru (veličina jetre i resorpcija žumanceta) uočeni pri koncentraciji od 10 µg/mL. Efekti na kardiovaskularni sistem nisu uočeni pri koncentracijama od 1 do 10 µg/mL. Ispitivanje D,L-sulforafana na embrionima zebrica je pokazalo da se štetni efekti pojavljuju pri veoma niskim koncentracijama, što ukazuje na potrebu za daljim ispitivanjem toksikološkog potencijala ovog molekula.

**KLJUČNE REČI:** D,L-sulforafan, toksičnost, zebrica, srednja smrtna koncentracija LC50



## TOXICITY TESTING OF D,L-SULFORAPHANE IN A ZEBRAFISH MODEL

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Sulforaphane (SFN) belongs to the group of isothiocyanates and is present in many cruciferous plants, especially broccoli. The positive properties of this bioactive molecule in the form of extracts have been shown in numerous studies and include antibacterial, cardioprotective, and neuroprotective effects, antioxidant and immunomodulatory effects, as well as positive effects in various cancers.

However, the potential for harmful effects of SFN has not been sufficiently investigated, particularly for chemically obtained D,L-sulforaphane. This study aimed to investigate the toxicity of D,L-sulforaphane on zebrafish (*Danio rerio*) model. Wild (AB) strain embryos and zebrafish transgenic lines with fluorescently labeled liver cells (Tg(fabp:EGFP)) and endothelial cells of blood vessels (Tg(fli1:EGFP)) were used, treated with different concentrations of SFN (1 to 20 µg/mL).

The survival of embryos, developmental toxicity, hepatotoxicity and cardiotoxicity were monitored for five days. A concentration of 20 µg/mL of D,L-sulforaphane caused the death of all embryos, while the median lethal concentration (LC50) was found to be 14.2 µg/mL. D,L-sulforaphane exhibited toxic effects at concentrations higher than 3 µg/mL, primarily on the development of the swim bladder (4 µg/mL), and growth and development of embryos (4.58 µg/mL), while harmful effects on the liver (liver size and yolk resorption) were observed at a concentration of 10 µg/mL. Effects on the cardiovascular system were not observed at concentrations from 1 to 10 µg/mL. The investigation of D,L-sulforaphane on zebrafish embryos showed that harmful effects occur at very low concentrations, indicating the need for further investigation of toxicological potential of this molecule.

**KEYWORDS:** D,L-sulforaphane, toxicity, zebrafish, mean lethal concentration LC50



## TOKSIKOLOŠKE KARAKTERISTIKE PARACETAMOLA

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Paracetamol je sintetski neopioidni derivat p-aminofenola, jedan od najčešće korišćenih analgoantipiretika. Pored neosporno potvrđenog terapijskog efekta, paracetamol može ispoljiti štetne efekte, pre svega dozno-zavisnu hepatotoksičnost. Može biti uzročnik trovanja, najčešće slučajnih, kao rezultat prekoračenja doze usled neadekvatne primene, čemu posebno doprinosi podatak da je paracetamol lako dostupan OTC lek. Preporučena maksimalna dnevna doza za paracetamol iznosi 4 g, a za pacijente sa povećanim rizikom od hepatotoksičnosti 2 g. Toksikinetika paracetamola je veoma kompleksna. Ap-sorpciju paracetamola u slučaju per os primene doza većih od maksimalnih dnevnih doza, karakteriše brza resorpcija iz želuca i gornjeg dela GIT-a i stvaranje toksičnog metabolita N-acetil-p-benzohinonimin (NAPQI) u jetri.

Pri primeni terapijskih doza NAPQI se konjuguje sa glutationom i nastaje acetaminofen-3-merkaptorna kiselina koja se izlučuje urinom. Međutim, pri predoziranju dolazi do saturacije enzima konjugacije (glukuroniltransferaze i sulfotransferaze), ubrzane potrošnje zalihe glutationa jetri, i povećane sinteze NAPQI. NAPQI se kovalentno vezuje za sulfhidrilne grupe enzima jetre, posebno one koji učestvuju u homeostazi kalcijuma što je ujedno glavni razlog hepatotoksičnosti paracetamola. Hepatotoksičnost paracetamola se ispoljava u vidu teškog oštećenja jetre sa centrolobularnom nekrozom i masnom degeneracijom, a ponekad i sa akutnom tubularnom nekrozom. Zapažaju se i tzv. premošćavanja nekroze („bridging“) sa inflamatornim ćelijama kao i hipertrofičnim Kupffer-ovim ćelijama ispunjenim lipofuscinom. Slično patološko oštećenje se javlja i u epitelu proksimalnih tubula bubrega. Deacetilacijom paracetamola u jetri se stvara i mala količina p-aminofenola koji može biti odgovoran za methemoglobinemiju pri hroničnom trovanju paracetamolom.

**KLJUČNE REČI:** Paracetamol, hepatotoksičnost, štetna dejstva



## TOXICOLOGICAL CHARACTERISTICS OF PARACETAMOL

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Paracetamol is a synthetic non-opioid derivative of p-aminophenol, a widely used analgesic-antipyretic drug. In addition to the indisputably confirmed therapeutic effect, paracetamol can induce adverse effects, primarily dose-dependent hepatotoxicity. Poisoning with paracetamol is most often accidental as a result of exceeding the dose, as paracetamol is an easily available OTC drug. The recommended maximum daily dose for paracetamol is 4 g, and for patients with an increased risk of hepatotoxicity, 2 g. The toxicokinetic of paracetamol is complex. Excess production of the toxic metabolite N-acetyl-p-benzoquinonimine (NAPQI) in the liver which is caused by exceeded maximum dose is exacerbated by rapid absorption from the GIT. When using therapeutic doses, NAPQI is conjugated with glutathione to form acetaminophen-3-mercapturic acid, which is excreted in the urine.

However, in case of overdose, there is the saturation of conjugation enzymes (glucuronyltransferase and sulfotransferase), accelerated consumption of liver glutathione stores, and increased synthesis of NAPQI. NAPQI binds covalently to the sulfhydryl groups of liver enzymes, especially those involved in calcium homeostasis, which is also the main cause of the hepatotoxicity of paracetamol. Hepatotoxicity of paracetamol manifests in the form of severe liver damage with centrilobular necrosis and fatty degeneration, and occasionally with acute tubular necrosis. Histologically “bridging necrosis” can be seen, with inflammatory cells and hypertrophic Kupffer cells with lipofuscin. Similar pathological damage occurs in the epithelium of the proximal kidney tubules. Deacetylation of paracetamol in the liver produces a small amount of p-aminophenol, which can be the cause of methemoglobinemia in chronic poisoning.

**KEYWORDS:** paracetamol, hepatotoxicity, adverse effects



# UTJECAJ POJEDINAČNOG I ZAJEDNIČKOG TRETMANA MIKOTOKSINIMA OKRATOKSINOM A I CITRININOM NA ORGANSKE ANIONSKE, KATIONSKE I OSTALE FIZIOLOŠKI VAŽNE PRIJENOSNIKE TE NAKUPLJANJE OVIH MIKOTOKSINA U BUBREGU

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Nefrotoksični mikotoksini okratoksin A (OTA) i citrinin (CTN) zajednički kontaminiraju hranu i krmivo. Proizvode ih plijesni iz rodova *Aspergillus* i *Penicillium* uključujući vrstu *Penicillium verrucosum* koja proizvodi oba mikotoksina. U našem su istraživanju muški štakori soja Wistar 21 dan pojedinačno i zajednički tretirani s OTA (125 i 250 µg/kg b.w.) i CTN-om (2 mg/kg b.w.) dok je jedna grupa osim ova dva mikotoksina dobila i resveratrol (RSV, 20 mg/kg b.w.). Rezultati akumulacije ovih toksina u bubrezima iz naših prijašnjih istraživanja potaknuli su nas na istraživanje membranskih proteina uključenih u njihov prijenos kroz membranu te fiziologiju bubrega. Pomoću imunokemijskih metoda istraživali smo ekspresiju/lokalizaciju organskih anionskih i kationskih prijenosnika, Na<sup>+</sup>/K<sup>+</sup>-ATP-azu, kanale vode, Na-glukozne prijenosnike i β-aktin.

Nakupljanje toksina u bubregu mjereno je pomoću HPLC-a. Pojedinačni i zajednički tretman s OTA i CTN uzrokuje smanjenje ekspresije organskih anionskih i kationskih prijenosnika (Oat1, Oat2, Oat5, Oct1) ovisno o dozi dok na fiziološki značajne proteine (Na<sup>+</sup>/K<sup>+</sup>-ATP-azu, Aqp1, Aqp2, Sglt1, Sglt2 i β-aktin) nije bilo utjecaja. Nakon tretmana s OTA+CTN+RSV ekspresija većine proteina (Oat1, Oat2, Oat5, Oct1, Oct2, Sglt1, β-aktin, i glikoziliranih izoformi proteina Aqp1 je bila smanjena. U toj je skupini koncentracija OTA u bubregu bila tri puta veća u odnosu na životinje tretirane mikotoksinima pojedinačno ili zajednički. Subkronična izloženost štakora pojedinačnom ili zajedničkom tretmanu s OTA i CTN prvenstveno utječe na izlučivanje OTA iz bubrega dok RSV pojačava nefrotoksični učinak ovih mikotoksina, posebno OTA. (Projekt je financirala Hrvatska zaklada za znanost: IP-09-2014-5982).

**KLJUČNE REČI:** in vivo, imunohistokemija, membranski prijenosnici, bioakumulacija, prijenosnici otpjenih tvari, western blot





## RENAL ACCUMULATION AND EFFECTS OF OCHRATOXIN A AND CITRININ MYCOTOXINS ON ORGANIC ANION, CATION AND OTHER PHYSIOLOGICALLY RELEVANT TRANSPORTERS AFTER INDIVIDUAL AND COMBINED ORAL EXPOSURE IN RATS

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Nephrotoxic mycotoxins ochratoxin A (OTA) and citrinin (CTN) commonly contaminate food and feed. They are produced by *Aspergillus* and *Penicillium* mould species including *Penicillium verrucosum* which can produce both mycotoxins. In our study adult male Wistar rats were treated with OTA (125 and 250 µg/kg b.w.) and CTN (2 mg/kg b.w.), with their combinations and with OTA (250 µg/kg b.w.), CTN and resveratrol (RSV; 20 mg/kg b.w.) for 21 days. Accumulation of these toxins in rat kidneys observed in our previous *in vivo* experiments made us focus on studying the effects of these mycotoxins on membrane proteins involved in their transport and maintenance of renal physiology. We used immunochemical methods to investigate expression/localization of organic anion and cation transporters, sodium-potassium ATPase, water channels, sodium-glucose cotransporters and β-actin.

Renal bioaccumulation of toxins was measured by HPLC. Results showed that individual/combined mycotoxins OTA and CIT generally led to selective and dose-dependent expression decrease of organic anion and cation transporters (Oat1, Oat2, Oat5, Oct1) whereas other physiologically relevant proteins (Na<sup>+</sup>/K<sup>+</sup>-ATPase, Aqp1, Aqp2, Sgl1, Sgl2 and β-actin) were largely unaffected. Majority of studied proteins (Oat1, Oat2, Oat5, Oct1, Oct2, Sgl1, β-actin, and Aqp1 glycosylated isoforms) had decreased expression in animals treated with OTA+CTN+RSV. Consequently, renal concentration of OTA in those animals was three times higher compared to animals treated with individual/combined mycotoxins. Thus, subchronic exposure of rats to individual/combined OTA and CTN primarily impairs the renal excretion of OTA while RSV enhances the nephrotoxic effects of studied mycotoxins leading to hazardous increase of OTA in rat kidneys. (Croatian Science Foundation project IP-09-2014-5982).

**KEYWORDS:** *in vivo*, immunohistochemistry, membrane transporters, renal bioaccumulation, solute carriers, western blot



## SUBAKUTNA TOKSIČNOST FLUORIDA U KALCIFIKOVANIM TKIVIMA WISTAR PACOVA

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Preventivna upotreba fluorida (F-) ima pozitivan uticaj na zubna tkiva. Međutim, prekomeran unos F- može doprineti nastanku lokalne i/ili sistemske toksičnosti. Cilj studije bio je ispitivanje mehanizama subakutne toksičnosti rastućih koncentracija F- kod pacova u tkivima zuba i femura. Za potrebe eksperimenta, 30 mužjaka Wistar pacova podeljeno je u 6 grupa (n=5) i tokom 28 dana imali su ad libitum pristup vodi sa česme ili vodi sa rastućim koncentracijama F- (10, 25, 50, 100 i 150 mg/l). Sekutići, molari i levi femur izolovani su pomoću odgovarajućih klješta i podeljeni na 3 dela za određivanje koncentracija F-, bioelemenata (cinka (Zn), bakra (Cu) i gvožđa (Fe)) i histološke analize. PROASTweb70.1 softver korišćen je za analizu odnosa doze i efekta F-.

Rezultati ove studije pokazali su da subakutna ekspozicija povišenim dozama F- dovodi do značajno povišenih vrednosti koncentracija F- u femuru i zubima pacova doznih grupa sa 100 i 150 mg/l F-. Fluoridi su uticali na disbalans koncentracija Zn i Cu, dok morfološka oštećenja u kalcifikovanim tkivima nisu uočena. Potvrđena je dozna zavisnost za efekat F- za smanjenje koncentracija Cu u femuru (BMDL 1,6 mg F-/kg t.m.) i smanjenje koncentracija Zn u zubima (BMDL 21,0 mg F-/kg t.m.). Na osnovu rezultata ove studije možemo zaključiti da subakutna ekspozicija povišenim dozama F- dovodi do disbalansa bioelemenata i taloženja F- u zubima i femuru eksperimentalnih pacova. S obzirom da su ljudi i životinje svakodnevno izloženi F-, ova studija je značajna za buduću procenu zdravstvenog rizika ekspozicije fluoridima.

**KLJUČNE REČI:** fluoridi, zubi, femur, bioelementi, benchmark doza



## SUBACUTE FLUORIDE TOXICITY IN CALCIFIED TISSUES OF WISTAR RATS

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Prophylactic fluoride (F<sup>-</sup>) use has positive effects on tooth structure. However, excessive F<sup>-</sup> consumption could cause local and/or systematic intoxication. The aim of the present study was to examine subacute toxicity of increasing F<sup>-</sup> concentrations in the teeth and femur of rats. The experiment was conducted on 30 male Wistar rats for 28 days, divided into six groups (n=5), and during 28 days animals had ad libitum access to tap water or water with F<sup>-</sup> solutions (10, 25, 50, 100 and 150 mg/L F<sup>-</sup>). The teeth and left femur were extracted and processed for the determination of F<sup>-</sup> tissue concentrations, bioelements (zinc (Zn), copper (Cu), and iron (Fe)), and histological analyses. PROASTweb 70.1 was used for the determination of the dose-response F<sup>-</sup> relationship.

The results confirmed a significant increase of F<sup>-</sup> concentrations in the femur and teeth among groups with 100 and 150 mg/L F<sup>-</sup>. Fluorides caused Zn and Cu disbalance, while histological changes were not observed. The dose-dependent changes were confirmed for decreased Cu concentrations in the femur (BMDL 1.6 mg F<sup>-</sup>/kg b.w.) and decreased Zn concentrations in the teeth (BMDL 21.0 mg F<sup>-</sup>/kg b.w.). According to the results of the present study, subacute exposure to increased F<sup>-</sup> concentrations leads to bioelements disbalance and F<sup>-</sup> deposition in the teeth and femur of experimental rats. Since human and animal populations are daily exposed to F<sup>-</sup>, this dose-response study is valuable for future health risk assessment regarding fluoride exposure.

**KEYWORDS:** fluorides, teeth, femur, bioelements, benchmark dose.



# ANTIOKSIDANTNI EFEKAT SOJINIHZOFLAVONA I PROBIOTIKA NA OKSIDATIVNI STRES PACOVA IZLOŽENIH UGLJEN TETRAHLORIDU

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**UVOD:** Zdravstvene prednosti sojinih izoflavona se često pripisuju njihovoj antioksidantnoj aktivnosti. Kako crevni mikrobiom ima veliki uticaj na metabolizam izoflavona, probiotici bi mogli povećati njihovu bioraspoloživost i in vivo antioksidantni potencijal.

**CILJ:** Cilj je bio da se proceni antioksidantna aktivnost sojinih izoflavona, probiotika i njihove kombinacije na oksidativni stres izazvan ugljen tetrahloridom (CCl<sub>4</sub>) kod pacova.

**METODOLOGIJA:** Wistar pacovi su raspoređeni u sedam grupa po šest životinja: grupa I – kontrola; II – izoflavoni soje (50 mg/kg); III – probiotici (10<sup>9</sup> CFU/kg); IV – CCl<sub>4</sub> i.p. (1 mg/kg); V-VII – nakon doze CCl<sub>4</sub>, hranjene izoflavonima, probioticima i njihovom kombinacijom, redom, tokom 15 dana, nakon čega su životinje žrtvovane. U serumu su određivani ukupni antioksidantni status (TAS), alanin aminotransferaza (ALT) i aspartat aminotransferaza (AST). U tkivima jetre i bubrega merena je lipidna peroksidacija (TBARS), aktivnosti katalaze (CAT) i glutation-S-transferaze (GST).

**REZULTATI:** Poređenjem rezultata dobijenih u CCl<sub>4</sub> grupi (IV) sa tretiranim grupama (V-VII), pokazalo se da su izoflavoni statistički značajno (p=0,003) povećali TAS. Probiotici (p

**ZAKLJUČAK:** Kod većine analiziranih parametara, kombinacija izoflavona i probiotika se pokazala kao najsnažnija u ublažavanju oksidativnog stresa izazvanog CCl<sub>4</sub>.

**KLJUČNE REČI:** izoflavoni, probiotici, pacovi, ugljen tetrahlorid, oksidativni stress



## PROFESIONALNA TOKSIKOLOGIJA / OCCUPATIONAL TOXICOLOGY

### ANALIZA INCIDENCE MALIGNIH OBOLJENJA PLUĆA U REPUBLICI SRBIJI U PROIZVODNIM DELATNOSTIMA

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Analizom incidence malignih oboljenja pluća zaposlenih u proizvodnim delatnostima dobićemo podatke koji su neophodni u prevenciji malignih oboljenja pluća u odnosu na profesionalnu izloženost. Kako u Srbiji postoji nedovoljno registrovanje profesionalnih malignih bolesti, u Institutu za medicinu rada Srbije je u prethodnih 10 (deset) godina utvrđeno samo 2 (dva) slučaja profesionalnih malignih bolesti pluća, prezentovanje ovih podataka bi ukazalo na veličinu ovog problema u Srbiji. Cilj rada bio je analiza značaja povezanosti rada u proizvodnim delatnostima sa incidencom malignih oboljenja pluća u Srbiji. Istraživanje je sprovedeno retrospektivnom analizom Nacionalnog registra za rak iz koga su izdvojene dijagnoze sa šiframa: C34; C38.4; C39.9; C45. Podaci su analizirani u odnosu na delatnost, starost, navike pušenja i utvrđenu dijagnozu. Statistička analiza podataka obavljena je uz pomoć programa SPSS.

**KLJUČNE REČI:** rak pluća, zanimanja, medicina rada, profesionalni rak



# ANALYSIS OF THE INCIDENCE OF MALIGNANT LUNG DISEASES IN THE REPUBLIC OF SERBIA IN PRODUCTION ACTIVITIES

OCCUPATIONAL  
TOXICOLOGY

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By analyzing the incidence of malignant lung diseases among employees in production activities, we will obtain data that are necessary for the prevention of malignant lung diseases in relation to professional exposure. As there is insufficient registration of occupational malignant diseases in Serbia, only 2 (two) cases of occupational malignant lung diseases were determined in the Serbian Institute of Occupational Health in the previous 10 (ten) years, the presentation of these data would indicate the magnitude of this problem in Serbia. The goal of the work: Analysis of the significance of the connection between work in production activities and the incidence of malignant lung diseases in Serbia.

The research was conducted through a retrospective analysis of the National Cancer Registry, from which the diagnoses with codes: C34; C38.4; C39.9; C45. The data were analyzed in relation to the activity, age, smoking habits and established diagnosis. Statistical data analysis was performed with the help of the SPSS program.

**KEYWORDS:** lung neoplasms, occupations, occupational health, occupational cancer



## TOKSIČNOST SMJEŠE TOLUENA, KSILENA, ETANOLA I ACETALDEHIDA:

### IN SILICO TOKSIKOGENOMIČKA ANALIZA

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Iako su vrlo toksični za razne organe i organske sisteme, organski rastvarači se još uvijek široko koriste u brojnim industrijama. Profesionalna izloženost smješi organskih rastvarača je češća od izlaganja samo jednom rastvaraču. In silico metodologije su postale važne u istraživanju odnosa između hemikalija i gena, hemijskih interakcija, molekularnih puteva i bioloških procesa. Ovo istraživanje ima za cilj da pruži dokaze o hepatotoksičnom potencijalu smješe organskih rastvarača toluena, ksilena, etanola i acetaldehida i pokaže značaj in silico toksikogenomičke analize podataka u određivanju mogućih mehanizama toksičnosti smješe.

Za prikupljanje podataka korišteni su Comparative Toxicogenomics Database (CTD), GeneMania i ToppGene Suite. Rezultati istraživanja su pokazali da testirani rastvarači ostvaruju hepatotoksični potencijal putem 4 zajednička gena (CAT, CXCL8, IL6, PTGS2). Fizičke interakcije (77,64%) bile su najistaknutije interakcije između gena, dok je koekspresija prisutna u 8,01%, kolokalizacija u 3,63%, a genetske interakcije u 2,87%. Analiza genske ontologije otkrila je biološke procese na koje utiče ispitivana smješa (odgovor na oksidativni stres, odgovor na lipide, ćelijski odgovor na jedinjenja koja sadrže kiseonik, ćelijska detoksikacija oksidanasa, ćelijska detoksikacija vodonikovog peroksida, metabolički proces azotnog oksida, metabolički proces reaktivnih vrsta azota, odgovor faktora nekroze tumora). Aktivnost peroksidaze, aktivnost oksidoreduktaze, antioksidativna aktivnost, aktivnost prostaglandin-endoperoksid sintaze i vezivanje hema bile su bitne molekularne funkcije koje doprinose razvoju bolesti. Rezultati daju novi uvid u molekularne mehanizme uključene u hepatotoksičnost, ističući ulogu oksidativnog stresa kao jednog od mehanizama toksičnosti smješe organskih rastvarača.

**KLJUČNE REČI:** organski rastvarači, profesionalna izloženost, oksidativni stres, toksičnost smješe



# TOXICITY OF A MIXTURE OF TOLUENE, XYLENE, ETHANOL AND ACETALDEHYDE: *IN SILICO* TOXICOGENOMIC DATA-MINING

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Although highly toxic to various organs and systems of organs, organic solvents are still widely used in multiple industries. Occupational exposure to a mixture of organic solvents is more common than exposure to a single solvent. In silico methodologies have become important in researching the relationship between chemicals and genes, chemical interactions, molecular pathways and biological processes. This study aims to provide evidence for the hepatotoxic potential of a mixture of organic solvents toluene, xylene, ethanol and acetaldehyde and show the significance of in silico toxicogenomic data mining in determining possible mechanisms of mixture toxicity.

The Comparative Toxicogenomics Database (CTD), GeneMania and ToppGene Suite were used for data-mining. The results showed the tested solvents achieved their hepatotoxic potential via 4 common genes (CAT, CXCL8, IL6, PTGS2). Physical interactions (77,64%) were the most prominent interaction between the genes, while co-expression was present at 8,01%, co-localization at 3,63% and genetic interactions at 2,87%. Gene ontology analysis revealed biological processes affected by the investigated mixture (response to oxidative stress, response to lipid, cellular response to oxygen-containing compound, cellular oxidant detoxification, cellular detoxification of hydrogen peroxide, nitric oxide metabolic process, reactive nitrogen species metabolic process, response to tumor necrosis factor). Peroxidase activity, oxidoreductase activity, antioxidant activity, prostaglandin-endoperoxide synthase activity, and heme binding were the essential molecular functions contributing to disease development. Our results provide new insight into the molecular mechanisms involved in hepatotoxicity, highlighting the role of oxidative stress as one of the mechanisms of organic solvents' mixture toxicity.

**KEYWORDS:** organic solvents, occupational exposure, oxidative stress, mixture toxicity





## KORELACIJA PARAMETARA OKSIDATIVNOG STRESA I FUNKCIJE JETRE KOD RADNIKA ZAPOSLENIH U INDUSTRIJI OBUĆE

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Profesionalna izloženost smješi organskih rastvarača predstavlja povećani rizik od razvoja hepatotoksičnosti kod zaposlenih u industriji obuće. Oksidativni stres je predložen kao jedan od najvažnijih patofizioloških mehanizama hepatotoksičnosti organskih rastvarača. Cilj ovog istraživanja bio je utvrditi vezu između parametara oksidativnog stresa i pokazatelja funkcije jetre izmerenih u serumu radnika zaposlenih u industriji obuće. Studija je obuhvatila 30 radnika zaposlenih u industriji obuće i 30 zdravih ispitanika, administrativnih radnika raspoređenih u kontrolnu grupu. Grupa izloženih radnika je imala značajno veće vrijednosti bilirubina, aspartat aminotranferaze (AST), alanin aminotranferaze (ALT), gama glutamiltransferaze (GGT) u poređenju sa kontrolnom grupom ( $p < 0,001$ ).

Takođe, dobijene vrijednosti parametara oksidativnog stresa (superoksid anjon radikal ( $O_2^-$ ), totalni oksidativni status (TOS), prooksidativno-antioksidativni balans (PAB)) i antioksidativne zaštite (totalni antioksidativni status (TAS), koncentracija ukupnih sulfhidrilnih grupa (-SH grupe), aktivnost superoksid-dismutaze (SOD)) kod izloženih radnika su bili značajno povećani u odnosu na kontrolnu grupu ( $p < 0,001$ ). Korelaciona analiza je pokazala snažnu pozitivnu povezanost TOS i AST, ALT i GGT, -SH grupa i bilirubina, dok je negativna povezanost pokazana između  $O_2^-$  i bilirubina ( $p < 0,01$ ). Godine radnog staža u industriji obuće su bile u snažnoj pozitivnoj korelaciji sa  $O_2^-$ , i negativnoj korelaciji sa ukupnim -SH grupama ( $p < 0,01$ ). Rezultati ove studije pokazuju da su navedeni parametri oksidativnog stresa povećani kod radnika u industriji obuće i snažno koreliraju sa parametrima funkcije jetre.

**KLJUČNE REČI:** profesionalna izloženost, smješa organskih rastvarača, hepatotoksičnost, oksidativni stres, antioksidativna zaštita



## CORRELATION OF OXIDATIVE STRESS PARAMETERS AND LIVER FUNCTION IN WORKERS EMPLOYED IN THE SHOE INDUSTRY

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Occupational exposure to mixture of organic solvents presents an increased risk of hepatotoxicity in shoe industry workers. Oxidative stress has been proposed as one of the most important pathophysiological mechanisms of hepatotoxicity caused by organic solvents. This study aimed to determine the relationship between oxidative stress and liver function parameters measured in serum of shoe industry workers. The study included 30 workers employed in the shoe industry and 30 healthy unexposed subjects. Group of exposed workers had significantly higher bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (GGT) values than control group ( $p < 0.001$ ).

Also, obtained values of oxidative stress parameters (superoxide anion radical (O<sub>2</sub><sup>-</sup>), total oxidative status (TOS), prooxidative-antioxidant balance (PAB)) and antioxidant defence (total antioxidant status (TAS), the concentration of sulfhydryl groups (-SH groups), superoxide dismutase activity (SOD)) in exposed workers were significantly increased compared to the control group ( $p < 0.001$ ). Correlation analysis showed a strong positive association between TOS and AST, ALT and GGT, -SH groups and bilirubin, while a negative association between O<sub>2</sub><sup>-</sup> and bilirubin was obtained ( $p < 0.01$ ). Employment duration was strongly correlated with O<sub>2</sub><sup>-</sup> but negatively correlated with -SH groups ( $p < 0.01$ ). This study shows that oxidative stress parameters are increased in workers in the shoe industry and strongly correlate with liver function parameters.

**KEYWORDS:** occupational exposure, mixture of organic solvents, hepatotoxicity, oxidative stress, antioxidant defense



## REGULATORNA TOKSIKOLOGIJA / REGULATORY TOXICOLOGY

### ZAKONSKA REGULATIVA U OBLASTI ZDRAVSTVENE ISPRAVNOSTI IGRAČAKA

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Predmeti opšte upotrebe uređuju se Zakonom o predmetima opšte upotrebe ("Službeni glasnik RS", br. 25 od 3. aprila 2019., 14 od 7. februara 2022.). Igračke, kao predmet opšte upotrebe, jesu proizvodi koji su projektovani ili namijenjeni za igru djece do 14 godina. Pravilnikom o bezbjednosti igračkaka („Službeni glasnik RS“, broj 78 od 1. novembra 2019.) utvrđuju se pravila o bezbjednosti igračkaka i njihovom slobodnom kretanju na tržištu, u svrhu obezbjeđenja visokog nivoa zaštite života i zdravlja ljudi. Ovaj pravilnik primjenjuje se na: funkcionalne igračke, igračke za vodu, igračke za slobodnu aktivnost, hemijske igračke, mirisne ploče za igru i kozmetičke komplete.

U proizvode koji se ne smatraju igračkama spadaju: ukrasni predmeti za svečanosti i proslave; proizvodi, namijenjeni kolekcionarima, starosti preko 14 godina; sportska oprema, uključujući koturaljke, rolere i skejt bord, koja je namijenjena djeci tjelesne mase preko 20 kg; skuteri i druga prevozna sredstva, namijenjena za sportske aktivnosti ili za prevoz na javnim putevima ili javnim stazama; oprema za upotrebu u dubokoj vodi i oprema za podučavanje plivanja djece, kao što su sjedišta za plivanje i pomoćna sredstva za plivanje; slagalice sa više od 500 dijelova i drugi. Prisustvo alergeni mirisa kao što su: alil izotiocijanat, difenilamin, eugenol i mnogi drugi, dozvoljeno je pod uslovom da oni ne prelaze količinu od 100 mg/kg. Pojedini alergeni mirisi kao što su: farnesol, benzil benzoat, anisil alkohol, moraju biti navedeni na etiketi ili ambalaži ili uputstvu igračke, ako su dodati igračkama u koncentracijama, većim od 100 mg/kg.

**KLJUČNE REČI:** igračka, Pravilnik, bezbjednost, alergeni mirisi



## LEGISLATION IN THE FIELD OF HEALTH SAFETY OF TOYS

REGULATORY  
TOXICOLOGY

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Items of general use are regulated by the Law on Items of General Use ("Official Gazette of RS", No. 25 of April 3, 2019, 14 of February 7, 2022). Toys, as an object of general use, are products designed or intended for play by children up to 14 years of age. The Rulebook on the Safety of Toys ("Official Gazette of RS", No. 78 of November 1, 2019) establishes the rules on the safety of toys and their free movement on the market, in order to ensure a high level of protection of human life and health. This regulation applies to: functional toys, water toys, leisure toys, chemical toys, scented game boards and cosmetic kits.

Products that are not considered toys include: decorative items for festivities and celebrations; products, intended for collectors, aged over 14 years; sports equipment, including inline skates, rollerblades and skateboards, intended for children weighing over 20 kg; scooters and other means of transport, intended for sports activities or for transport on public roads or public paths; equipment for use in deep water and equipment for teaching children to swim, such as swimming seats and swimming aids; puzzles with more than 500 pieces and others. The presence of allergenic fragrances such as: allyl isothiocyanate, diphenylamine, eugenol and many others, is allowed provided that they do not exceed the amount of 100 mg/kg. Some allergenic fragrances such as: farnesol, benzyl benzoate, anisyl alcohol, must be listed on the label or packaging or instructions of the toy, if they are added to toys in concentrations higher than 100 mg/kg.

**KEYWORDS:** toy, Regulations, safety, allergenic smells



## PRAVILNIK O KOZMETIČKIM PROIZVODIMA - IZVEŠTAJ O BEZBEDNOSTI KOZMETIČKOG PROIZVODA

REGULATORY  
TOXICOLOGY

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Prema propisima u Republici Srbiji, kozmetički proizvodi se svrstavaju u grupu predmeta opšte upotrebe i kao takvi su regulisani odredbama Zakona o predmetima opšte upotrebe. Prema ovom zakonu odgovorno lice obezbeđuje da je kozmetički proizvod prošao procenu bezbednosti na osnovu relevantnih informacija predstavljajući na tržište, kao i da je izrađen izveštaj o bezbednosti kozmetičkog proizvoda. Na osnovu člana 16. stav 1. tačka 3) Zakona o predmetima opšte upotrebe, ministar zdravlja donosi Pravilnik o kozmetičkim proizvodima. U okviru ovog pravilnika propisana je i dokumentacija od značaja za dokazivanje bezbednosti kozmetičkog proizvoda. Deo ove dokumentacije je dosije sa informacijama o proizvodu u okviru kojeg se nalazi i izveštaj o bezbednosti kozmetičkog proizvoda. Ovaj izveštaj mora da sadrži informacije o bezbednosti kozmetičkog proizvoda i procenu bezbednosti kozmetičkog proizvoda. Procena bezbednosti kozmetičkog proizvoda se zasniva na proceni bezbednosti sastojaka, a procenjuje se pojava akutnih lokalnih i sistemskih efekata. Procenu izvodi lice koje poseduje javnu ispravu u oblasti visokog obrazovanja iz oblasti farmacije, medicine, toksikologije ili sličnih ekvivalentnih disciplina.

**KLJUČNE REČI:** zakon, pravilnik, izveštaj, procena bezbednosti



## REGULATION ON COSMETIC PRODUCTS - COSMETIC PRODUCT SAFETY REPORT

REGULATORY  
TOXICOLOGY

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According to the regulations in the Republic of Serbia, cosmetic products are classified as items of general use and as such are regulated by the provisions of the Law on Items of General Use. According to this law, the responsible person ensures that the cosmetic product has undergone a safety assessment based on relevant information before placing it on the market, as well as that a report on the safety of the cosmetic product has been prepared. On the basis of Article 16, paragraph 1, item 3) of the Law on Items of General Use, the Minister of Health adopts the Rulebook on Cosmetic Products.

Within this rulebook, the documentation important for proving the safety of the cosmetic product is prescribed. Part of this documentation is a Product Information File, which includes a Safety Report of the cosmetic product. This report must contain information on the safety of the cosmetic product and a safety assessment of the cosmetic product. The safety assessment of the cosmetic product is based on the safety assessment of the ingredients, and the occurrence of acute local and systemic effects is assessed. The assessment is performed by a person who has a public document in the field of higher education in the field of pharmacy, medicine, toxicology or similar equivalent disciplines.

**KEYWORDS:** law, rulebook, safety report, safety assessment



## REPRODUKTIVNA TOKSIKOLOGIJA / REPRODUCTIVE TOXICOLOGY

### EFEKTI ORALNO PRIMENJENOG ARSENA NA BROJ MLADUNACA MIŠEVA

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U radu su prikazani rezultati kumulativnog uticaja arsen(III)-oksida na broj mladunaca miševa praćenih kroz tri uzastopne generacije. Miševi su podeljeni u tri grupe, jednu kontrolnu i dve ogleadne grupe. Kontrolna grupa je dobijala vodu iz vodovodne mreže, prva ogleadna grupa je pila vodu sa koncentracijom rastvorenog arsen(III)-oksida od 10,6 mg/L, dok je druga ispitivana grupa pila vodu sa koncentracijom rastvorenog arsen(III)-oksida od 106 mg/L što je deset puta veća koncentracija arsena u odnosu na prvu ogleadnu grupu.

Koncentracije arsen(III)-oksida primenjivane u eksperimentu predstavljaju vrednosti koje su preračunate sa humane vrednosti na vrednost animalnog modela a prema koncentraciji koja je registrovana u podzemnim vodama Zrenjanina. Brojani su jednodnevni živorođeni mladunci ženki miševa. Brojanje je vršeno u periodu od 75 dana (period od 75 dana predstavlja vreme od dana odvajanja ženki iz kaveza sa mužjacima do prvog legla ženki). U svakoj generaciji, ženke su bile izložene samo jednom reproduktivnom ciklusu. Prosečan broj mladunaca se smanjio u obe eksperimentalne grupe kroz tri uzastopne generacije u odnosu na kontrolnu grupu. Studija je pokazala da arsen(III)-oksid u primenjenim koncentracijama utiče na broj mladunaca.

**KLJUČNE REČI:** arsen (III)-oksid, broj mladunaca, reproduktivna toksikologija, Zrenjanin



## THE EFFECTS OF ORALLY ADMINISTERED ARSENIC ON THE NUMBER OF MICE CUBS

REPRODUCTIVE  
TOXICOLOGY

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The article presented the results of the cumulative influence of arsenic (III)-oxide on the number of young mice observed over three consecutive generations. The mice were divided into three groups, one control and two study groups. The control group received water from the water supply network, the first studied group drank water with a dissolved concentration of 10.6 mg/l arsenic(III)-oxide, while the second studied group received a ten times higher concentration of 106 mg/l arsenic(III)-oxide.

The concentration represents, values converted from human to animal model, which identified in groundwater of Zrenjanin. In this study, one-day-old live young of a female were counted. The number of young per female mouse was determined over a 75-day period (the 75-day period denotes the time from the day of separation in the cage with males to the first litter of females). In each generation, females were exposed to only one reproductive cycle. The average number of juveniles decreased in both experimental groups over the three consecutive generations compared to the control group. The study showed that arsenic(III)-oxide at the applied concentrations affected the number of young.

**KEYWORDS:** arsenic (III)-oxide, number of young, reproductive toxicology, Zrenjanin





## UČINCI SUBKRONIČNE IZLOŽENOSTI TEMBOTRIONU NA ANTIOKSIDACIJSKI STATUS I KONCENTRACIJU ESENCIJALNIH ELEMENATA U TESTISIMA I EPIDIDIMISIMA ODRASLIH ŠTAKORA

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Tembotrion je član triketonske klase herbicida koji se obično koristi za suzbijanje korovnih biljaka. Dobiva se kemijskim putem iz prirodnog fitotoksina (leptospermona) i dizajniran je da bude ekološki prihvatljiv. Iako se smatra dobrom zamjenom za konvencionalne pesticide, nedavna istraživanja ukazuju da izloženost tembotrionu može imati štetne učinke na zdravlje ljudi. Cilj ovog istraživanja bio je procijeniti učinke peroralne izloženosti niskim dozama tembotriona na antioksidacijski status i koncentraciju esencijalnih elemenata u testisima i epididimisima odraslih štakora soja Wistar.

Štakori su tijekom 28 dana svakodnevno izlagani dozama tembotriona koje su usporedive s trenutno predloženim zdravstveno utemeljenim referentnim vrijednostima (0,0007 (Acceptable Operator Exposure Level - AOEL), 0,0013 (Residual Exposure Level - REL) ili 0,7 (1000×AOEL) mg/kg tjelesne mase/dan). Iako je kod štakora izloženih tembotrionu uočeno značajno povećanje tjelesne mase, nije primijećen učinak na masu testisa i epididimisa. U usporedbi s negativnom kontrolom, tretman najvećom dozom tembotriona rezultirao je značajnim povećanjem razine reduciranog glutationa i ukupnog antioksidacijskog statusa (TAS) u epididimisu, dok je u testisima TAS značajno smanjen. Štakori izloženi tembotrionu imali su značajno nižu koncentraciju Ca i Na u testisima te Ca u epididimisu u usporedbi s kontrolnim životinjama. S druge strane, u tkivu testisa štakora izloženih tembotrionu u dozi od 0,0013 mg/kg tjelesne mase/dan izmjerena je značajno viša koncentracija Fe. S obzirom da su tako niske doze tembotriona proizvele mjerljive biološke učinke u testisima i epididimisima štakora, preporučljiva je daljnja procjena ovog široko korištenog herbicida i njegovog učinka na reproduktivno zdravlje.

**KLJUČNE RIJEČI:** antioksidacijski status, esencijalni elementi, pesticidi, reproduktivno zdravlje



# EFFECTS OF SUB-CHRONIC EXPOSURE TO TEMBOTRIONE ON ANTIOXIDANT STATUS AND CONCENTRATION OF ESSENTIAL ELEMENTS IN TESTES AND EPIDIDYMIS OF ADULT RATS

REPRODUCTIVE  
TOXICOLOGY

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Tembotrione is a member of triketone class of herbicides usually used for post-emergence weed control. It is derived chemically from a natural phytotoxin (leptospermon) and designed to be “eco-friendly”. Although considered as a good alternative to conventional pesticides, there are reports indicating its adverse health effects. The aim of this study was to assess the effects of exposure to low doses of tembotrione on antioxidant parameters and concentration of essential elements in testes and epididymis of adult Wistar rats. Rats were orally treated with doses comparable to currently proposed health-based reference values (0.0007 (AOEL), 0.0013 (REL) or 0.7 (1000×AOEL) mg/kg b.w./day) for 28 consecutive days.

Although a significant increase in animal body mass was observed in tembotrione-treated rats, exposure to the herbicide had no effect on the mass of testes/epididymis. In epididymis, treatment with the highest dose of tembotrione significantly increased the levels of reduced glutathione and total antioxidant status (TAS), while in the testes TAS significantly decreased when compared to negative control. Rats treated with tembotrione had a significantly lower concentration of Ca and Na in testes and Ca in epididymis in comparison to control animals. On the other hand, significantly higher concentration of Fe was measured in testicular tissue of rats treated with 0.0013 mg/kg b.w./day of tembotrione. The fact that such low doses had the potential to produce measurable biological outcomes calls for further evaluation of this widely used herbicide and its effect on reproductive health.

**KEYWORDS:** antioxidant status, essential elements, pesticides, reproductive health



## PROTECTIVE EFFECTS OF BIOCHANIN-A AGAINST CHROMIUM AND ARSENIC-INDUCED REPRODUCTIVE DAMAGE IN THE EXPERIMENTAL MODEL

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One of the most frequent causes of reproductive damage in humans is heavy metal exposure. The different heavy metals and metalloids that are exposed to in industrial and ecological settings have terrible effects on the reproductive system, leading to infertility. Chromium and arsenic at moderate to low concentrations may have an impact on a number of reproductive factors, including semen quality. As a substitute for synthetic drugs for treating reproductive disorders brought on by environmental toxins, research has recently concentrated on the unique features of natural compounds and their constituents.

One such compound is biochanin-A which is a phytoestrogen and is sold as a dietary supplement. Hence, the aim of this study was to observe the beneficial effects of biochanin-A against combined chromium and arsenic-induced reproductive toxicity in male Swiss albino mice. Potassium dichromate (75 ppm) and sodium meta-arsenite (100 ppm) were given orally along with biochanin-A (50 mg/kg) intraperitoneally for a period of 15 days. After the completion of the experiment, the testis was removed and the following parameters were assessed. The results showed that body weight and organ weight (testis) were found to decline in the intoxicated group of mice as compared to the control. With the administration of biochanin-A, the above parameters were altered along with the increase in the number of motile sperms. It was also seen that the biochemical parameters were also affected by the intoxication as a result, protein carbonyl content (PCC) was found to increase with a simultaneous decrease in activities of reduced glutathione (GSH) and total thiol (TT). Concurrently, it was found that the biochanin-A-administered group was able to mitigate the altered parameters. The outcomes show that this natural compound (biochanin-A), by reducing the generated oxidative stress, has curative potential against the combined chromium and arsenic-activated reproductive damage.

**KEYWORDS:** heavy metal, reproductive damage, chromium, arsenic, biochanin-A



## SLOBODNE TEME / FREE TOPICS

### KONTROVERZNE SMRTI SRPSKOG SREDNJEG VEKA -CAR DUŠAN NEMANJIĆ

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Hilandarski medicinski kodeks svedoči o slučajevima zločinačkih trovanja među srpskim velikašima. Iznenađna smrt najvećeg srpskog vladara srednjeg veka cara Dušana Nemanjića („Dušan Silni”, 1308-1355) se najverovatnije može povezati sa trovanjem. Prva pretpostavka o uzroku njegove smrti, koja se nalazi u šest vizantijskih izvora, jeste da je car Dušan umro od posledica malarije. Međutim, mnogi istraživači se slažu, da se malarija ne može smatrati bolešću koja će uzrokovati iznenadnu smrt, posebno se pozivajući na fizičko zdravlje i dobru kondiciju cara Dušana. Drugi mogući uzrok, da je preminuo zbog kardiovaskularne bolesti većina istoričara odbacuje, zbog izričite zabrane neumerenosti u hrani i piću navedene u Zakoniku cara Dušana iz 1349. godine, kojim su bile predviđene i stroge kazne za nepridržavanje.

Usled navedenih nepotvrđenih kontroverzi, neposredno pre nego što su mošti cara Dušana prenete u crkvu Svetog Marka, 25. maja 1968. godine, izvršena je spektrometrijska analiza kostiju na Prirodno-matematičkom fakultetu u Beogradu, koja je utvrdila prisustvo velike količine olova. Pored toga, u jednom spisu iz manastira Dečani, zabeleženo je da je car Stefan Dušan otrovan od strane Mlečana, iz osвете zbog odbijanja Dušana da im ustupi osvojene gradove Klis i Skradin. Po drugim zapisima, kao Dušanov trovač navodi se njegov vidar koji je na trovanje nagovoren od strane vizantijske uhode Mavroduka. Upravo se Vizantija najčešće i dovodi u vezu sa iznenadnom smrću cara Dušana. U tom periodu u Konstantinopolju je bila izuzetno zastupljena trgovina otrovima, pa su toksična sredstva bila lako dostupna, što potkrepljuje sumnje.

**KLJUČNE REČI:** smrt Dušana Silnog, trovanje, kontroverze



## CONTROVERSIAL DEATHS OF THE SERBIAN MIDDLE AGES – EMPEROR DUŠAN NEMANJIĆ

FREE TOPICS

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The Chilandar medical codex testifies to cases of criminal poisoning among Serbian noblemen. The sudden death of the greatest Serbian monarch of the Middle Ages, Emperor Dušan Nemanjić ("Dušan the Mighty", 1308-1355), can most likely be linked to the poisoning. The first premise about the cause of his death, which is found in six Byzantine sources, is that Emperor Dušan died from malaria. However, many researchers agree that malaria cannot be considered the cause of sudden death, referring to Emperor Dušan's good health.

Another possible cause, that he died due to cardiovascular disease, is rejected by most historians, due to the explicit prohibition of immoderation in food and drink stated in The Code of Stephan Dušan from 1349, the unique law-code book. Spectrometric analysis of the bones, performed at the Faculty of Science and Mathematics in Belgrade in 1968 (just before his relics were transferred to the St Mark's Church in Belgrade), discovered lead in a considerable amount, which only supports the poisoning controversy. In addition, in a document from the Dečani monastery, it was recorded that Emperor Stefan Dušan was poisoned by the Venetians, due to the conflict over the conquered territories of Klis and Skradin.

According to other records, Dušan's poisoner is mentioned as his medic under the order of the Byzantine Empire. It is Byzantium that is most often associated with the sudden death of Emperor Dušan. In that period, the trade in poisons was extremely prevalent in Constantinople, so poisons were easily available, which supports the suspicions.

**KEYWORDS:** Dušan the mighty death, poisoning, controversies



## SRPSKA SREDNJOVEKOVNA TOKSIKOLOGIJA: TOKSIKOLOŠKI SPIS U HILANDARSKOM MEDICINSKOM KODEKSU

FREE TOPICS

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Prvi naučni spis iz oblasti toksikologije u srpskoj medicini nalazi se u najdragocenijem spomeniku srpske medicinske kulture Hilendarskog medicinskog kodeksu. Cilj ovog rada je da predstavi Toksikološki spis Hilendarskog medicinskog kodeksa, otkrivenog polovinom 20. veka u biblioteci manastira Hilandara. Toksikološki spis obuhvata znanja antičke medicine, postavke Hipokrata, Galena, ali i učitelja medicinskih škola u Salernu i Monpeljeu, prepise medicinskih spisa srednjovekovnih pisaca, kao i odlomke originalnih dela. Prevodioci spisa, kao i njegovi sastavljači bili su Srbi, učeni lekari koji su za veliki broj termina, tj. stručnih izraza dali nazive na staroslovenskom jeziku, postavljajući temelj srpskoj medicinskoj terminologiji i pokazujući da su izuzetno dobro poznavali materiju koju su prenosili na naš jezik.

Budući da se na više mesta u spisu pominje Galen, pretpostavlja se da ovaj spis sadrži odlomke iz poznatog Galenovog dela „O otrovima“. Međutim, pominju se i drugi autori salernsko-monpeljeske škole. Sam spis sadrži pet odeljaka koji nose sledeće nazive: Ovde počinje opis svih otrova koji se upotrebljavaju peroralno; Ovde se govori o poznavanju toksičnih osobina otrova; Ovde se govori o lečenju onih koji su se otrovali; Ovde se govori o prostim (jednostavnim) lekovima protiv otrova i Ovde se govori o lekovima koji se dobijaju iz ruda. Sagledavajući sadržaje odeljaka Toksikološkog spisa može se zaključiti da je u vreme nastanka Hilendarskog medicinskog kodeksa u Srbiji poznavanje otrova bilo zasnovano na tadašnjim opštim naučnim saznanjima o toksičnosti i otrovima, kao i postupcima prilikom lečenja otrovanih osoba i protivotrovima poznatim u Evropi pred kraj srednjeg veka.

**KLJUČNE REČI:** toksikologija, srednji vek, Srbija, Hilendarski medicinski kodeks



## SERBIAN MEDIEVAL TOXICOLOGY: TOXICOLOGICAL RECORD IN THE HILANDAR MEDICAL CODEX

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The first scientific document in the toxicology field in Serbian medicine is found in the most precious monument of Serbian medical culture, Hilandar Medical Codex. This paper aimed to present the Toxicology File of Hilandar Medical Codex, discovered in the middle of the 20th century in the library of Hilandar monastery. This file includes the knowledge of ancient medicine, the statements of Hippocrates, Galen, as well as the teachers of the medical schools in Salerno and Montpellier, copies of medical records of medieval writers and fragments of original works.

The translators/compliers of the document were Serbs, doctors, who named the professional terms in Old Slavic language, laying the foundation of Serbian medical terminology and showing a great knowledge of the material they were translating. Since Galen is mentioned in several places in the document, it is assumed that it contains passages from his famous work "On poisons". However, other authors of Salerno-Montpellier school are also mentioned. The document itself contains five sections: Description of all poisons that are used orally; Toxic properties of poison; Treating the poisoned ones; Simple medicines against poisons and Medicines obtained from ores. Looking at the contents of the sections of the Toxicological File, it can be concluded that, at the time of the creation of the Hilandar Medical Code in Serbia, knowledge of poisons was based on the general scientific knowledge of toxicity and poisons, as well as procedures for treating poisoned persons and antidotes known in Europe towards the end of the Middle Ages.

**KEYWORDS:** toxicology, middle ages, Serbia, Hilandar medical codex



## EDUKACIJA OPŠTE POPULACIJE O TOKSIČNOSTI: MEETOX PROJEKAT

FREE TOPICS

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Skoro svakodnevno se u sredstvima javnog informisanja mogu čuti informacije o toksičnim supstancama i njihovom prisustvu u našem neposrednom okruženju, vazduhu, vodi, hrani, kao i proizvodima široke potrošnje, što izaziva zabrinutost među širom javnošću i, generalno, potrošačima. Vrlo često se među ovakvim informacijama može uočiti neadekvatno korišćenje terminologije i poistovećivanje različitih pojmova. Dodatno, informacione tehnologije i društvene mreže često pružaju neproverene informacije u pogledu toksičnosti, od kojih su brojne netačne i nisu zasnovane na naučnim saznanjima.

O svojstvu toksičnosti se ne uči pre univerzitetskog obrazovanja, a toksikologija se izučava samo na pojedinim fakultetima, zdravstvenim i biohemijsko-prehrambeno-tehnološkim. Stoga je cilj projekta u okviru Erasmus+ programa malih partnerstava za obrazovanje odraslih da ispita razumevanje informacija o toksičnosti hemikalija kod opšte populacije i sprovede edukaciju o osnovnim elementima toksičnosti razvojem programa obuke radi bezbednog svakodnevnog korišćenja hemikalija i predmeta opšte upotrebe. Projekat će omogućiti razumevanje šta znači kada je neka hemikalija toksična i kako se bezbedno može koristiti u određenim proizvodima, zatim, bolje razumevanje novih informacija naučnih istraživanja i saznanja o toksičnim efektima, kao i razlozima za uvođenje ograničenja i zabrana za korišćenje hemikalija, kao i značaj čitanja etiketa i deklaracija proizvoda i ispravno donošenje odluka o bezbednom korišćenju proizvoda, a u cilju zaštite zdravlja ljudi. Koordinator dvogodišnjeg projekta pod nazivom „Upoznaj toksičnost – živi bezbedno” je Udruženje toksikologa Srbije, a partnerske institucije su Univerzitet u Beogradu – Farmaceutski fakultet iz Srbije, Hrvatsko toksikološko društvo i Institut za medicinska istraživanja i medicinu rada iz Hrvatske.

**KLJUČNE REČI:** toksičnost, edukacija, opšta populacija, Erasmus+ program, MeeTox projekat





## EDUCATION OF THE GENERAL POPULATION ABOUT TOXICITY: MEETOX PROJECT

FREE TOPICS

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Almost every day in the media, information about toxic substances and their presence in our immediate environment (air, water, food, and consumer products) can be heard. This causes concern among the general public and consumers. Very often, when presenting such information, inadequate use of terminology and identification of different concepts can be observed. Additionally, information technology and social networks often provide unverified information regarding toxicity, many of which are inaccurate and not based on scientific knowledge. The property of toxicity is not learned before university education and toxicology is studied only at certain faculties, health-based and biochemical-food-technological.

Erasmus+ Small Partnerships Program for Adult Education aims to examine the general population's understanding of chemical toxicity and provide education on the basic elements of toxicity by developing a training program on safe daily use of chemicals and consumer products. The project will provide an understanding of chemical toxicity and how they can be safely used in certain products; a better understanding of new information from scientific research and knowledge about toxic effects; reasons for introducing restrictions and bans on the use of chemicals, as well as the importance of reading labels and product declaration and correct decision-making on the safe use of products. The coordinator of the two-year project "Meet the toxicity – live safely" is Serbian Society of Toxicology, while partner institutions are the University of Belgrade – Faculty of Pharmacy from Serbia and Croatian Society of Toxicology and Institute for Medical Research and Occupational Medicine from Croatia.

**KEYWORDS:** toxicity, education, general population, Erasmus+ program, MeeTox project



## KOLIKO OPŠTA POPULACIJA POZNAJE I RAZUME TOKSIČNOST: PILOT ISPITIVANJE

FREE TOPICS

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U vreme kada broj hemikalija koje se svakodnevno koriste raste iz dana u dan, a mnoge od njih poseduju toksična svojstva, raste i zabrinutost šire javnosti. Osnovna saznanja o svojstvu toksičnosti nisu obuhvaćena školskim planovima i programima. Toksikologija se izučava jedino na pojedinim fakultetima, prvenstveno zdravstvenih struka, i to kao zaseban predmet, a u najširem obimu na studijama farmacije, kao i studijskim programima biohemijsko-prehrambeno-tehnoloških fakulteta. Dodatno, napredak tehnologija i široka dostupnost informacija putem društvenih mreža često vodi netačnom informisanju u pogledu toksičnosti hemikalija i potencijalnog rizika koje mogu imati po zdravlje ljudi.

Cilj ovog rada je bio da se ispita znanje i stavovi opšte populacije u pogledu toksičnosti supstanci koje se nalaze u predmetima opšte upotrebe. U istraživanju je učestvovalo 427 ispitanika, među njima znatno više osoba ženskog pola i duplo više ispitanika nemedicinske struke u odnosu na ispitanike sa medicinskim obrazovanjem. Zabrinutost u vezi sa sveprisutnošću toksičnih supstanci se ne razlikuje između ispitanika medicinske i nemedicinske struke. Ispitanici uglavnom ne razlikuju posedovanje toksičnog potencijala neke supstance u odnosu na rizik od pojave štetnih efekata koji se može javiti usled korišćenja iste. Samo nešto više od polovine ispitanika smatra da se neka supstanca, ukoliko ima potencijal da izazove toksičan efekat, može bezbedno koristiti u proizvodima opšte upotrebe. Dobijeni rezultati ukazuju na neophodnost edukacije i informisanja šire javnosti o toksičnom potencijalu supstanci i naučne zasnovanosti bezbednog korišćenja proizvoda, odnosno predmeta opšte upotrebe koji sadrže toksične supstance, što je cilj dvogodišnjeg evropskog Erasmus+ projekta „Upoznaj toksičnost – živi bezbedno“ (MeeTox).

**KLJUČNE REČI:** razumevanje toksičnosti, opšta populacija, predmeti opšte upotrebe, MeeTox projekat



## HOW MUCH THE GENERAL POPULATION KNOWS AND UNDERSTANDS TOXICITY: A PILOT STUDY

FREE TOPICS

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Nowadays, when the number of chemicals used on a daily basis, many of which possess toxic properties, is constantly increasing, so is the concern of the general population. Basic knowledge about toxicity is not included in school curricula. Toxicology is studied only at certain faculties, primarily health professions-based, as a separate subject, and in the broadest scope at pharmacy studies and study programs of biochemical-food-technology faculties. The advancement of technologies and wide availability of information through social networks often leads to inaccurate information regarding toxicity of chemicals and potential risk they may have on human health.

This work aimed to examine the knowledge and attitudes of the general population regarding toxicity of substances, i.e. chemicals found in consumer products. 427 respondents participated in the research (significantly more women and twice as many non-medical respondents than respondents with medical education). Concerns about the ubiquity of toxic substances did not differ between medical and non-medical respondents. Respondents generally did not differentiate between the possession of a substance's toxic potential and risk of adverse effects that may occur as a result of its use. Slightly more than half of the respondents believed that a substance, if it has toxic potential, can be safely applied in products of general use. These results indicate the necessity of educating and informing the general public about toxic potential of substances and scientific basis of products' safe use, which is the goal of the two-year European Erasmus+ project "Meet the toxicity – live safely" (MeeTox).

**KEYWORDS:** understanding toxicity, general population, consumer products, MeeTox project



## THE EFFECTS OF DISTANCE AND WARMING TEMPERATURE ON INTENSITY OF EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELD FROM FORCED-AIR WARMING SYSTEM

FREE TOPICS

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Electronic devices, increasingly used in operating rooms, heavily depend on mains electricity. This may significantly increase occupational exposure to extremely low frequency electromagnetic field. As a possible carcinogen as per International Agency for Research on Cancer, Swedish Confederation of Professional Employee recommended it should be below 0.2  $\mu$ T (micro Tesla). Forced air warming system is used to prevent hypothermia. This study was to investigate the effect of distance and warming temperature on extremely low frequency electromagnetic field intensity from Bair-Hugger Warming Unit, in order to find the minimum safe distance from the device. The forced-air warming unit was hung on the side of an operating table in an empty operating room, with all other electronic devices turned off. With three different warming temperatures, electromagnetic field was measured at 15, 30, and 45 cm from the anterior surface of the device.

Data was collected at a sampling time of 0.5 times per second for 10 minutes. Kruskal-Wallis one-way analysis was used. Increasing warming temperature and reducing the distance from the device resulted in a significant increase in electromagnetic field intensity ( $p < 0.05$ ). At 45 cm in particular, low and medium warming temperatures showed significantly lower intensity compared to high temperature ( $p < 0.05$ ). Exposure below 0.2  $\mu$ T was achieved only when it was at 45 cm regardless of temperature. Health workers should be aware of occupational exposure to extremely low frequency electromagnetic field. It is suggested to stay at least 45 cm away from Bair-Hugger Warming Unit.

**KEYWORDS:** extremely low frequency electromagnetic field, operating room, air warmer



## TOKSIKOLOGIJA SMEŠA / MIXTURES TOXICOLOGY

### BIOKOMPATIBILNOST LIPOFILNIH EUTEKTIČKIH RASTVARAČA NA ĆELIJSKOJ LINIJI ASTROCITOMA 1321N1 IN VITRO

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Korišćenje eutektskih rastvarača (engl. deep eutectic solvents, DES) predstavlja atraktivan pristup u ekstrakciji kanabidiola (CBD) iz konoplje. Može da zameni druga ekstrakciona sredstva koja mogu da ispolje potencijalne citotoksične efekte. Efikasni i bezbedni postupci ekstrakcije postaju sve važnije imajući u vidu široku primenu ulja CBD-a kao sastojka raznih farmaceutskih proizvoda i nutraceutika. Cilj ove studije je bio da se ispita biokompatibilnost novorazvijenog lipofilnog DES-a na ćelijskoj liniji humanog astrocitoma 1321N1.

Posle 24 h in vitro izlaganja različitim zapreminama rastvarača, procenili smo vitalnost ćelija pomoću MTS testa, dok je nivo primarnog oštećenja DNK meren testom alkalnog kometa. Dobijeni rezultati nisu pokazali statistički značajnu razliku u vijabilnosti ćelija u odnosu na kontrolu u svim uzorcima. Takođe, izlaganje DES-u nije izazvalo statistički značajne promene vrednosti %DNK u repu kometa (%DNK <0,03) u poređenju sa kontrolom u svim ispitivanim uzorcima. Naši nalazi su pokazali prihvatljivu biokompatibilnost testiranog DES-a, kako u pogledu vitalnosti ćelije, tako i u pogledu stabilnosti genoma ćelija 1321N1. Ovo ukazuje na značajan potencijal DES-a za dalja istraživanja i njegovu moguću primenu kao rastvarača za ekstrakciju CBD, ali i drugih jedinjenja koja se mogu koristiti u prehrambenoj, farmaceutskoj i kozmetičkoj industriji.

**KLJUČNE REČI:** eutektski rastvarači, biokompatibilnost, primarna oštećenja DNK

**ZAHVALNICA:** Ovaj rad je finansijski podržan od strane Hrvatske zaklade za znanost broj HrZZ-UIP-2017-05-7260.

# BIOCOMPATIBILITY OF LIPOPHILIC DEEP EUTECTIC SOLVENTS ON THE ASTROCYTOMA CELL LINE 1321N1 *IN VITRO*

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The use of deep eutectic solvents (DES) represents an attractive approach in the extraction of cannabidiol (CBD) from hemp. It may replace other extraction media, which could exert potential cytotoxic effects. Effective and safe extraction procedures become ever more important considering the wide application of CBD oil as an ingredient of various pharmaceuticals and nutraceuticals. The aim of this study was to investigate the biocompatibility of a newly developed lipophilic DES on the human astrocytoma cell line 1321N1. After 24 h of *in vitro* exposure to different volumes of the solvent, we evaluated cell viability using the MTS assay, while the level of primary DNA damage was measured using the alkaline comet assay. The obtained results showed no statistically significant difference in cell viability compared to the control in all of the samples.

Also, exposure to DES did not provoke statistically significant changes in the value of %DNA in the comet tail (%DNA <0.03) compared to the control in all of the tested samples. Our findings demonstrated an acceptable biocompatibility of the tested DES, both on cell viability and on the genome stability of 1321N1 cells. This suggested significant potential of DES for further research and its possible application as a solvent for the extraction of CBD as well different compounds that can be used in the food industry, pharmacy and cosmetics.

**KEYWORDS:** deep eutectic solvents, biocompatibility, primary DNA damage

**ACKNOWLEDGEMENTS:** This work was financially supported by the Croatian Science Foundation number HrZZ-UIP-2017-05-7260.

# CITOTOKSIČNOST POLIBROMOVANIH DIFENILETRA I POLIHLOOROVANIH BIFENILA NA ČELIJE HUMANOG NEUROBLASTOMA (SH-SY5Y) I BUBREGA (HEK293)

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Polihlorovani bifenili (PCB) i polibromovani difeniletri (PBDE) pripadaju grupi jedinjenja poznatih kao perzistentni organski zagađivači (POP) koje karakteriše visoka postojanost, lipofilnost, potencijal bioakumulacije i toksičnost. Iako potiču iz različitih izvora, akumuliraju se u istim delovima okoliša i u ljudima. U ovoj studiji, ćelije humanog neuroblastoma (SH-SY5Y) i bubrege (HEK293) bile su izložene tokom 24 sata sledećim mešavinama kongenera:  $\Sigma$ PCB (CB-28, -52, -101, -118, -138, -153, -180) i  $\Sigma$ PBDE (BDE-28, -47, -99, -100, -153, -154, -183) u ukupnim koncentracijama od 265 i 12 ng mL<sup>-1</sup>, respektivno. Ove vrednosti su bile maksimalne koncentracije svake grupe zagađivača detektovane u pojedinačnim uzorcima ljudskog mleka prikupljenim u Zagrebu, Hrvatska tokom kampanje uzorkovanja 2019/2020. Primitili smo da i  $\Sigma$ PBDE i  $\Sigma$ PCB imaju sličan efekat na vitalnost ćelija HEK293, dok su SH-SY5Y ćelije bile više pogođene  $\Sigma$ PBDE. Ipak, primećeno smanjenje održivosti od 12% nije smatrano statistički značajnim u korišćenoj analizi.

Međutim, kada su ova dva tipa ćelija bila izložena kombinaciji  $\Sigma$ PBDE i  $\Sigma$ PCB (265 + 12 ng mL<sup>-1</sup>), smanjenje vitalnosti je bilo približno 23% i statistički značajno u poređenju sa kontrolom. Naš rezultat je pokazao da, kada se primenjuju zajedno, ove dve klase zagađivača mogu delovati sinergistički i poremetiti ćelijsku homeostazu. Ovaj nalaz izaziva zabrinutost u pogledu izloženosti ljudi mešavinama takvih bioakumulativnih ekoloških ksenobiotika i imajući u vidu da su testirane koncentracije zaista otkrivene u ljudskom mleku. Dalja detaljna istraživanja o mogućim štetnim efektima su od najveće važnosti.

**KLJUČNE REČI:** polihlorovani bifenili, polibromovani difeniletri, ćelije humanog neuroblastoma (SH-SY5Y), ćelije bubrege (HEK293), citotoksičnost

**ZAHVALNICA:** Ovaj rad je podržan projektima Hrvatske zaklade za znanost UIP-2017-05-6713 (DeValApp) i UIP-2017-05-7260 (CellToxTargets).

# CYTOTOXICITY OF POLYBROMINATED DIPHENYL ETHERS AND POLYCHLORINATED BIPHENYLS ON HUMAN NEUROBLASTOMA (SH-SY5Y) AND KIDNEY (HEK293) CELLS

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Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) belong to a group of compounds known as persistent organic pollutants (POPs) characterized by high persistence, lipophilicity, bioaccumulation potential, and toxicity. Although they originate from different sources, they accumulate in the same environmental compartments and in humans. In this study, human neuroblastoma (SH-SY5Y) and kidney (HEK293) cells were exposed for 24h to the following mixtures of congeners:  $\Sigma$ PCB (CB-28, -52, -101, -118, -138, -153, -180), and  $\Sigma$ PBDE (BDE-28, -47, -99, -100, -153, -154, -183) in total concentrations of 265 and 12 ng mL<sup>-1</sup>, respectively.

These values were the maximum concentrations of each pollutant group detected in individual human milk samples collected in Zagreb, Croatia during the sampling campaign 2019/2020. We observed that both  $\Sigma$ PBDEs and  $\Sigma$ PCBs had a similar effect on HEK293 cell viability, while SH-SY5Y cells were more affected by  $\Sigma$ PBDEs. Still, the observed decrease of 12% in viability was not considered statistically significant by the analysis used. However, when these two cell types were exposed to a combination of  $\Sigma$ PBDEs and  $\Sigma$ PCBs (265 + 12 ng mL<sup>-1</sup>), the decrease in viability was approximately 23% and statistically significant in comparison to control. Our result indicated that, when applied together, these two pollutant classes could act synergistically and disturb cell homeostasis. This finding raises concern with regard to human exposure to mixtures of such bioaccumulative environmental xenobiotics and considering that the tested concentrations were actually detected in human milk. Further detailed research on possible adverse effects is of the utmost importance.

**KEYWORDS:** polychlorinated biphenyls, polybrominated diphenyl ethers, human neuroblastoma (SH-SY5Y) cells, kidney (HEK293) cells, cytotoxicity

**ACKNOWLEDGEMENTS:** This work was supported by Croatian Science Foundation projects UIP-2017-05-6713 (DeValApp) and UIP-2017-05-7260 (CellToxTargets).





## UTICAJ KOMBINACIJE IMUNOMODULATORA NA POVEĆANJE EFIKASNOSTI IMUNOTERAPIJE KARCINOMA

MIXTURES  
TOXICOLOGY

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Incidenca karcinoma u svetu je u stalnom porastu i, prema procenama Svetske zdravstvene organizacije, mogao bi u predstojećim godinama postati vodeći uzrok smrtnosti. Iako se beleže značajna dostignuća u unapređenju lečenja pacijenata obolelih od karcinoma, kako razvojem bioloških lekova (CAR-T ćelija i PD-1/PD-L1 inhibitora), tako i kombinovanjem dostupne terapije sa imunomodulatorskim agensima, još uvek nije poznato kako bi kombinacije imunomodulatora mogle doprineti povećanju efikasnosti imunoterapije karcinoma. Cilj ovog rada bio je istražiti imunomodulatorni potencijal različitih supstanci i predložiti koja bi kombinacija mogla najbolje da ispolji pozitivan efekat uz minimalno ispoljavanje negativnih efekata.

Pretragom različitih literaturnih navoda referenciranim u brojnim indeksnim bazama uočeno je da brojne fitohemikalije pokazuju imunomodulatornu aktivnost, među kojima se izdavaća sulforafan (SFN), izotiocijanata poreklom iz kupusastog povrća, najviše brokolija. In vitro rezultati pokazuju da SFN inhibira rast i proliferaciju ćelija tumora urinarnog trakta koje su rezistentne na konvencionalnu terapiju, ukazujući na značajan antitumorski potencijal. Sa druge strane, inaktivisana bakterija *Pseudomonas aeruginosa* (PA-MSHA) pokazuje slične efekte na rezistentnim ćelijama raka dojke i karcinoma pluća. Stoga, kombinacija imunomodulatora SFN i PA-MSHA predstavlja obećavajuću antitumorsku strategiju. Međutim, potencijal za izazivanje štetnih efekata, kako SFN tako i PA-MSHA je nedovoljno ispitan i veoma je malo istraživanja o njihovom toksičnom potencijalu prilikom pojedinačne primene, dok nema ispitivanja o njihovom kombinovanom dejstvu. Stoga, neophodno je ispitati uticaj pojedinačne i kombinovane primene SFN i PA-MSHA na poboljšanje efikasnosti imunoterapije karcinoma, sa posebnim osvrtom na bezbednost njihove primene, pojedinačne ili kombinovane (Srbija-Kina projekat: 451-03-1203/2021-09).

**KLJUČNE REČI:** sulforafan, *Pseudomonas aeruginosa*, kombinovana terapija, biološki lekovi, bezbednost



## IMPROVING ANTI-CANCER IMMUNOTHERAPY EFFICACY BY COMBINING IMMUNE MODULATORS

MIXTURES  
TOXICOLOGY

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The incidence of cancer worldwide is constantly increasing and, according to the World Health Organization's estimates, could become the leading cause of death in the coming years. While significant advances have been made in improving cancer treatment through biological drug development (CAR-T cells and PD-1/PD-L1 inhibitors) as well as combining available therapies with immunomodulatory agents, it is still unknown how combinations of immunomodulators could contribute to increasing cancer immunotherapy effectiveness.

This study aimed to investigate the immunomodulatory potential of different substances and suggest which combination could best exhibit a positive with minimal negative effects. By searching literature indexed in numerous databases, it was found that numerous phytochemicals exhibit immunomodulatory activity, among which sulforaphane (SFN), an isothiocyanate derived from cruciferous vegetables, mainly broccoli, stands out. In vitro results show that SFN inhibits the growth and proliferation of resistant urinary tract tumor cells, indicating significant anti-tumor potential. On the other hand, inactivated *Pseudomonas aeruginosa* bacteria (PA-MSHA) show similar effects on resistant breast cancer and lung cancer cells. Thus, the combination of these immunomodulators represents a promising anti-tumor strategy. However, there is very little research on the toxic potential of SFN and PA-MSHA individually, while there are no studies on their combined effect. Therefore, it is necessary to examine the influence of the individual and combined use of SFN and PA-MSHA on the improvement of the effectiveness of cancer immunotherapy, with a special focus on the safety of their use, either individually or in combination (Serbia-China project: 451-03-1203/2021-09).

**KEYWORDS:** sulforaphane, *Pseudomonas aeruginosa*, combined therapy, biological drugs, safety



## INHIBICIJA NRF2 SIGNALNOG PUTA U JETRI PACOVA NAKON SUBHRONIČNE IZLOŽENOSTI SMEŠI TOKSIČNIH METALA

MIXTURES  
TOXICOLOGY

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Nuklearni eritroidni faktor 2 (Nrf2) je jedan od ključnih odbrambenih faktora protiv različitih patoloških procesa, kao što su oksidativno oštećenje i karcinogeneza. Nedavno je primećeno da se izloženost ljudi niskim dozama toksičnih metala može povezati sa razvojem različitih bolesti. Cilj ove studije bio je ispitati efekat smeše toksičnih metala (Cd, As, Hg, Pb, Cr, Ni) na koncentraciju Nrf2 u tkivu jetre Wistar pacova nakon 90 dana ekspozicije. Životinje su podeljene u 8 grupa: 2 kontrolne i 6 tretiranih (mužjaci (M), n=20/ ženke (F), n=20).

Oralnom gavažom aplikovani su vodeni rastvori smeša toksičnih metala u tri različite doze preračunate na osnovu prethodno sprovedene studije humanog biomonitoringa (doze koje odgovaraju: medijanama izmerenih koncentracija – M1/F1; 95.-om percentilu izmerenih koncentracija – M2/F2; izračunatoj donjoj granici pouzdanosti Benchmark doze za efekte na nivoe hormona: TSH, fT4, fT3, insulin i testosteron – M3/F3). Životinje su žrtvovane i sakupljeni su uzorci jetre. Koncentracija Nrf2 određena je pomoću komercijalnog ELISA kita (Fine Biotech, Vuhan, Kina). Statistički značajno smanjenje koncentracije Nrf2 u tkivu jetre mužjaka pacova uočeno je kod svih tretiranih grupa u odnosu na kontrolu, dok je kod ženki značajno sniženje Nrf2 primećeno jedino kod F3 grupe, i to u poređenju sa kontrolnom, F1 i F2 grupama. Dobijeni rezultati ukazuju na sposobnost smeše metala da inhibiraju Nrf2 signalni put, snižavajući koncentraciju ovog faktora, što može predstavljati jedan od mehanizama njihove toksičnosti.

**KLJUČNE REČI:** toksični metali, Nrf2 signalni put, Wistar pacovi, mehanizam toksičnosti

**ZAHVALNICA:** Ovo istraživanje je podržao Fond za nauku Republike Srbije, PROMIS, Grant No 6066532, DecodExpo projekat.



## TOXIC METAL MIXTURE INHIBITED NRF2 SIGNALING PATHWAY IN RAT LIVER AFTER SUBCHRONIC EXPOSURE

MIXTURES  
TOXICOLOGY

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Nuclear erythroid factor 2 (Nrf2) is one of the key defence factors against various pathological processes, including oxidative damage and carcinogenesis. Recently, human exposure to low doses of toxic metals has been associated with the development of various diseases. The aim of this study was to examine the effect of a mixture of toxic metals (Cd, As, Hg, Pb, Cr, Ni) on Nrf2 concentration in liver tissue of Wistar rats after 90-day exposure. The animals were divided into 8 groups: 2 control and 6 treated (male (M) n=20/ female (F) n=20).

Aqueous solutions of toxic metal mixtures were administrated in three different doses calculated on the basis of previously conducted human biomonitoring study (doses based on: median concentrations – M1/F1; 95th percentile concentrations – M2/F2; calculated lower confidence limit of the Benchmark dose for effects on hormone levels: TSH, fT4, fT3, insulin and testosterone – M3/F3). Animals were sacrificed and liver samples were collected. The concentration of Nrf2 was determined by commercial ELISA kit (Fine Biotech, Wuhan, China). A statistically significant decrease in the concentration of Nrf2 in the liver tissue of male rats was observed in all treated groups compared to the control, while in females a significant decrease in Nrf2 was observed only in the F3 group compared to the control, F1 and F2 groups. The obtained results indicate the ability of the metal mixture to inhibit the Nrf2 signaling pathway, lowering the concentration of this factor, which can be proposed as one of the mechanisms of their toxicity.

**KEYWORDS:** toxic metals, Nrf2 signaling pathway, Wistar rats, toxicity mechanism

**ACKNOWLEDGEMENT:** This research was supported by the Science Fund of the Republic of Serbia, PROMIS, Grant No 6066532, DecodExpo project.



## PHLORETIN MITIGATES ARSENIC AND CHROMIUM-INDUCED REPRODUCTIVE DAMAGE BY ACTIVATING THE NRF2 PATHWAY AND INHIBITING THE OXIDATIVE STRESS

MIXTURES  
TOXICOLOGY

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Heavy metal pollution of the environment has lately been connected to escalating ecological and global concerns for public health. Human exposure has also greatly grown as a result of an inevitable rise in their use in numerous industrial, agricultural, domestic, and technical applications. The most frequent and pervasive environmental toxins that have adverse health consequences are arsenic and chromium. It is hardly surprising that natural products have made a considerable contribution to the growth of human pharmaceutical collections over time. They are the main source of innovative scaffolds for developing powerful drugs to address a variety of health ailments.

Hence, the objective of this study was to examine the salutary effects of phloretin against combined arsenic and chromium-induced reproductive toxicity in male Swiss albino mice. Sodium meta-arsenite (100 ppm) and potassium dichromate (75 ppm) were given orally in conjunction with intraperitoneal administration of phloretin (50 mg/kg) for a period of two weeks. It was observed that the number of non-motile sperms was higher in the arsenic and chromium co-exposed intoxicated group along with a decrease in organ (testis) weight and testis somatic index as compared to the control group. Several biochemical indices were assessed and the result showed that lipid peroxidation (LPO) levels were found to increase, together with the reduced levels of glutathione s-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) in the co-exposed intoxicated mice. The aforementioned altered parameters were reversed by the administration of phloretin and thereby preserving cellular homeostasis along with the upregulation in the nuclear factor erythroid 2-related factor 2 (Nrf2) gene expression. The results demonstrate the therapeutic properties of this bioactive molecule (phloretin) against the combined arsenic and chromium-activated reproductive toxicity by mitigating the induced oxidative stress.

**KEYWORDS:** heavy metal, arsenic, chromium, natural compound, phloretin, Nrf2



## ISTRAŽIVANJE MOLEKULARNIH MECHANIZAMA TOKSIČNOSTI SMEŠE PFAS JEDINJENJA: TOKSIKOGENOMSKA ANALIZA PODATAKA

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Zbog svoje rasprostranjenosti u životnoj sredini, perzistentnosti i toksičnih efekata, per- i polifluoroalkil supstance (PFAS) predstavljaju rizik po zdravlje ljudi. Cilj ovog istraživanja bio je proceniti molekularne mehanizme toksičnosti smeše 4 najzastupljenija PFAS jedinjenja (perfluorooxanske kiseline (PFOA), perfluorooktan sulfonata (PFOS), perfluorononanske kiseline (PFNA) i perfluoroheksan sulfonata (PFHxS)) toksikogenomskom analizom podataka. Komparativna toksikogenomska baza podataka (engl. Comparative Toxicogenomic Database, <http://ctdbase.org>), GeneMANIA (<https://genemania.org>), ToppGene Suite (<https://toppgene.cchmc.org>) i CytoHubba (<https://apps.citoscapes.org/apps/cytohubba>) korišćeni su za in silico analizu. PFOA, PFOS, PFNA i PFHxS su interagovali sa 3814, 3448, 2077 i 581 genom, redom, dok je 309 gena bilo zajedničko za sve PFAS.

Većina ovih gena bila je u koekspresiji (45,07%), dok 5 najvažnijih gena u celom setu predstavljaju strukturne komponente ribozoma, kodiraju ribozomske proteine i učestvuju u citoplazmatskoj translaciji (RPL13, RPL10, RPL18, RPLP0 i RPS15). Najvažnije bolesti povezane sa smešom PFAS uključuju poremećaje ish-rane i metabolizma, poremećaje gastrointestinalnog trakta i jetre, kao i metabolizma lipida. Biološki procesi uključuju ćelijski i lipidni metabolički proces, kao i odgovor na stimulus i biološku regulaciju, molekularne funkcije uključuju procese vezivanja (vezivanje proteina, jona, organskog cikličkog jedinjenja i heterocikličnih jedinjenja) i katalitičku aktivnost, dok molekularni putevi uključuju PPAR signalizaciju, metabolizam lipida i lipoproteina, metabolizam masnih kiselina, triacilglicerola i ketonskih tela, meta-put nuklearnih receptora i beta-oksidaciju masnih kiselina. Dobijeni rezultati o molekularnim mehanizmima toksičnosti smeše PFAS, uključujući dejstvo na metaboličke procese, a posebno metabolizam lipida, daju osnovu za dalja in vitro i in vivo laboratorijska ispitivanja.

**KLJUČNE REČI:** PFAS jedinjenja, toksikogenomika, in silico predviđanje, genomski biomarkeri, mehanizmi toksičnosti



## EXTRACTING MOLECULAR MECHANISMS OF TOXICITY OF PFAS MIXTURE: TOXICOGENOMIC DATA MINING

MIXTURES  
TOXICOLOGY

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Because of their widespread dispersion in the environment, persistency and toxic effects, per- and polyfluoroalkyl substances (PFAS) pose a risk to human health. This research aimed to assess molecular mechanisms of toxicity of the mixture of 4 most prevalent PFAS (perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonate (PFHxS)) by conducting toxicogenomic data-mining. Comparative Toxicogenomics Database (<http://ctdbase.org>), GeneMANIA (<https://genemania.org>), ToppGene Suite (<https://toppgene.cchmc.org>), and CytoHybba (<https://apps.cytoscape.org/apps/cytohubba>) were used for the in silico analysis. PFOA, PFOS, PFNA and PFHxS interacted with 3814, 3448, 2077 and 581 genes, respectively, while 309 genes were mutual for all PFAS.

The majority of these genes was in co-expression (45.07%), while 5 most important genes in the whole set are structural components of ribosomes which encode ribosomal proteins and are involved in cytoplasmic translation (RPL13, RPL10, RPL18, RPLP0 and RPS15). Most important diseases connected with PFAS mixture included nutritional and metabolic, digestive system, liver and diseases, as well as lipid metabolism. Biological processes included cellular, metabolic, and lipid metabolic process, response to stimulus and biological regulation, molecular functions included binding processes (protein binding, ion binding, organic cyclic compound binding, heterocyclic compound binding) and catalytic activity, while pathways included PPAR signaling, metabolism of lipids and lipoproteins, fatty acid, triacylglycerol, and ketone body metabolism, nuclear receptors meta-pathway and fatty acid beta-oxidation. The obtained results on the molecular mechanisms of PFAS mixture toxicity, including disturbances of metabolic processes, especially lipid metabolism, provide a basis for further in vitro and in vivo laboratory tests.

**KEYWORDS:** PFAS compounds, toxicogenomics, in silico prediction, genomic biomarkers, mechanisms of toxicity



## TOKSIKOLOŠKA PROCENA RIZIKA / TOXICOLOGICAL RISK ASSESSMENT

### ULOGA BIOMONITORINGA U PROCENI RIZIKA

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Humani biomonitoring (HBM) uvodi koncept i svest o tome kako se mere i analiziraju nivoi hemikalija, zagađivača i drugih supstanci u ljudskom telu radi procene izloženosti i uticaja na zdravlje. HBM je naučna disciplina koja koristi biološke uzorke, kao što su krv, urin, kosa i druga tkiva, da bi se utvrdilo koliko su ljudi izloženi štetnim supstancama. Cilj HBM-a je stvaranje preciznih i pouzdanih informacija o nivou izloženosti štetnim supstancama u opštoj populaciji i informisanje javnih zdravstvenih politika i strategija za zaštitu zdravlja. Analiza bioloških uzoraka se obično vrši korišćenjem osetljivih analitičkih metoda kako bi se osigurala tačnost rezultata.

HBM je važan za praćenje trendova izloženosti i procenu uticaja na zdravlje, kao i za informisanje javnosti i usvajanje politika koje će smanjiti izloženost štetnim supstancama. Rezultati HBM istraživanja se takođe koriste za razvoj preventivnih strategija i smanjenje rizika po ljudsko zdravlje. Koncepti i metodologija HBM odnose se na naučni pristup i tehnike koje se koriste za merenje nivoa hemikalija, zagađivača i drugih supstanci u ljudskom telu, uključujući krv, urin, kosu i druga tkiva. Koncept HBM zasniiva se na ideji da se ljudsko telo može koristiti kao „biološki indikator“ za praćenje izloženosti štetnim supstancama i procenu potencijalnog uticaja na zdravlje ljudi. Metodologija HBM uključuje prikupljanje i analizu bioloških uzoraka, kao što su krv ili urin, kako bi se odredili nivoi supstanci prisutnih u telu.

**KLJUČNE REČI:** humani biomonitoring, koncept, metodologija





## THE ROLE OF BIOMONITORING IN RISK ASSESSMENT

TOXICOLOGICAL  
RISK ASSESSMENT

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Human biomonitoring (HBM) introduces the concept and awareness of how the levels of chemicals, pollutants and other substances in the human body are measured and analyzed to assess exposure and health effects. HBM is a scientific discipline that uses biological samples, such as blood, urine, hair and other tissues, to determine how much people are exposed to harmful substances. The goal of HBM is to create accurate and reliable information on the level of exposure to harmful substances in the general population and to inform public health policies and health protection strategies. Analysis of biological samples is usually performed using sensitive analytical methods to ensure the accuracy of the results.

HBM is important for monitoring exposure trends and assessing health impacts, as well as informing the public and adopting policies that will reduce exposure to harmful substances. The results of HBM research are also used to develop preventive strategies and reduce risks to human health. The concepts and methodology of human biomonitoring refer to the scientific approach and techniques used to measure the levels of chemicals, pollutants, and other substances in the human body, including blood, urine, hair, and other tissues. The HBM concept is based on the idea that the human body can be used as a "biological indicator" to monitor exposure to harmful substances and assess the potential impact on human health. The HBM methodology involves the collection and analysis of biological samples, such as blood or urine, to determine the levels of substances present in the body.

**KEYWORDS:** biomonitoring, concept, methodology



# PROCENA KARCINOGENOG RIZIKA OD IZLOŽENOSTI ODRASLIH I DECE BENZO(A)PIRENU PUTEM ZAGAĐENOG VAZDUHA U KRITIČNIM PODRUČJIMA REPUBLIKE SRBIJE

TOXICOLOGICAL  
RISK ASSESSMENT

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Tokom prethodnih godina, Republika Srbija svrstava se među najzagađenije balkanske i evropske zemlje. Dominantna zagađujuća materija su suspendovane čestice koje u urbanim područjima čine toksični metali i organska jedinjenja kao što su policiklični aromatični ugljovodoni (PAU). Glavni izvori PAU su sagorevanje čvrstih goriva i emisija iz saobraćaja. Kao indikator izloženosti PAU koristi se benzo(a)piren (B(a)P), s obzirom da je njegov udeo u smeši ovih jedinjenja i do 20%. B(a)P je svrstan u 1. grupu karcinogena jer izaziva karcinom pluća, a mnoge studije ukazuju da B(a)P iz zagađenog vazduha ima najveći doprinos u ukupnom karcinogenom potencijalu PAU, od 50% do 67%. Cilj rada bila je procena karcinogenog rizika od inhalacione izloženosti odraslih i dece B(a)P putem zagađenog vazduha u pet odabranih kritičnih područja Srbije.

Vrednosti srednjih godišnjih koncentracija B(a)P tokom 2020. i 2021. godine su preuzete iz Izveštaja Agencije za zaštitu životne sredine Srbije za merne stanice u Subotici, Smederevu, Boru, Valjevu i Kragujevcu. Prilikom procene rizika korišćena je metodologija Američke agencije za zaštitu životne sredine. Neprihvatljiv rizik po zdravlje izložene populacije predstavljaju ILCR vrednosti više od 1.0E-06. Izračunate ILCR vrednosti prelaze ovu vrednost, i za odrasle i za decu, na stanicama Subotica – OŠ „Sonja Marinković“ (1,49E-06; 1,82E-06) i Valjevo (1,79E-06; 2,18E-06). Iako su rizici na preostalim mernim stanicama bili prihvatljiviji za obe razmatrane populacije, svaka izračunata vrednost za decu bila je viša od vrednosti za odrasle, ukazujući na viši rizik od izloženosti dece. Dobijeni rezultati ukazuju na potrebu preduzimanja mera u cilju smanjenja emisije B(a)P u vazduhu u Republici Srbiji.

**KLJUČNE REČI:** benzo(a)piren, suspendovane čestice, zagađenje vazduha, procena rizika



# CARCINOGENIC RISK ASSESSMENT FROM ADULTS' AND CHILDREN'S EXPOSURE TO BENZO(A)PYRENE FROM THE POLLUTED AIR IN CRITICAL SITES OF THE REPUBLIC OF SERBIA

TOXICOLOGICAL  
RISK ASSESSMENT

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Republic of Serbia is considered as one of the most polluted Balkan and Europe country due to particulate pollution. In urban areas, toxic metals and polycyclic aromatic hydrocarbons (PAHs) are the main compounds of particulate matter. The most researched PAH is benzo(a)pyrene (B(a)P), which makes up the majority of the mixture of PAHs (approximately 20%) and has the highest carcinogenic potential of all PAHs in polluted air (between 50% and 67%). It is classified as human carcinogen, related to lung cancer development. Combustion of fossil fuels and vehicle emission are main anthropogenic sources. The aim of this study was to assess inhalation carcinogenic risk from B(a)P exposure of adults and children through the polluted air in five critical sites of Serbia.

Mean yearly concentrations of B(a)P for 2020-2021 were used from Reports of the Serbian Environmental Protection Agency, for measuring stations in Subotica, Smederevo, Bor, Valjevo and Kragujevac. The USEPA method was used to calculate incremental lifetime cancer risk (ILCR) values and the risk was classified as acceptable ( $1.0E-06$ ). Obtained ILCR values were acceptable for all measuring station and both for adults and children, except for Subotica – primary school "Sonja Marinkovic" ( $1.49E-06$ ;  $1.82E-06$ ) and Valjevo ( $1.79E-06$ ;  $2.18E-06$ ). Although obtained acceptable risk for both considered populations at other three measuring stations, obtained ILCR values for children were one order of magnitude higher than for adults, which suggest higher risk for children's health. Obtained results are showing that actions for reduction of air pollution in Serbia should be taken.

**KEYWORDS:** benzo(a)pyrene, particulate matter, air pollution, risk assessment



# PROCENA RIZIKA OD OSTATAKA PESTICIDA U GROŽĐU I VINU IZ ORGANSKE I KONVENCIONALNE PROIZVODNJE VINOVE LOZE

TOXICOLOGICAL  
RISK ASSESSMENT

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Priklom analize ostataka pesticida u 250 uzoraka crnog i belog vinskog grožđa iz konvencionalne i organske proizvodnje detektovano je 20 ostataka pesticida. Na osnovu koncentracija detektovanih ostataka dobijenih u ovom istraživanju urađena je procena izloženosti populacije preko grožđa. Ulazni elementi korišćeni za procenu izloženosti su sledeći: populacione grupe, podaci o prosečnoj telesnoj masi, podaci o konzumiranoj količini grožđa, podaci o sadržaju ostataka pesticida u grožđu.

Procena hronične izloženosti ostacima pesticida sprovedena je korišćenjem prosečnih detektovanih koncentracija u grožđu. Takođe je određivana i izloženost pri akutnom unosu za pesticide sa definisanim akutnim referentnim dozama (ARfD). Doprinos crnog grožđa TDU prevazilazi 1% u slučaju difenokonazola: 38,7%, 19,3%, 11,6%, 7,5% i 5,1%, redom za odojčad, predškolsku decu, decu, adolescente i odrasle. U slučaju belog grožđa, unos difenokonazola je od 0,5% (odrasli) do 4,0% (odojčad), a slede tebukonazol, ciprodinil i propikonazol (0,4 – 3,0%, 0,17 – 1,3%, 0,13 – 1,0%, redom). Procena akutnog unosa za pesticide koji prekoračuju ARfD pokazuje da je pri konzumiranju crnog grožđa akutni referentni unos difenokonazola prekoračen 6 puta (adolescenti), odnosno 4 puta (odrasli), dok za odojčad iznosi 21%. U grupi predškolske dece i dece unos difenokonazola dostiže 61% i 37% ARfD, redom. Unos tebukonazola prekoračuje ARfD: za crno grožđe u grupi adolescenata (145%), i za belo grožđe kod odojčadi, adolescenata i odraslih (103%, 532% i 361%, redom). Rizik od ostataka pesticida u grožđu nije prihvatljiv za odojčad, adolescente i odrasle u Republici Srbiji, u slučaju grožđa sa ostacima difenokonazola i tebukonazola.

**KLJUČNE REČI:** ostaci pesticida, grožđe, procena rizika



# RISK ASSESSMENT OF PESTICIDE RESIDUES IN GRAPES AND WINE FROM ORGANIC AND CONVENTIONAL VINE PRODUCTION

TOXICOLOGICAL  
RISK ASSESSMENT

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Pesticide residues were analyzed in 250 samples of black and white wine grapes from conventional and organic production and 20 pesticide residues were detected. Based on the concentrations of residues obtained in this research, an assessment of population exposure through grapes was made. The following input elements used for exposure assessment are: population groups, data on average body weight, amount of grape consumption, concentration of pesticide residues. Assessment of chronic exposure to pesticide residues was carried out using average detected concentrations in grapes.

Acute intake exposure was also determined for pesticides with defined acute reference dose (ARfD). The contribution of red grapes to the TDI exceeds 1% in the case of difenoconazole: 38.7%, 19.3%, 11.6%, 7.5% and 5.1%, for infants, preschool children, children, adolescents and adults, respectively. In the case of white grapes, the intake of difenoconazole 0.5% (adults) to 4.0% (infants), followed by tebuconazole, cyprodinil and propiconazole (0.4 – 3.0%, 0.17 – 1.3%, 0.13 – 1.0%, respectively). In case of black grapes consumption, the acute reference intake of difenoconazole was exceeded 6 times (adolescents) and 4 times (adults), while for infants it was 21%. In the group of preschool children and children, intake of difenoconazole reaches 61% and 37% of ARfD, respectively. Tebuconazole intake exceeded the ARfD: for black grapes in the adolescent group (145%), and for white grapes in infants, adolescents and adults (103%, 532% and 361%, respectively). The risk of pesticide residues in grapes is not acceptable for infants, adolescents and adults in the Republic of Serbia in the case of grapes with difenoconazole and tebuconazole residues.

**KEYWORDS:** pesticide residues, wine grapes, risk assessment



# RIZIK OD TOKSIČNIH METAL(OID)A I BENZO(A)PIRENA NA SUSPENDOVANIM ČESTICAMA PM10 U VAZDUHU GRADA NOVOG SADA

TOXICOLOGICAL  
RISK ASSESSMENT

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Toksični metal(oid)i i policiklični aromatični ugljovodonici na suspendovanim česticama (PM) u vazduhu mogu izazvati ozbiljne negativne zdravstvene efekte. Cilj ove studije je sagledavanje kompozicionih varijacija kroz vreme i prostor, u smislu koncentracija olova, kadmijuma, arsena, nikla i benzo(a)pirena (BaP) i procena zdravstvenog rizika. PM10 su uzorkovane tokom 2019-2022 godine na pet lokacija u Novom Sadu i 3225 rezultujućih uzoraka je podvrgnuto ICP-MS (metal(oid)i) i GC-MS analizi (BaP). Ne-karcinogeni rizik, izražen kao indeks opasnosti (HI) dobijen sumiranjem količnika opasnosti individualnih metal(oid)a, kretao se od 0.27 na osnovnoj ruralnoj do 0.37 na prigradsko-saobraćajnoj lokaciji, sa prosečnom vrednošću od 0.33 (usrednjavanje svih lokacija), ukazujući na odsustvo ozbiljnog rizika.

Doprinos metal(oid)a opadao je sledećim redosledom: Ni (72.0%), As (18.2%), Cd (9.2%), Pb (0.6%). Karcinogeni rizik za decu kretao se od 6.5E-07 (osnovna ruralna) do 9.1E-07 (prigradsko-saobraćajna), sa prosekom na 8.4E-07, ukazujući na veoma nizak rizik. Karcinogeni rizik za odrasle varirao je od 2.6E-06 (osnovna ruralna) do 3.6E-06 (prigradsko-saobraćajna i industrijska), sa prosekom od 3.4E-06, ukazujući na nizak rizik. Metal(oid) sa najvećim doprinosom je bio As, praćen Ni, Pb i Cd. Sa druge strane, karcinogeni rizik uzrokovan prisustvom BaP je bio zanemarljiv i za decu (od 6.2E-08 do 1.5E-07, prosek 9.7E-08) i za odrasle (od 2.5E-07 do 5.8E-07, prosek 3.9E-07). Mada se procenjeni rizik čini prihvatljivim, potrebno je naglasiti da drugi putevi izloženosti (ingestija i dermalni kontakt) kao i drugi izvori (hrana i voda) mogu značajno doprineti ukupnom unosu široko rasprostranjenih kontaminanata.

**KLJUČNE REČI:** monitoring vazduha, procena rizika, Novi Sad



# HEALTH RISK OF TOXIC METAL(OID)S AND BENZO(A)PYRENE ON PM 10 PARTICULATE MATTER IN THE CITY OF NOVI SAD AIR

TOXICOLOGICAL  
RISK ASSESSMENT

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The toxic metal(oid)s and aromatic polycyclic hydrocarbons present on particulate matter (PM) in air could cause serious adverse health effects. The current study aimed to investigate PM10 compositional variations in time and location, in terms of concentrations of lead, cadmium, arsenic, nickel and benzo(a)pyrene (BaP), and to conduct health risk assessment. Therefore, PM10 were sampled during 2019-2022 at five monitoring sites in Novi Sad and resulting 3225 samples were subjected to ICP-MS (metal(oid)s) and GC-MS analysis (BaP). With regard to non-carcinogenic risk, hazard index (HI), obtained as a sum of hazard quotients (HQ) of individual metal(oid)s, ranged from 0.27 on basic rural to 0.37 on suburban-traffic site, with mean at 0.33 (averaging over all sites), revealing no serious health risk. Contributions of the metal(oid)s decreased in the following order: Ni (72.0%), As (18.2%), Cd (9.2%), Pb (0.6%).

Carcinogenic risk for children ranged from 6.5E-07 (basic rural) to 9.1E-07 (suburban-traffic), with mean at 8.4E-07, showing very low risk. On the other hand, carcinogenic risk for adults varied from 2.6E-06 (basic rural) to 3.6E-06 (suburban-traffic and industrial), with mean at 3.4E-06, indicating low risk. The metal(oid) with the highest contribution was As (66.8%), followed by Ni, Pb and Cd. Regarding BaP, carcinogenic risk was negligible for both children (from 6.2E-08 to 1.5E-07, mean 9.7E-08) and adults (from 2.5E-07 to 5.8E-07, mean 3.9E-07). Although estimated risk appeared to be acceptable, it should be emphasized that other exposure pathways (ingestion and dermal) as well as sources (food and water) could significantly contribute to the total burden of ubiquitous environmental contaminants.

**KEYWORDS:** air monitoring, risk assessment, Novi Sad



## PROCJENA IZLOŽENOSTI DJECE ALERGENIMA PREKO IGRAČAKA

TOXICOLOGICAL  
RISK ASSESSMENT

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Alergeni su tip antigena koji proizvodi snažan imuni odgovor u kojem se imuni sistem bori protiv uočene prijetnje. Toksikološka svojstva ftalata, kao alergena, uključuju: endokrine, neurološke i hepatotoksične efekte. Koncentracije sedam ftalata u dječijim igračkama, kupljenim u Krajstčercu na Novom Zelandu pružile su podatke za procjenu izloženosti koja izvodi hazard indekse (HI) za oralne i dermalne puteve izloženosti kod djece. Od 49 analiziranih igračaka, 65% je sadržavalo najmanje jedan ftalat u koncentraciji >0,1% mase; a 35% je sadržavalo smješu ftalata u pojedinačnim koncentracijama >0,1%. HI od 3,4 izveden je za izloženost četiri ftalata, povezana sa reproduktivnim i efektima na rast i razvoj. HI od 0,3 izveden je za grupu ftalata, povezanih sa hepatotoksičnim efektima.

Olovo kao alergen je teratogen i karcinogen i akumulira se u kostima. Najčešći izvori olova su: stare stambene zgrade u kojima je korištena olovna boja tokom gradnje, zagađeni vazduh u blizini industrije i voda i hrana. Više koncentracije mogu se pojaviti i u kravljem mlijeku. Trovanje olovom isključivo je hronično i zavisi od doze kojoj je pojedinac izložen. Od 100 analiziranih dječijih igračaka, 23 igračke u svom sastavu sadržavale su koncentracije olova koje su više od dozvoljenih. Granične vrijednosti olova u suvom, lomljivom, praškastom ili savitljivom materijalu za igračke su 13,5 mg/kg; u tečnom ili ljepljivom materijalu – 3,4 mg/kg; u materijalu, ostruganom sa površine igračke – 160 mg/kg. Roze i jeftine igračke imaju više koncentracije olova od ostalih igračaka.

**KLJUČNE REČI:** ftalati, olovo, izloženost, hazard indeks





## ASSESSMENT OF CHILDREN'S EXPOSURE TO ALLERGENS THROUGH TOYS

TOXICOLOGICAL  
RISK ASSESSMENT

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Allergens are a type of antigen that produces a strong immune response in which the immune system fights against a perceived threat. The toxicological properties of phthalates, as an allergen, include: endocrine, neurological and hepatotoxic effects. Concentrations of seven phthalates in children's toys purchased in Christchurch, New Zealand provided data for an exposure assessment deriving hazard indices (HI) for oral and dermal routes of exposure in children. Of the 49 analyzed toys, 65% contained at least one phthalate in a concentration >0.1% by mass; and 35% contained a mixture of phthalates in individual concentrations >0.1%. An HI of 3.4 was derived for exposure to four phthalates associated with reproductive and developmental effects. An HI of 0.3 was derived for the group of phthalates associated with hepatotoxic effects.

Lead as an allergen is teratogenic and carcinogenic and accumulates in bones. The most common sources of lead are: old residential buildings where lead paint was used during construction, polluted air near industry and water and food. Higher concentrations can also occur in cow's milk. Lead poisoning is exclusively chronic and depends on the dose to which the individual is exposed. Out of 100 analyzed children's toys, 23 toys in their composition contained concentrations of lead that were higher than allowed. Limit values of lead in dry, brittle, powdery or flexible material for toys are 13.5 mg/kg; in liquid or sticky material - 3.4 mg/kg; in the material scraped from the surface of the toy - 160 mg/kg. Pink and cheap toys have higher concentrations of lead than other toys.

**KEYWORDS:** phthalates, lead, exposure, hazard index



## ETIL KARBAMAT U RAKIJAMA OD VOĆA - PROCENA RIZIKA ZA ZDRAVLJE

TOXICOLOGICAL  
RISK ASSESSMENT

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Najnovija istraživanja Svetske zdravstvene organizacije pokazala su da se svake godine 3 miliona smrtnih ishoda javi kao direktna posledica konzumiranja alkoholnih pića, zbog prisustva jedinjenja koja mogu imati potencijalno karcinogeni efekat, među kojima je i etil karbamat (EC). Ciljevi rada su određivanje sadržaja EC u uzorcima rakija od različitih vrsta voća, poređenje dobijenih vrednosti sa limitom preporučenim od strane Međunarodne alijanse za odgovorno konzumiranje alkohola i komparativna procena zdravstvenog rizika izazvanog konzumiranjem voćnih rakija. Analiza 134 uzorka alkoholnih pića sa tržišta Republike Srbije sprovedena je primenom GC-MS.

Rizik je procenjen izračunavanjem margine izloženosti (MOE) i karcinogenog rizika tokom životnog veka, na osnovu faktora nagiba za oralnu toksičnost (1), odnosno bezbedne doze (2). U 95% uzoraka sadržaj EC je bio iznad limita od 0,4 mg/L. Uočen je statistički značajno viši sadržaj EC u rakijama od šljive. Za muškarce, 98% uzoraka je pokazalo MOE vrednosti ispod dozvoljenog limita od 10000 pri prosečnoj potrošnji alkohola, odnosno 100% kod hroničnog visokog unosa alkohola, dok je za žene to iznosilo od 79% pri prosečnoj potrošnji do 99% za visoki unos. Zavisno od primenjenog pristupa proceni, utvrđeno je da već pri prosečnoj potrošnji: (1) nijedan uzorak nema zanemarljiv karcinogeni efekat, nezavisno od pola, odnosno (2) više od 88% uzoraka predstavlja rizik po zdravlje žena, odnosno 99% po zdravlje muškaraca. Istraživanje je pokazalo prisustvo značajnih koncentracija EC u voćnim rakijama. Potrebno je primeniti mere u toku proizvodnje u cilju smanjenja nastanka EC i zakonski regulisati maksimalno dozvoljenu koncentraciju etil karbamata u rakijama.

**KLJUČNE REČI:** etil karbamat, MOE, karcinogeni rizik, voćne rakije



## ETHYL CARBAMATE IN FRUIT SPIRITS - HEALTH RISK ASSESSMENT

TOXICOLOGICAL  
RISK ASSESSMENT

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The latest World Health Organization research revealed that 3 million deaths every year result from harmful use of alcohol, due to the presence of compounds, including ethyl carbamate (EC), that can have potentially carcinogenic effect. The aims of this study are determination of EC content in fruit spirits, comparison of the obtained values with the limit proposed by the International Alliance for Responsible Alcohol Consumption and comparative health risk assessment caused by the fruit spirits consumption. Fruit spirit samples (134, Republic of Serbia) were analyzed using GC-MS. Risk was assessed using the margin of exposure (MOE) and excess lifetime cancer risk approach, based on the oral slope factor (1) or virtually safe dose (2). The EC content was above limit of 0.4 mg/L in 95% of samples. Higher content in plum spirits was statistically significant.

For men, 98% of the samples showed MOE values below the limit of 10 000 for average consumption, i.e., 100% for chronic heavy drinkers, while for women it ranged from 79% for average consumption to 99% for chronic heavy drinkers. Depending on the approach, already at average consumption: (1) no sample had a negligible cancer risk, regardless of gender; (2) more than 88% of the samples represented a risk to women's health, i.e., 99% to men's health. Research has shown a significant presence of EC in fruit spirits. It is necessary to implement measures during production to prevent the occurrence of EC, as well as legally regulate the maximum allowed concentration of EC in fruit spirits.

**KEYWORDS:** ethyl carbamate, MOE, carcinogenic risk, fruit spirits



## ODREĐIVANJE PRISUSTVA AKRILAMIDA U UZORCIMA PRŽENE KAFE I PROCENA RIZIKA NA ZDRAVLJE POTROŠAČA NA TRŽIŠTU REPUBLIKE SRBIJE

TOXICOLOGICAL  
RISK ASSESSMENT

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Istraživanje je imalo za cilj da ispita koncentraciju akrilamida u prženoj kafi i uradi procenu rizika dnevnog konzumiranja kafe po zdravlje ljudi. Uzorcima kafe iz maloprodajnih objekata dodat je akrilamidom-D3 kao interni standard. Uzorci kafe su ekstrahovani heksanom i vodom na 40 °C, a ekstrakt je prečišćen preko CHROMABOND® ABC18 SPE kolona.

Prečišćeni ekstrakti su analizirani validovanom LC-MS/MS metodom u SRM režimu praćenjem prelaza 72 na 55,2 i 44,3 m/z za akrilamid i 75 na 58,1 m/z za akrilamid-D3. LOQ je bio 50 µg/kg, dok su detektovani nivoi akrilamida bili u intervalu od 155 do 540 µg/kg. Imajući u vidu da stanovnici Srbije piju više od četiri šoljice pržene kafe dnevno (5 kg kafe/osobi/godišnje), izračunati su i procenjeni dnevni unos (EDI) i podnošljivi dnevni unos (TDI). Utvrđeno je da se EDI kreće između 0,03 i 0,10 mg/kg telesne težine/dan, dok TDI iznosi 2,6 µg/kg telesne težine/dan. Rezultati ove studije, ističu bezbednu potrošnju pržene kafe prema referentnim nivoima za prisustvo akrilamida u namirnicama (EU/2017/2158). Zdravstveni rizik usled konzumiranja ispitivane kafe je zanemarljiv.

**KLJUČNE REČI:** akrilamid, kafa, procena rizika



## DETERMINATION AND HEALTH RISK ASSESSMENT OF ACRYLAMIDE LEVELS IN COFFEE SAMPLES ON THE MARKET OF THE REPUBLIC OF SERBIA

TOXICOLOGICAL  
RISK ASSESSMENT

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This research aimed to investigate acrylamide concentration in roasted coffee and human health risk assessment of daily consumed coffee. Collected coffee samples from retail stores were spiked with D3-labeled acrylamide as an internal standard. Coffee samples were extracted by hexane and water at 40 °C and cleaned up by CHROMABOND® ABC18 SPE. The cleaned-up extracts were analyzed using LC-MS/MS in SIM mode by monitoring at m/z 72 to 55.2 and 44.3 for acrylamide and m/z 75 to 58.1 for acrylamide-D3, with a previously validated method.

The LOQ was 50 µg/kg, while the levels of acrylamide ranged from 155 to 540 µg/kg. Having in mind that Serbian residents drink more than four cups of roasted coffee a day (5 kg of coffee/per person/year), estimated daily intake (EDI), and tolerable daily intake (TDI) were also calculated. EDI was established to range between 0.03 and 0.10 µg/kg of body weight/day, with TDI of 2.6 µg/kg of body weight/day, respectively. The results of the present study, therefore, highlight the safe consumption of roasted coffee according to the benchmark levels for the presence of acrylamide in foodstuffs (EU/2017/2158). The health risk due to the consumption of investigated coffee is negligible.

**KEYWORDS:** acrylamid, roasted coffee, risk assessment



## RIZIK PO ZDRAVLJE ŠKOLSKE DECE S OBZIROM NA UKUPNU IZLOŽENOST PBDE- IMA SADRŽANIM U PRAŠINI IZ ŠKOLA I DOMAĆINSTAVA

TOXICOLOGICAL  
RISK ASSESSMENT

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Polibromovani difeniletri (PBDE) su klasa perzistentnih organskih zagađivača koji se koriste kao usporivači plamena za zaštitu od požara građevinskih materijala i raznih potrošačkih proizvoda. Oni su prvenstveno zagađivači u zatvorenom prostoru i akumuliraju se u prašini, koja je, uz hranu, glavni izvor izloženosti ljudi PBDE-ima. PBDE su izazvali zabrinutost javnosti zbog dokaza o njihovom štetnom uticaju na zdravlje ljudi. Školska deca (7-14 g) provode značajan deo dana (~5 h) u osnovnoj školi što može značajno doprineti ukupnom unosu PBDE-a. Nivoi 7 kongenera PBDE utvrđeni su u prašinama prikupljenim u osnovnim školama (N = 11) i u domaćinstvima (N = 30) u Zagrebu, Hrvatska.

Ukupne procenjene vrednosti dnevnog unosa (EDI) za  $\Sigma$ 7PBDE detektovane u prašini iz domova i škola bile su  $3,27 \times 10^{-3}$  ng kg<sup>-1</sup> dan<sup>-1</sup> u centralnom i  $1,16$  ng kg<sup>-1</sup> dan<sup>-1</sup> u najgorem slučaju. Doprinos unosa PBDE-a iz domova bio je za red veličine veći nego iz škola u centralnom scenariju, dok je u najgorem slučaju doprinos oba mikrookruženja bio jednak iako deca provode više vremena u domaćinstvima. Izračunate vrednosti indeksa opasnosti (HI) koji predstavljaju nekancerogeni rizik su  $\ll 1$  što ukazuje da unos PBDE-a putem gutanja prašine iz škola i domaćinstava predstavlja nizak rizik za školsku decu. Ipak, treba voditi računa da ovo nisu jedini zagađivači prisutni u prašini kao i da gutanje prašine nije jedini put izloženosti.

**KLJUČNE REČI:** PBDE-ovi, školska deca, kućna prašina, školska prašina, zdravstveni rizik



## SCHOOL CHILDREN'S HEALTH RISK CONSIDERING OVERALL EXPOSURE TO PBDES CONTAINED IN DUST FROM SCHOOLS AND HOUSEHOLDS

TOXICOLOGICAL  
RISK ASSESSMENT

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Polybrominated diphenyl ethers (PBDEs) are a class of persistent organic pollutants used as flame retardants for the fire protection of construction materials and various consumer products. They are primarily indoor pollutants and accumulate in dust, which, along with food, is the main source of human exposure to PBDEs. PBDEs have caused public concern because of evidence of their harmful effects on human health. School children (7-14 y) spend a significant part of their day (~5 h) in elementary school which can significantly contribute to the total intake of PBDEs.

The levels of 7 PBDE congeners were determined in dusts collected in elementary schools (N =11) and households (N = 30) in Zagreb, Croatia. The total estimated daily intake (EDI) values for the  $\Sigma$ 7PBDEs detected in dust from homes and schools were  $3.27 \times 10^{-3}$  ng kg<sup>-1</sup> day<sup>-1</sup> at the central and 1.16 ng kg<sup>-1</sup> day<sup>-1</sup> at the worst case scenario. The contribution of PBDEs intake from homes was one order of magnitude higher than from schools in the central case scenario, while in the worst case scenario the contribution of both microenvironments was equal even though children spent more time in households. The calculated values of hazard indexes (HI) presenting non-carcinogenic risk were  $\ll 1$  indicating that PBDE uptake via ingestion of dust from schools and households posed a low risk for school children. Nevertheless, care should be taken given that these are not the only contaminants present in dust as well as that dust ingestion is not the only exposure pathway.

**KEYWORDS:** PBDEs, school children, household dust, school dust, health risk



## ANALIZA REZULTATA KONCENTRACIJE FENOLA U URINU NA OSNOVU KONTROLISANOG UNOSA ODREĐENIH NAMIRNICA I ŽIVOTNOG STILA

TOXICOLOGICAL  
RISK ASSESSMENT

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Kod osoba koje nisu profesionalno ili iz životne sredine izložene benzenu koncentracija fenola u urinu može biti povišena. Cilj ovog rada je da se utvrdi u kojoj meri konzumiranje određenih namirnica uz životne navike, poput pušenja, konzumiranja alkohola i lekova utiču na rezultate koncentracije fenola u urinu. Četrdeset dobrovoljaca, 20 žena i 20 muškaraca, starosne dobi 15-58 godina, podeljenih u četiri grupe su učestvovali u ispitivanju: grupa-1 je konzumirala kikiriki dva dana pre sakupljanja uzorka urina, grupa-2 jedan dan pre, grupa-3 tokom oba dana pre uzorkovanja i grupa-4 je bila kontrolna. Korišćen je upitnik za dobijanje podataka o životnim navikama: pušački status, konzumiranje alkohola i upotreba lekova. Za procenu koncentracije fenola u urinu korišćena je semikvantitativna metoda sa Gibbs-ovim reagensom.

U kontrolnoj grupi 7/10 rezultata bilo je negativno (<1,00 mmol/l referentni nivo za procenu izloženosti benzenu), dok su tri rezultata bila pozitivna zbog uticaja pasivnog pušenja, povremene konzumacije alkohola i primene amoksicilina. U grupama ispitanika koji su konzumirali kikiriki dobijeni su sledeći pozitivni rezultati: 4/10 u 1. i 2. grupi i 5/10 u 3. grupi sa dužim konzumiranjem kikirikija. Uz pozitivan rezultat u ovim grupama većina ispitanika puši ili je pasivno izložena duvanskom dimu i povremeno konzumira alkohol. Potvrđeno je da se povišeni rezultati koncentracije fenola u urinu mogu dobiti kod osoba koje nisu profesionalno ili iz životne sredine izloženi benzenu. Duža konzumacija kikirikija, primena amoksicilina, aktivan ili pasivan pušački status i povremeno konzumiranje alkohola mogu imati uticaja na rezultat koncentracije fenola u urinu.

**KLJUČNE REČI:** benzen, fenoli, kikiriki, pušenje





## ANALYSIS OF THE RESULT OF THE CONCENTRATION OF PHENOL IN URINE BASED ON THE CONTROLLED INPUT OF CERTAIN FOOD AND LIFESTYLE

TOXICOLOGICAL  
RISK ASSESSMENT

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Phenol urine concentration could be positive even in people not exposed to benzene (professionally or from the environment). The aim of this work was to determine the extent to which consumption of certain foods and lifestyle habits (smoking, consuming alcohol and drugs) affect the results of phenol concentration in urine. Forty volunteers (20 female + 20 male), aged between 15 and 58, divided into four groups participated in the study: group 1 consumed peanuts two days, group 2 one day before the urine sample collection and group 3 during two days before sampling. Group 4 was control. Life habits were obtained by questionnaire: smoking status, alcohol consumption and use of drugs.

Semi-quantitative method with Gibbs reagent was used to assess the concentration of phenol in urine. In control group 7/10 results were negative (<1.00 mmol/l – reference level for the evaluation of benzene exposure), while three results were positive, due to passive smoking, occasional alcohol consumption and amoxicillin. In groups who consumed peanuts: 4/10 positive results in group 1 and 2, 5/10 in group 3 (prolonged consumption of peanuts). In positive results in groups of subjects who consumed peanuts, most respondents smoked/were passively exposed to tobacco smoke and occasionally consumed alcohol. It was confirmed that elevated results of phenol concentration in urine can be obtained in people not exposed to benzene, professionally or from the environment. Length of peanuts consumption can have an effect on the estimated concentration of phenol in urine.

**KEYWORDS:** benzene, phenols, peanuts, smoking



# RIZIK OD AROMATIČNIH UGLJOVODONIKA (BTEX) U AMBIJENTALNOM VAZDUHU GRADA NOVOG SADA

TOXICOLOGICAL  
RISK ASSESSMENT

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Izloženost aromatičnim ugljovodonicima, benzenu, toluenu, etilbenzenu i ksilenima (BTEX), može imati negativne efekte po zdravlje ljudi. Stoga je cilj ove studije bilo sagledavanje kompozicionih varijacija BTEX u ambijentalnom vazduhu grada Novog Sada i procena zdravstvenog rizika, na osnovu GC-MS analize 2626 uzoraka vazduha (24h) sa prigradsko-saobraćajne i urbano-saobraćajne lokacije tokom 2019-2022 godine. Nekarcinogeni rizik, iskazan kao indeks opasnosti (HI) dobijen sumiranjem količnika opasnosti povezanih sa referentnom inhalatornom koncentracijom individualnih BTEX jedinjenja, iznosio je 0,07 na prigradsko-saobraćajnoj i 0,1 na urbano-saobraćajnoj lokaciji, sa opštim prosekom od 0,09 koji ne izaziva zabrinutost.

Kombinovani doprinos benzena i ksilena činio je preko 99% vrednosti HI, sa odnosom individualnih doprinosa od 2,4 i 1,4 u korist benzena, redom na prigradsko- i urbano-saobraćajnoj lokaciji. Karcinogeni rizik, izračunat na osnovu inhalacionih jedinica rizika benzena i etilbenzena, bio je viši na urbano- nego na prigradsko-saobraćajnoj lokaciji, 2,3E-06 naspram 1,9E-06 u slučaju dece i 9,4E-06 naspram 7,8E-06 u slučaju odraslih, ukazujući na nizak rizik. Preovladjujući pokretač karcinogenog rizika bio je benzen, sa 8,7 puta većim impaktom, kako zbog veće izloženosti, tako i zbog većeg karcinogenog potencijala u odnosu na etilbenzen. Mada je procenjeni rizik povezan sa inhalatornim unosom BTEX iz ambijentalnog vazduha prihvatljiv, potrebno je razmotriti izuzetnu brojnost toksikanata koji imaju potencijal da ugroze ljudsko zdravlje, prisutnih ne samo u vazduhu već i u drugim medijumima životne sredine. Stoga je veoma izražena potreba za obezbeđenjem kvaliteta vazduha/životne sredine, primenom sveobuhvatnog pristupa koji angažuje društvo u celosti.

**KLJUČNE REČI:** monitoring, procena rizika, Novi Sad



## HEALTH RISK OF AROMATIC HYDROCARBONS (BTEX) IN THE CITY OF NOVI SAD AMBIENT AIR

TOXICOLOGICAL  
RISK ASSESSMENT

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The exposure to aromatic hydrocarbons, benzene, toluene, ethyl benzene and xylenes (BTEX), could have adverse health impact. Therefore, current study aimed to investigate compositional variations of BTEX in the ambient air of the City of Novi Sad and to assess consequent health risk, based on GC-MS analysis of 2626 air samples (24h) obtained on suburban-traffic and urban-traffic sites during 2019-2022. The non-carcinogenic risk, presented as hazard index (HI) obtained as sum of hazard quotients related to reference inhalation concentrations of individual BTEX compounds, was 0.07 on suburban-traffic and 0.1 on urban-traffic site, with overall mean at 0.09, showing no health risk.

Combined benzene and xylenes contribution to HI was more than 99% (with contributions ratio of 2.4 and 1.4, in favor of benzene, on suburban- and urban-traffic sites, respectively). The carcinogenic risk, based on benzene and ethyl benzene inhalation risk units, was higher on urban- than on suburban-traffic site, 2.3E-06 vs. 1.9E-06 in case of children and 9.4 E-06 vs. 7.8E-06 in case of adults, indicating low risk. The main driver was benzene, showing 8.7-fold higher impact, due to both higher exposure and higher inherent carcinogenic potential in comparison with ethyl benzene. Albeit the estimated risk related to the inhalation exposure to BTEX was acceptable, one should consider a vast multitude of toxicants having potential to endanger human health, present not only in the air but also in other environmental media. Hence, there is an urge to ensure the air/environment quality, using a comprehensive approach engaging the entire society.

**KEYWORDS:** monitoring, risk assessment, Novi Sad



## ZDRAVSTVENI RIZIK ODOJČETA S OBZIROM NA UKUPNU IZLOŽENOST PBDE- IMA IZ MAJČINOG MLEKA I KUĆNE PRAŠINE

TOXICOLOGICAL  
RISK ASSESSMENT

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Polibromovani difeniletri (PBDE) su lipofilna organska jedinjenja koja se koriste kao usporivači plame-  
na u građevinskim materijalima i svakodnevним proizvodima za domaćinstvo. Oni se akumuliraju u  
prašini čije je gutanje jedan od najvažnijih puteva izloženosti PBDE-ima za ljude pored ishrane. Oni se  
bioakumuliraju u tkivima bogatim lipidima i izazivaju štetne zdravstvene efekte. Zbog toga je majčino  
mleko najčešći i praktičniji alat koji se koristi za ljudski biomonitoring, posebno odojčadi kojoj se na  
ovaj način PBDE direktno prenose. Sprovedena je integrisana studija o nivoima 7 specifičnih PBDE kon-  
genera u uzorcima ljudskog mleka (N = 21) prvotkinja koje žive u Zagrebu i okolini i odgovarajućim  
uzorcima prašine iz njihovog domaćinstva.

Primećeno je da je izloženost odojčadi PBDE-ima putem gutanja kućne prašine približno četiri puta  
veća u poređenju sa majkama. Ukupne procenjene vrednosti dnevnog unosa (EDI) za  $\Sigma$ 7PBDE detekto-  
vane u majčinom mleku i kućnoj prašini kretale su se u rasponu od 0,9 do 41 ng kg<sup>-1</sup> dan<sup>-1</sup> u centralnom  
i najgorem scenariju zbog činjenice da je unos PBDE-a preko humanog mleka daleko značajniji (medi-  
jan 99 %) u poređenju sa unosom putem gutanja prašine (medijan ~1 %). Dobijene vrednosti indeksa  
opasnosti (HI) koji predstavljaju nekancerogeni rizik su  $\ll 1$ , što ukazuje na nizak rizik usled unosa PB-  
DE-a. S obzirom na osetljiv period razvoja odojčadi i unos raznih zagađivača kasnije u životu, važno je  
da unos ovih jedinjenja u ovom najranijem uzrastu ne predstavlja značajan rizik.

**KLJUČNE REČI:** PBDE-ovi, majčino mleko, kućna prašina, izloženost odojčeta, zdravstveni rizik



# INFANT'S HEALTH RISK CONSIDERING OVERALL EXPOSURE TO PBDES FROM MOTHER'S MILK AND HOUSEHOLD DUST

TOXICOLOGICAL  
RISK ASSESSMENT

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Polybrominated diphenyl ethers (PBDEs) are lipophilic organic compounds used as flame retardants in building materials and everyday household products. They accumulate in dust, whose ingestion is one of the most important routes of exposure to PBDEs for humans besides diet. They bioaccumulate in lipid-rich tissues and cause adverse health effects. This is why breast milk is the most common and practical tool used for human biomonitoring, especially of infants to whom in this way PBDEs are directly transferred. An integrated study on levels of 7 specific PBDE congeners in human milk samples (N = 21) from primipara mothers living in Zagreb and its surroundings and matched dust samples from their household was conducted. It was observed that infants' exposure to PBDEs via house dust ingestion is approximately four times higher compared to mothers.

Total estimated daily intake (EDI) values for the  $\Sigma$ 7PBDEs detected in breast milk and house dust ranged from 0.9 to 41 ng kg<sup>-1</sup> day<sup>-1</sup> at both the central and worst case scenario due to the fact that the intake of PBDEs via human milk is far more significant (median 99 %) compared to intake via dust ingestion (median ~1 %). The obtained values of hazard indexes (HI) presenting non-carcinogenic risk were <<1, indicating low risk due to PBDEs uptake. Considering the sensitive period of infants' development and the intake of various pollutants later in life, it is important that the intake of these compounds at this earliest age does not pose a significant risk.

**KEYWORDS:** PBDEs, breast milk, house dust, infant's exposure, health risk



## IZAZOVI I MOGUĆNOSTI UPOTREBE PAPAVER SOMNIFERUM L. U PREHRAMBENOJ INDUSTRIJI I TOKSIKOLOGIJI

TOXICOLOGICAL  
RISK ASSESSMENT

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Papaver somniferum L. je biljka opijumskog maka koja se konzumira kao hrana. Veliki broj sorti maka se koristi za proizvodnju farmaceutskih alkaloida opijuma i za proizvodnju semena za ishranu. Seme je bogato linoleinskom i oleinskom kiselinom- mononezasićenom masnom kiselinom, koja pomaže u snižavanju nivoa LDL (Low Density Lipoprotein - lipoproteina male gustine) u krvi i povećanje nivoa HDL (High Density Lipoprotein - lipoproteina velike gustine), što je veoma značajno za zdravlje kardiovaskularnog sistema. Seme ne sadrži opijate, ali može biti kontaminirano njima tokom berbe, jer njegov lateks sadrži brojne alkaloide (morfijum, tebain, kodein, papaverin, noskapin).

Opijumski alkaloidi i njihovi polusintetski i sintetski derivati (heroin), oksimorfon, oksikodon, hidroksikodon, dihidrokodein) imaju značaj u toksikologiji i farmaciji, kao opioidni analgetici i sredstva zloupotrebe. Analgetički efekat se postiže dejstvom agonista prevenstveno na  $\mu$ -receptore, dok  $\kappa$  i  $\delta$ -receptori uglavnom smanjuju aktivnost puteva koji prenose informacije o bolu do centralnog nervnog sistema. Trovanja opioidnim analgeticima su posledica predoziranja heroinom i drugim opioidima. Konzumiranje većih količina prehrambenih proizvoda koji sadrže mak često može dati pozitivne rezultate testa na droge u urinu, što predstavlja izazove u razlikovanju uzimanja semena maka kao hrane od davanja opijata.



Izloženost tebainu konzumiranjem hrane koja sadrži mak može predstavljati zdravstveni rizik. Maksimalno dozvoljena koncentracija kodeina (MAC) u urinu je 300 ng/mL. Prag upotrebe hrane koji sa sigurnošću ne bi doveo do pozitivnih testova na lekove trenutno nije dostupan.

**KLJUČNE REČI:** proizvodnje hrane, zdravlje, koncentracije opijumskih alkaloida, opijati maka, toksičnost

**ZAHVALNICA:** Istraživanje je podržalo Ministarstvo prosvete, nauke i tehnološkog razvoja Republike Srbije (Broj granta: 451-03-47/2023-01/200032) i Projekat broj PR-166-Srbija i Prizma Projekat 6770 PALraFES (2023-2027): Očuvanje poljoprivrednog zemljišta ruralnih područja u funkciji energetske stabilnosti.



## CHALLENGES AND POSSIBILITIES OF USING PAPAVER SOMNIFERUM L. IN THE FOOD INDUSTRY AND TOXICOLOGY

TOXICOLOGICAL  
RISK ASSESSMENT

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Papaver somniferum L. is the opium poppy plant consumed as food. There are a large number of poppy varieties that are used to obtain opium alkaloids for pharmaceutical purposes and to produce seeds for use as food. Poppy seeds are rich in linoleic and oleic acid, a monounsaturated fatty acid that helps lower LDL (low density lipoprotein) levels in the blood and increases HDL (high density lipoprotein) levels, which are very important for cardiovascular health. Poppy seeds do not contain opiates, but can be contaminated with them when harvested, as the poppy latex contains numerous alkaloids (morphine, thebaine, codeine, papaverine, and noscapine).

Opium alkaloids and their semisynthetic and synthetic derivatives (heroin, oxymorphone, oxycodone, hydrocodone, dihydrocodeine) are of multiple importance in toxicology and pharmacy as opioid analgesics and abuse agents. The analgesic effect is achieved by the action of agonists primarily on  $\mu$ -receptors, while  $\kappa$ - and  $\delta$ -receptors mainly reduce the activity of pathways that transmit pain information to the central nervous system. Poisoning with opioid analgesics is usually the result of an overdose of heroin, methadone, and other opioids. Consumption of large amounts of poppy-containing foods often results in positive urine test results, which can make it difficult to distinguish between poppy as food and opioid use.





Exposure to thebaine through consumption of poppy-containing foods could pose a health risk. MAC-The Maximum Allowable Concentration of codeine in urine is 300 ng/mL. A threshold for food consumption that would not be certain to result in positive drug tests is not currently available.

**KEYWORDS:** food production, health, opium alkaloids, poppy opiates, toxicity

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## PROCENA IZLOŽENOSTI KOZMETIČKIM SASTOJcima IZ KOZMETIČKIH PROIZVODA

TOXICOLOGICAL  
RISK ASSESSMENT

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Kozmetički proizvodi se svakodnevno upotrebljavaju tokom celog života, što znači da je izlaganje supstancama iz kozmetičkih proizvoda ponavljano, višestruko, u dužem vremenskom periodu i da može doći do bioakumulacije. Izloženost ljudi kozmetičkim sastojcima se izračunava na osnovu deklariranih funkcija i upotrebe supstance kao kozmetičkog sastojka, količine u odgovarajućim kategorijama kozmetičkih proizvoda i učestalosti njihove upotrebe.

Kada se utvrde svojstva kozmetičkog sastojka čija se bezbednost ispituje, kao i mogući scenario izloženosti, potrebno je identifikovati doze koje će biti upotrebljene za procenu izloženosti, tj. bezbednosti. Identifikacija hazarda može ukazati ili na sistemske efekte, za koje je neophodno izvesti SED (Systemic Exposure Dose) – sistemska doza izloženosti, ili na lokalne efekte, što zahteva poređenje sa lokalnom eksternom dozom –LED (Local External Dose).

Tamo gde je to potrebno, vrednosti procene izloženosti dobijene konzervativnim pristupom se preciziraju na višem nivou procene korišćenjem probabilističkog pristupa. Za razne vrste kozmetičkih proizvoda dermalni put je često najbitniji put izloženosti. Na zahtev SSCS-a (Scientific Committee on Consumer Safety, naučni komitet za bezbednost potrošača) Cosmetic Europe je sprovela veliku studiju upotrebe za najbitnije kategorije kozmetičkih proizvoda među korisnicima u različitim državama EU. Na osnovu ove studije probabilističkom metodom su dobijene vrednosti za 12 kategorija proizvoda. Ove vrednosti se mogu koristiti za procenu dermalne izloženosti na prvom nivou. Pojedinačna izloženost opisuje izloženost kozmetičkom sastojku u jednoj kategoriji proizvoda jednim putem unosa. Zbirna izloženost predstavlja zbir pojedinačnih izloženosti tako da ona opisuje izloženost iz svih kategorija proizvoda u kojima se kozmetički sastojak upotrebljava i svih značajnih puteva izloženosti.

**KLJUČNE REČI:** izloženost, SED, LED



## ASSESSMENT OF EXPOSURE TO COSMETIC INGREDIENTS FROM VARIOUS COSMETIC PRODUCTS

TOXICOLOGICAL  
RISK ASSESSMENT

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Cosmetic products are used daily throughout life, which means that exposure to substances from cosmetic products is repeated, multiple, over a long period of time and that bioaccumulation can occur. Human exposure to cosmetic ingredients is calculated based on the declared functions and use of the substance as a cosmetic ingredient, the amount in the respective categories of cosmetic products and the frequency of their use. Once the properties of the cosmetic ingredient whose safety is being tested are determined, as well as the possible exposure scenario, it is necessary to identify the doses that will be used for the assessment of exposure, i.e. safety.

Hazard identification can indicate either systemic effects, for which it is necessary to report SED (Systemic Exposure Dose), or local effects, which requires comparison with local external dose – LED. Where necessary, exposure estimate values obtained from a conservative approach are refined at a higher level of assessment using a probabilistic approach. For various types of cosmetic products, the dermal route is often the most important route of exposure. At the request of the SSCS (Scientific Committee on Consumer Safety), Cosmetic Europe conducted a large study of usage for the most important categories of cosmetic products among users in different EU countries. Based on this study, values for 12 product categories were obtained using the probabilistic method. These values can be used to estimate dermal exposure at the first level. Single exposure describes exposure to a cosmetic ingredient in a single product category through a single route of exposure. Aggregate exposure is the sum of individual exposures so that it describes exposure from all product categories in which the cosmetic ingredient is used and all significant routes of exposure.

**KEYWORDS:** exposure, SED, LED



## ANALIZA RIZIKA OD UNOSA HLORITA PUTEM VODE ZA PIĆE IZ LOKALNOG VODOVODA FABRIKE

TOXICOLOGICAL  
RISK ASSESSMENT

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Hloriti predstavljaju sporedni proizvod dezinfekcije u tretmanu vode hlordioksidom, te je određivanje njihove koncentracije u vodi za piće važno za procenu njihovog uticaja na zdravlje. Oralno izlaganje značajnim količinama hlorita može dovesti do iritacije digestivnog trakta i povećanja nivoa methemoglobina u krvi, što dovodi do smanjenog vezivanja kiseonika za hemoglobin. Hloriti nisu klasifikovani kao kancerogen. Cilj je bio da se utvrdi da li u lokalnom vodovodu fabrike postoji zdravstveni rizik za 600 zaposlenih koji su izloženi hloritima putem vode za piće. Definisani su: dnevna izloženost hloritima izražena po kilogramu telesne mase, potrošnja vode dnevno za datu starosnu grupu i pol, učestalost ekspozicije, prosečna telesna masa zaposlenih, dnevni unos.

U posmatranom periodu (januar 2019 - decembar 2022.) u ukupno 98 uzoraka vode određena je koncentracija hlorita (opseg: 0,006-0,55 mg/L, srednja vrednost 0,16 mg/L) i upoređena sa maksimalno dozvoljenom koncentracijom (0,20 mg/L). Analiza hlorita vršena je na jonskom hromatografu Metrohm 930 IC Flex, metodom EPA 300.1. Za izračunavanje hazardnog indeksa (HI) određivani su: koncentracija hlorita, broj radnih dana u godini, prosečan preporučeni dnevni unos vode za zaposlene starosne grupe 19-65 godina, prosečna telesna masa zaposlenih. Dobijena vrednost HI za srednju vrednost koncentracije hlorita za muškarce iznosi 0,30, a za žene 0,11. Za maksimalnu koncentraciju hlorita, HI za muškarce iznosi 1,02, a za žene 0,75. Usvojeno je da za HI>1 postoji mogućnost negativnih efekata na zdravlje kao rezultat dugotrajne izloženosti što se u ovom slučaju odnosi na grupu zaposlenih muškog pola, pri maksimalnoj vrednosti hlorita od 0,55 mg/L koja je registrovana u 1% uzoraka vode.

**KLJUČNE REČI:** hloriti, uticaj na zdravlje, hazardni indeks, lokalni vodovod



## RISK ANALYSIS OF CHLORITE INTAKE WITH DRINKING WATER FROM THE FACTORY'S LOCAL WATER SUPPLY

TOXICOLOGICAL  
RISK ASSESSMENT

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Chlorites are a side product of disinfection in the treatment of water with chlorine dioxide, so determining their concentration in drinking water is important for assessing their impact on health. Chlorites are not classified as carcinogenic. Oral exposure to relatively large amounts of chlorite can lead to irritation of the digestive tract and increased levels of methemoglobin in the blood, which reduces the ability of oxygen to bind with hemoglobin. The aim was to determine whether there is a health risk in the factory's local water supply for 600 workers who are exposed to chlorites through drinking water.

The following are defined: daily exposure to chlorites expressed per kilogram of body weight, daily water consumption for a given age group and gender, frequency of exposure, average body weight of employees, daily intake. In the observed period (January 2019 - December 2022), the concentration of chlorite was determined in a total of 98 water samples (range: 0.006-0.55 mg/L, mean value 0.16 mg/L) and compared with the maximum allowed concentration (0,20 mg/L). The analysis of chlorite was performed on a Metrohm 930 IC Flex ion chromatograph, using the EPA 300.1 method. To calculate the hazard index (HI), the following were determined: chlorite concentration, number of working days in a year, average recommended daily intake of water for employees in the age group 19-65 years, average weight of workers. The obtained value of HI for the mean value of chlorite concentration for men is 0.30, and for women 0.11. For the maximum chlorite concentration, the HI for men is 1.02 and for women 0.75. It was accepted that for  $HI > 1$  there is a possibility of negative health effects as a result of long-term exposure, which in this case refers to the group of male workers, at the maximum chlorite value of 0.55 mg/L which was registered in 1% of the samples.

**KEYWORDS:** chlorites, impact on health, hazard index, local water supply



## VETERINARSKA TOKSIKOLOGIJA / VET MEDICINE TOXICOLOGY

### INFLAMATORNI PROCESI U JETRI PACOVA SUBAKUTNO TRETIRANIH HLORPIRIFOSOM I PROTEKTIVNI EFEKAT VITAMIN B1

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Hlorpirifos (CPF) je organofosfatni insekticid koji pored inhibicije acetilholinesteraze, dovodi do indukcije oksidativnog stresa i inflamatornih procesa u jetri. Cilj istraživanja je ispitivanje inflamatornog odgovora jetre prilikom tretiranja pacova hlorpirifosom i regulacije ovog procesa primenom vitamina B1. Pacovi soja Wistar su podeljeni u tri grupe: kontrolnu, grupu tretiranu sa 30 mg/kg tm CPF i grupu tretiranu sa 30 mg/kg tm CPF i 100 mg/kg tm vitamina B1 tokom 7 dana. Određene su aktivnosti paraoksonaze 1 (PON-1), mijeloperoksidaze (MPO) i ATP-aza i koncentracije lipoproteina ApoA i ApoB, i interleukina 1 $\beta$  (IL-1 $\beta$ ) i 6 (IL-6). Imunoheimijskim metodama je određena ekspresija ciklooksigenaze 2 (COX-2) i ukupne ekstracelularno regulisane kinaze, tERK1,2. Patohistološke promene praćene su bojenjima: hematoksilin-eozin, Masson trihromatsko bojenje, perjodna kiselina Schiff-ov reagens i Gomori bojenje.

Dobijeni rezultati kod grupe tretirane CPF ukazuju na inflamatorne procese, promene redoks ravnoteže lipoproteinskih frakcija i narušenog energetskog metabolizma hepatocita. Međutim, nakon tretmana vitaminom B1 vrednosti ispitivanih parametara potvrđuju njegov antiinflamatorni i antioksidativni efekat efekat.

**KLJUČNE REČI:** hlorpirifos, inflamacija, ATPaze, vitamin B1



## INFLAMMATORY PROCESSES IN THE LIVER OF RATS SUBACUTELY TREATED WITH CHLORPYRIFOS AND THE PROTECTIVE EFFECT OF VITAMIN B1

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Chlorpyrifos (CPF) is an organophosphate insecticide that, in addition to inhibiting acetylcholinesterase, induces oxidative stress and inflammatory processes in the liver. The research aims to examine the liver's inflammatory response during the treatment of rats with chlorpyrifos and the regulation of this process with the use of vitamin B1. Wistar rats were divided into three groups: control, the group treated with 30 mg/kg bw CPF and the group treated with 30 mg/kg bw CPF and 100 mg/kg bw vitamin B1 for 7 days. The activities of paraoxonase 1 (PON-1), myeloperoxidase (MPO), and ATPase and concentrations of lipoproteins ApoA and ApoB, and interleukin 1 $\beta$  (IL-1 $\beta$ ) and interleukin 6 (IL-6) were determined.

The expression of cyclooxygenase 2 (COX-2) and total extracellularly regulated kinase, tERK1,2, was determined by immunochemical methods. Pathohistological changes were followed by staining: hematoxylin-eosin, Masson trichromatic staining, periodic acid Schiff's reagent, and Gomori staining. The results obtained in the group treated with CPF indicate inflammatory processes, changes in the redox balance of lipoprotein fractions, and impaired energy metabolism of hepatocytes. However, after treatment with vitamin B1, the values of the investigated parameters confirm its anti-inflammatory and antioxidant effects.

**KEYWORDS:** chlorpyrifos, inflammation, ATPase, vitamin B1



## VETERINARSKI LEKOVI KAO MOGUĆI ZAGAĐIVAČI ŽIVOTNE SREDINE

VET MEDICINE  
TOXICOLOGY

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Veterinarski lekovi su namenjeni pre svega za preveniranje i lečenje bolesti, odnosno za zaštitu zdravlja životinja. Osim toga, neki ove lekove, ilegalno koriste i za stimulaciju rasta, odnosno kao aditive u hrani. Pokazalo se da široka, a ponekad i neracionalna primena veterinarskih lekova ima za posledicu ne samo razvoj rezistencije (kada su u pitanju antimikrobni lekovi), već se isti (u manjim ili većim koncentracijama) mogu utvrditi i u životnoj sredini. U zemljištu, površinskim i podzemnim vodama najčešće se mogu detektovati antimikrobni lekovi, antiparazitici i antiinflamatorni lekovi.

Svi oni u manjem ili većem stepenu mogu delovati štetno na brojne organizme u životnoj sredini. U životnu sredinu oni dospavaju različitim putevima: tokom proizvodnje, u toku procesa primene, odnosno lečenja, ili u toku uklanjanja neiskorišćenih lekova, odnosno ambalaže. Potencijalni izvor kontaminacije životne sredine veterinarskim lekovima predstavlja svakako otpadni materijal (sa farmi životinja), koji se skuplja i potom (u obliku stajskog đubriva) nanosi na njive na kojima su zasejane određene poljoprivredne kulture.

Uticaj veterinarskih lekova na životnu sredinu zavisi od brojnih faktora, uključujući fizičko-hemijska svojstva, korišćenu količinu i način primene, vrstu tretmana i dozu, način sakupljanja, deponovanja i rukovanja stajnjakom, metabolizma unutar životinje, te brzine degradacije u stajnjaku. Podaci o ekotoksičnosti danas su dostupni za veliki broj veterinarskih lekova. Osim direktnog delovanja na organizme u zemljištu (mikroorganizmi, gliste) i vodi (alge, bakterije, biljke), poznato je da neki od ovih lekova mogu delovati indirektno i na ptice grabljivce i slepe miševе.

**KLJUČNE REČI:** veterinarski lekovi, antimikrobni lekovi, antiparazitici, antiinflamatorni lekovi, štetni efekti, životna sredina





## VETERINARY DRUGS AS POSSIBLE CONTAMINANTS OF ENVIRONMENT

VET MEDICINE  
TOXICOLOGY

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Veterinary medicines are primarily intended for the prevention and treatment of diseases, i.e. for the protection of animal health. In addition, some use these drugs illegally for growth stimulation, that is, as food additives. It has been shown that the wide and sometimes irrational application of veterinary drugs results not only in the development of resistance (in the case of antimicrobial drugs), but also in the environment (in lower or higher concentrations). Antimicrobials, antiparasitics and anti-inflammatory drugs can most often be detected in soil, surface and underground water.

All of them, to a lesser or greater degree, can have a harmful effect on numerous organisms in the environment. They reach the environment in different ways: during production, during the process of application, i.e. treatment, or during the removal of unused medicines, i.e. packaging. A potential source of environmental contamination with veterinary drugs is definitely waste material (from animal farms), which is collected and then applied (in the form of manure) to the fields where certain agricultural crops are sown. The impact of veterinary drugs on the environment depends on a number of factors, including physico-chemical properties, the amount used and the method of application, the type of treatment and dose, the method of collecting, depositing and handling manure, metabolism inside the animal, and the rate of degradation in manure. Ecotoxicity data are now available for a large number of veterinary drugs. In addition to direct action on organisms in the soil (microorganisms, earthworms) and water (algae, bacteria, plants), it is known that some of these drugs can act indirectly on birds of prey and bats.

**KEY WORDS:** veterinary drugs, antimicrobial drugs, antiparasitic drugs, anti-inflammatory drugs, adverse effects, environment



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