

**INSTITUTE FOR MEDICAL RESEARCH AND OCCUPATIONAL HEALTH**

# **Annual Report**



**ZAGREB, 2018**

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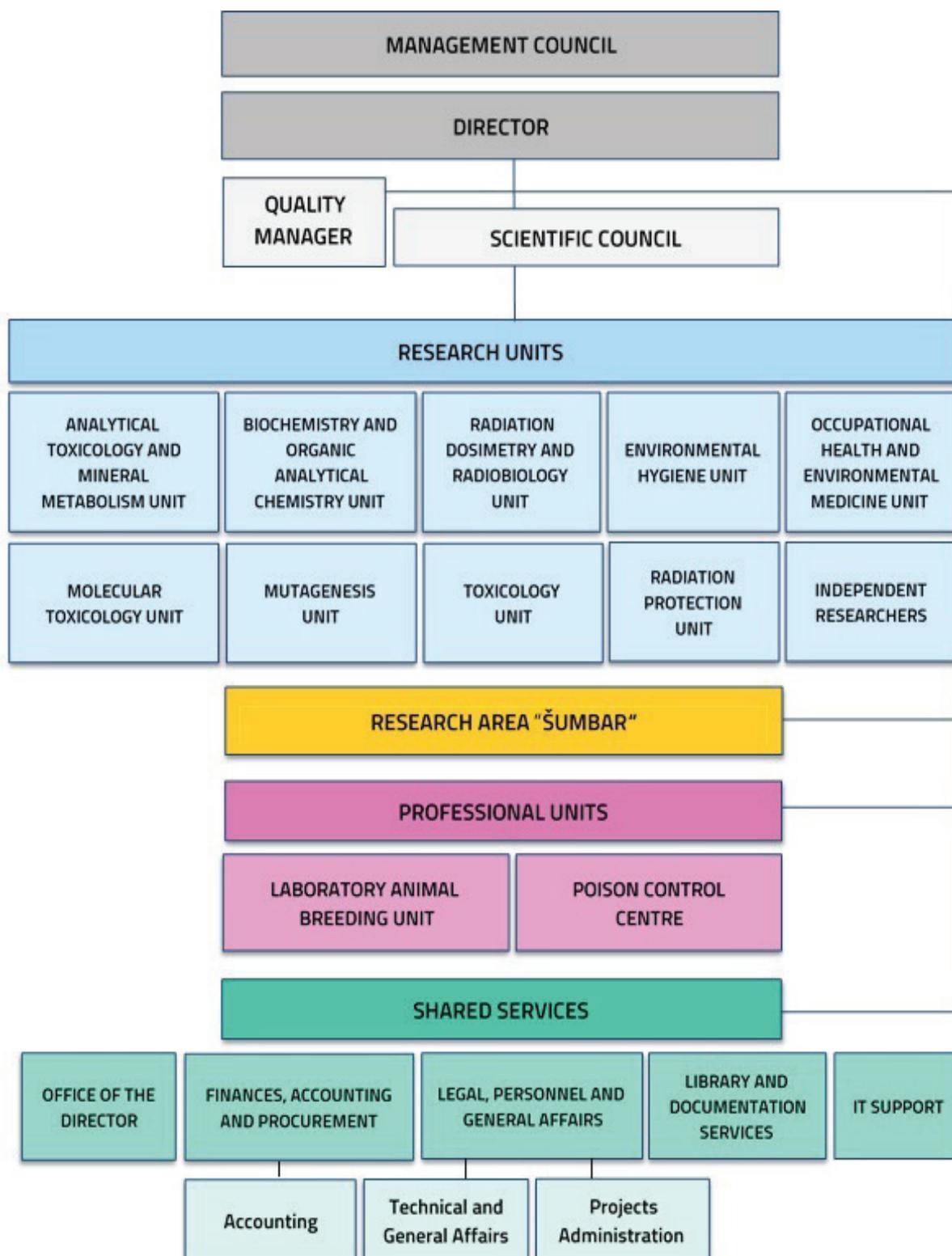
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## 1. ORGANISATION OF THE INSTITUTE

The Institute for Medical Research and Occupational Health (IMROH) was founded in 1947 in Zagreb and is currently the second largest public scientific and research institution in the Republic of Croatia functioning under the Ministry of Science and Education of the Republic of Croatia. The Institute has a leading role in Croatian research in the fields of molecular toxicology, allergotoxicology, protection from chemical weapons and radiological protection, environmental radiocontamination, air quality, distribution of metals and specific inorganic and organic environmental pollution, and human exposure to such forms of pollution. It continuously invests in the training of its scientific and professional staff, maintenance and upgrading of equipment, maintenance of premises and periodic intercomparisons of methods and their accreditation. The Institute is registered for conducting research and professional, educational, and publishing activity.

*Personnel and work place structure on 31 Dec 2017*

TOTAL NUMBER OF EMPLOYEES (2017)		155	%
Sources of funding	State budget	145	93
	IMROH	7	5
	Croatian Science Foundation	3	2
Sex	Women	115	74
	Men	40	26
Level of education	High	103	67
	Higher	11	7
	Middle	33	21
Academic title	Low	8	5
	PhD	71	46
	MSc	2	1
Teaching title	Full Professor	4	3
	Docent	4	3
Specialist title	Specialist in Epidemiology	1	<1
	Specialist in Occupational Medicine and Sports	2	1
Scientific work positions	Permanent Scientific Advisor	16	10
	Scientific Advisor	9	6
	Senior Scientific Associate	14	9
	Scientific Associate	17	11
	<b>Total</b>	<b>56</b>	<b>36</b>
Associate work positions	Postdoctoral	10	6
	Assistant	15	10
	<b>Total</b>	<b>25</b>	<b>16</b>
Professional work positions	Professional Advisor	2	1
	Senior Professional Associate	1	<1
	Professional Associate	10	6
	<b>Total</b>	<b>13</b>	<b>8</b>
Technician work positions	Senior Technician	15	10
	Technical Associate	15	10
	<b>Total</b>	<b>30</b>	<b>20</b>
Work positions in Shared Services	Division of the Director	1	<1
	Affairs Department of Finances, Accounting, and Procurement	7	4
	Department of Legal, Personnel, and General	18	12
	Division of Library and Documentation Services	2	1
	IT Support	3	2
<b>Total</b>	<b>31</b>	<b>20</b>	



*The organisational structure of IMROH*

## MANAGEMENT OF THE INSTITUTE

### MANAGEMENT COUNCIL

Prof Nikola Ružinski, PhD, Faculty of Mechanical Engineering and Naval Architecture, University of Zagreb (Chair)

Prof Stipan Jonjić, PhD, School of Medicine, University of Rijeka (Deputy Chair)

Božo Pavičin, Croatian Chamber of Economy

Zdenko Franić, PhD (Representative of the Institute's research staff)

Branka Roić, BEc (Representative of the Institute's professional staff)

### DIRECTOR

Prof Ana Lucić Vrdoljak, PhD

### DEPUTY DIRECTORS

Irena Brčić Karačonji, PhD

Prof Radovan Fuchs, PhD, DVM

## SCIENTIFIC COUNCIL

Snježana Herceg Romanić, PhD (Chair)

Davorka Breljak, PhD (Deputy Chair)

Verica Ferenčak (Secretary)

## ETHICS COMMITTEE

### CHAIR

Prim Jelena Macan, PhD, MD

### MEMBERS

Prof Radovan Fuchs, PhD, DVM

Maja Peraica, PhD, MD

Martina Piasek, PhD, MD

Prof Jure Zovko, PhD, Prof. Phyl., Faculty of Philosophy, University of Zadar

Mirela Deranja (Secretary)

## QUALITY MANAGER

Zdenko Franić, PhD

### 1.1. Ethics Committee

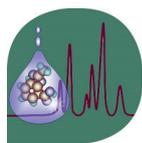
All of the submitted requests were considered according to the criteria set by the Code of Ethics of the Institute for Medical Research and Occupational Health, and applicants were given written opinions that were officially recorded. Altogether, 12 meetings and consultations via e-mail were held and ethical principles were considered in the following claims:

- submitting topics of doctoral theses with mentorship at the Institute (1 claim)
- supplemented approved research projects of the Croatian Science Foundation IP-06-2016 (2 claims)
- applications from the Institute's researchers to international scientific research projects (1 claim)
- proposals for in-house Institute projects implemented acquired from institutional financing (3 claims)
- project proposals submitted by the Institute's researchers as head investigators or associates to Croatian Science Foundation call IP-01-2018 (2 claims).

Requests for consideration of ethical aspects of research performed outside the Institute were considered from two profit research centres and one from the School of Veterinary Medicine, University of Zagreb.

## 2. RESEARCH UNITS

UNIT	CODE	HEAD	CONTACTS
Analytical Toxicology and Mineral Metabolism	604	Jasna Jurasović	Tel. +385 (1) 4682 530 e-mail: jurasovic@imi.hr
Biochemistry and Organic Analytical Chemistry	609	Snježana Herceg Romanić	Tel. +385 (1) 4682 553 e-mail: sherceg@imi.hr
Radiation Dosimetry and Radiobiology	608	Ivica Prlić	Tel. +385 (1) 4682 570 e-mail: iprlc@imi.hr
Environmental Hygiene	610	Gordana Pehneć	Tel. +385 (1) 4682 580 e-mail: gpehneć@imi.hr
Occupational Health and Environmental Medicine	615	Jelena Macan	Tel. +385 (1) 4682 600 e-mail: jmacan@imi.hr
Molecular Toxicology	606	Davorka Breljak	Tel. +385 (1) 4682 622 e-mail: dbreljak@imi.hr
Mutagenesis	616	Nevenka Kopjar	Tel. +385 (1) 4682 630 e-mail: nkopjar@imi.hr
Toxicology	603	Maja Peraica	Tel. +385 (1) 4682 640 e-mail: mperaica@imi.hr
Radiation Protection	602	Gordana Marović	Tel. +385 (1) 4682 650 e-mail: marovic@imi.hr
Independent Researcher	387	Aleksandra Fučić	Tel. +385 (1) 4682 522 e-mail: afucic@imi.hr
Independent Researcher	389	Ante Miličević	Tel. +385 (1) 4682 524 e-mail: antem@imi.hr
Independent Researcher	373	Jasmina Sabolović	Tel. +385 (1) 4682 526 e-mail: jsabolov@imi.hr



## 2.1. Analytical Toxicology and Mineral Metabolism Unit

### EMPLOYEES

#### HEAD:

Jasna Jurasović, PhD, permanent scientific advisor

#### RESEARCHERS

Martina Piasek, PhD, MD, permanent scientific advisor

Alica Pizent, PhD, permanent scientific advisor

Zorana Kljaković-Gašpić, PhD, scientific advisor

Nataša Brajenović, PhD, senior scientific associate

Irena Brčić Karačonji, PhD, senior scientific associate (Deputy Director)

Maja Lazarus, PhD, senior scientific associate

Assist Prof Ivana Vinković Vrček, PhD, senior scientific associate

Tatjana Orct, PhD, scientific associate

Blanka Tariba Lovaković, PhD, scientific associate

Anja Mikolić, PhD, postdoctoral researcher

Ankica Sekovanić, MSc, junior researcher, assistant

Antonija Sulimanec Grgec, MSc, junior researcher, assistant

Tanja Živković Semren, MSc, junior researcher, assistant

Andreja Jurič, MSc, assistant (from 15 Feb 2017)

Barbara Pem, MSc, assistant (from 15 Dec 2017)

#### TECHNICAL STAFF

Mladen Komesar, senior technician

Vesna Triva, senior technician

Snježana Mataušić, technician

Krešimir Nekić, technician

### RESEARCH

#### A. RESEARCH ACTIVITIES WITH INSTITUTIONAL FINANCING

##### A.1. *Exposure to metals and their effects in pregnancy and postnatal period* (MSES, 2007–2013)

The statistical data analysis attained within the research project financed by the Croatian Ministry of Science, Education and Sports (2007–2013/14) and carried out in healthy postpartum women with term vaginal deliveries (37–42 gestation weeks) is completed. The study evaluated the impact of main exposure sources to the toxic metals cadmium (Cd) and lead (Pb) through cigarette smoking and mercury (Hg), via seafood consumption on the concentrations of toxic metals (Cd, Pb, and Hg), essential elements iron (Fe), zinc (Zn), copper (Cu) and selenium (Se) in maternal blood, placenta and cord blood (by ICP-MS) as well as on steroid hormones progesterone and estradiol in the placenta (by IEMA). The participants were from the continental area (gave birth in Merkur University Hospital in Zagreb) and coastal Croatia (gave birth in General Hospital Zadar). In addition to this study, we assessed the influence of maternal gene polymorphism *MT2A* rs28366003 (by PCR-RLFP) on the concentrations of toxic and essential elements in the samples of mother-child pairs and measured the concentrations of metallothionein MT2 (by ELISA) and free thiol (–SH) groups (spectrophotometrically). The findings were linked with data on the study participants collected by questionnaire and the results were statistically analysed. Most of the results are comprised in

a doctoral thesis that was positively evaluated by the professional Commission at the Faculty of Pharmacy and Biochemistry of the University of Zagreb. In a research paper (96) were reported that in healthy postpartum women ( $n = 268$ ; mean age 29 years) who were active smokers compared to non-smokers we found higher Cd and Pb levels in all of the measured samples, Fe and Cu in cord blood and Zn in placenta. Maternal MT2 serum increased related to the smoking intensity. In non-smokers with genotype AG/GG (6 % of participants) vs. genotype AA (94 % of participants) Fe was significantly lower in the placenta. No effect of single nucleotide polymorphism (SNP) in gene *MT2A* c.-77 A>G (rs28366003) was found for any measured element in the maternal blood and placenta. Levels of MT2 in serum were lower, although not significantly, irrespective of maternal smoking. It was concluded that these results confirmed the relationship between cigarette smoking and increased Cd and Pb levels in maternal-placental-foetal unit together with new data on the concentrations of toxic and essential elements measured concomitantly in maternal blood, placenta and cord blood as well as maternal MT2 levels. New and original evidence has to do with the influence of *MT2A* -5A/G SNP and decreased placental Fe in non-smokers. For a final conclusion on the influence of this gene polymorphism on the element levels in mother-child pairs, future studies would require a larger number of participants divided across subgroups defined by the main source of exposure to a particular toxic metal.

#### **A.2. In-house scientific projects (Chapter 16.1.A.2.)**

- *Biomonitoring of contaminants using biomarkers in European brown bear (Ursus arctos)*  
Muscle, liver, and kidney cortex of brown bear from Croatia ( $n = 467$ ) were used as bioindicator tissues in the environmental assessment of toxic and essential element status. Among the three terrestrial carnivores inhabiting Croatia (bear, wolf, and lynx), the brown bear had the highest Cd and Pb levels, which even reached toxicologically relevant levels in the small part of the population, largely old females (Cd: 0.6 % and Pb: 1 % of the population). The age and sex of bears were important factors influencing element levels, especially Cd and Pb because of their accumulative nature (33). There were no sex differences in Cd accumulation with age among bears before their sexual maturity, while in adult males the accumulation pattern disappeared and differed significantly compared to adult females (312). Except sex, reproductive (mature vs. immature) and life stages (cub, yearling, subadult, and adult) were confirmed as important factors with impact on toxic metals in brown bear tissues. Toxic metal levels in mother-cub pairs were investigated and compared regarding the transplacental and lactational transfer of metals. Old females were the most burdened part of the population with regard to Cd and Pb, but it may be that the risk of transfer and adverse health effects in their cubs is higher for Pb (84). Similar levels of ochratoxin A were found in plasma of wild and captured bears (Kuterevo Sanctuary and ZOO) (269).
- *Exposure to cadmium and its effects during gestation and postnatal period: investigations in laboratory rats*  
Within the investigation on female rats (Wistar HsdBrlHan) exposed to a low dose of Cd ( $5 \text{ mg L}^{-1}$  in drinking water) during almost the entire gestation (20 days), all of the planned analyses are completed. We determined microelements Cd, Fe, Zn, and Cu (by ICP-MS) in the blood, liver, and kidney of mother rats (F0 generation) as well as in the placentas and fetuses on gestation day 20 and in blood, liver, kidney, and brain of 14-day-old (weaned) pups and at the onset of puberty at age of 47-54 days (F1 generation). In placental samples and serum of F0 and F1 female rats, steroid hormones progesterone and testosterone were determined (by ELISA).
- *Investigation of interactions between irinotecan and tetrahydrocannabinols on a rat experimental model using integrated biochemical, molecular biology, pathohistological, and analytical methods*  
We carried out a pilot study in which male Wistar rats were exposed to irinotecan ( $100 \text{ mg kg}^{-1}$ , administered once, *i. p.*), tetrahydrocannabinol (THC) ( $7 \text{ mg kg}^{-1}$ , administered once, for 3 and 7 days,

*p. o.*) and their combinations. Rats were killed 24 hours after the last application of THC and blood and organ samples were collected for various hematologic, biochemical, and molecular-biological analyses. Measurements of standard haematological and biochemical parameters (differential blood count and serum liver enzymes, bilirubin, creatinine, lipid profile, and glucose) were also performed. An analytical method (GC-MS) was developed to determine the mass concentration of THC and its metabolites in rat urine samples. The role of phytocannabinoids in cancer prevention was presented at the *FEBS Advanced Lecture Course on Oncometabolism* (298).

### A.3. Other research activities

The research on the quality and safety of targeted food groups regarding the content of essential and toxic elements in edible tissues is continued. We determined the levels of essential macroelements and microelements potassium (K), sodium (Na), sulphur (S), magnesium (Mg), Ca, as well as manganese (Mn), Fe, Zn, Cu, and Se in eight fish species typical for the coastal area of Croatia. Samples of muscle tissue ( $n = 323$ ) of oily fish (chub, horse mackerel, European anchovy and sardine) and lean fish (bogue, blotched picarel, European hake, and red mullet) caught in the eastern Adriatic Sea were analysed by ICP-MS after wet digestion of freeze-dried homogenates. We found lower S and higher K, Mg, Zn, Fe, and Cu levels in oily than in lean fish, with the highest levels in sardines. It was shown that, besides omega-3 fatty acids, small oily fish is also a valuable source of essential elements and a healthy food choice. The results were presented at the international scientific conference on trace elements in humans "TEMA-16, ISTERH 2017 and NTES 2017" held in Saint Petersburg, Russia (270). Furthermore, we prepared and analysed samples of muscle, liver, gills, and gonads of three fish species (Prussian carp, common carp, and common rudd) from the Vransko Lake and analysed the multielement analysis data and biometric parameters statistically.

Within the framework of the now terminated project "Monitoring of organic and inorganic pollutants in the environment of the Plitvice lakes", financed by the Public Institution "Plitvice Lakes National Park" until 2013, we prepared a publication on the impact of volatile and persistent organic pollutants, trace elements/heavy metals, and anthropogenic radionuclides in the aquatic part (water, sediment, fish) of the Plitvice Lakes National Park, in the period from 2011 to 2013.

In collaboration with the Department of Geology of the Faculty of Science, University of Zagreb, and the Radiation Protection Unit (IMROH), results of a health risk assessment from potentially toxic trace elements (Cd, Se) present in soil in the vicinity of the thermal power plant Plomin were presented at an international scientific conference (291).

A headspace solid-phase microextraction method (HS-SPME) followed by gas chromatography with mass spectrometric detection (GC-MS) was evaluated and optimised for analysis of urinary volatile organic metabolites. We investigated the influence of coating fibre material, incubation, and extraction temperatures and times, and salt addition on HS-SPME efficiency. Multivariate optimisation methods using reduced factorial and Doehlert matrix designs were applied. The optimised method was used for the investigation of urine samples' stability regarding different storage conditions (4, -20, and -80 °C) and freeze-thaw processes. The obtained results indicated that storing human urine up to six months at -80 °C with no more than two freeze-thaw cycles can be considered suitable for metabolomics studies (105).

The antimicrobial effect of strawberry tree (*Arbutus unedo* L.) water and methanol leaf extracts was tested on 15 uropathogens. The strongest antimicrobial activity of leaf extracts was detected for clinical strains of *Enterococcus faecalis*, which was probably associated with the ability of bacterial  $\beta$ -glucosidase, exerting strong activity in *E. faecalis*, to convert arbutin, the main phenolic compound in leaves, to hydroquinone responsible for the antimicrobial effect (27). The effects of strawberry tree water leaf extract, arbutin, and hydroquinone on haematological parameters and levels of primary DNA damage were investigated in white blood cells (WBC) of rats. Our findings suggest no

significant changes in the haematological parameters following prolonged exposure (4 weeks) to these compounds. DNA damage measured in the WBC of rats treated with all of the compounds was below 10 % of the DNA in the comet tail, which indicates low genotoxicity (79). To confirm the botanical origin of strawberry tree honey, a liquid–liquid extraction followed by gas chromatography–mass spectrometry was developed for the quantitative determination of homogentisic acid (HGA), the main phenolic compound in this honey. The optimized method was applied for determining the HGA content in nine strawberry tree honey samples from southern Dalmatia (10). A scientific paper discussing the cytotoxic, DNA damaging, and cytogenetic effects of hydroquinone (HQ) in human peripheral blood lymphocytes *in vitro* was published. The tested HQ concentrations (8, 140, and 280  $\mu\text{g ml}^{-1}$ ) produced relatively weak cytotoxicity in resting lymphocytes. HQ's marked genotoxic effects were detected using the alkaline comet assay. Our results on comet test suggested that the two higher HQ concentrations possibly led to cross-linking and adduct formation. Increased levels of DNA breakage measured following exposure to the lowest concentration suggested mechanisms related to oxidative stress and inhibition of topoisomerase II. The two latter concentrations completely blocked lymphocyte division and also led to erythrocyte stabilization and prevented their lysis (26). The nutritional and antioxidant properties of strawberry tree fruit were presented at the Food Safety and Quality Congress (214).

The concentration of 23 elements, total phenolic content, and antioxidant capacity were determined in nine samples of strawberry tree honey from locations in the southern Croatian Adriatic coast. Strawberry tree honey had a generally higher total phenolic content and antioxidant capacity compared to 14 other analysed unifloral honeys collected across Croatia. According to the estimated daily intake of essential elements, strawberry tree honey was shown to be nutritionally richer than the majority of unifloral honeys available in Croatia. The toxic element levels in investigated honey samples indicated pristine area origin (100).

Collaboration with the Faculty of Pharmacy and Biochemistry, University of Zagreb and with the Teaching Institute for Public Health „A. Štampar“ has continued. An investigation on the differences between organically and conventionally produced foods was performed. Levels of pesticides, mycotoxins, metals, and sulphites were measured in organically and conventionally produced Croatian wines. The obtained results were analysed and used for manuscript preparation, which was finally accepted for publication.

Within collaboration with the Faculty for Agronomy from Osijek, University of Graz, and Palacký University & Institute of Experimental Botany, Olomouc, a study on hormonal response of pepper plants to treatment with silver nanoparticles was finished and resulted in a publication (67).

## **B. RESEARCH PROJECTS FUNDED BY EXTERNAL SOURCES**

- *Organic pollutants in the environment – markers and biomarkers of toxicity* (OPENTOX, Chapter 16.1.A.1.)

Within the OPENTOX project, research on the effects of low-level pesticide doses on cytotoxic, genotoxic and biochemical parameters in HepG2 cell lines and blood and tissue samples of adult male Wistar rats continued. The activities of antioxidant enzymes SOD and GSH-Px were measured in cell line, blood, and tissue samples. Results of low doses of glyphosate in *in vitro* conditions using HepG2 cells monitored 4 and 24 hours after the initiation of exposure was published (28). Exposure of male Wistar rats to terbuthylazine resulted in a significant changes in antioxidant enzyme activities in blood samples and disturbance of oxidative/antioxidant balance after 28-day treatment (62, 292). A significant increase of glutathione peroxidase and superoxide dismutase activity in blood was observed in the rats after a 28-day treatment with chlorpyrifos, whereas no significant effect was found in brain tissue samples (81). A possible connection between radiofrequency exposure (RF) and development of oxidative stress was investigated by evaluating impairment in

the cellular oxidation-reduction balance in fibroblast cells V79 immediately after RF exposure (38). The results of optimisation of the analytical method for determining organochlorine compounds in human milk were presented at the EUROTOX congress (255). Z. Kljaković-Gašpić and co-workers from the Biochemistry and Organic Analytical Chemistry Unit prepared a publication on the content of persistent organic pollutants (OCPs, PCBs) and total mercury in archive samples of tuna from the Adriatic Sea.

- *Aging-related expression of membrane transporters in rat* (AGEMETAR, Chapter 16.1.A.1.)

We continued measurement of the activity of the enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) and trace element levels in tissues of laboratory rats after 21-month treatment with melatonin and resveratrol. Influence of *in vivo* perfusion on trace element concentrations in rat organs (by ICP-MS) was described. It was shown that the blood remained in organs may have significant influence on tissue concentrations of various elements in a sex-dependent manner (45). Results of sex differences and effect of gonadectomy in metallothionein expression and trace elements concentration in rat liver and kidney tissues were presented (266).

- *Assessment of daily exposure to metals and maternal individual susceptibility as factors of developmental origins of health and disease*, (METALORIGINS, Chapter 16.1.A.1.)
- *Interaction of metallic nanoparticles with sulphur-containing biomolecules – implications for nano-bio interface* (NanoFaceS, Chapter 16.1.A.1.)
- *The European upconversion network – from the design of photon-upconverting nanomaterials to biomedical applications* (UPCON, Chapter 16.2.A.5.)
- *Anti-Microbial Coating Innovations to prevent infectious diseases* (AMICI, Chapter 16.2.A.5.)
- *Possible early non-invasive biomarkers of chronic exposure to arsenic* (Chapter 16.2.A.10.)

## PROFESSIONAL SERVICES

Expert analyses of metals and metalloids in different samples of different origins (ICP-MS analysis) and drugs in human hair and urine samples (GC-MS analysis) were carried out upon request by various institutions, companies, and individuals.

A total of 319 analyses of specific indicators of exposure and effect to toxic metals/metalloids and essential trace element status in the human organism were performed. Most of the analyses were related to determining biological markers of Pb exposure [concentrations of Pb and erythrocyte protoporphyrin (EP) and activity of  $\delta$ -aminolevulinic acid dehydratase (ALAD) in blood] during the assessment of professional exposure in workers at different workplaces ( $n = 192$ ). Concentrations of Hg in urine, blood, and hair ( $n = 54$ ) and a wide range of elements (Al, As, Ca, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Se, Sn, Tl, and Zn) in those biological samples ( $n = 73$ ) were also determined. Based on a contract signed with the Institute of Public Health of Brod-Posavina County, analyses of Pb, Ni, Cr, V, Mn, and Tl in whole blood/serum, urine, and hair samples of 40 subjects, in total 720 analyses (289). Apart from analysis of human biological samples, Cd was measured in dried tobacco leaves (6 samples) and Tl in model solutions (6 samples).

Drugs of abuse from the amphetamine and opiate groups, methadone, and cocaine were determined in 31 hair samples (45 analyses in total). THC-COOH was analysed in one urine samples. Thirty-eight e-mail queries (infodroge@imi.hr) regarding the analysis of drugs of abuse were received.

*List of proficiency tests*

ORGANISER	TEST	AREA	DATE
Society of Hair Testing, Strasbourg, France	Proficiency Test 2017	Analysis of drugs of abuse in hair	7/2017 and 12/2017 (two times per year, three hair samples)
Société Française de Biologie Clinique (SFBC), Paris, France	Trace Elements External Quality Assurance Scheme – Occupational and Environmental Laboratory Medicine	Element analysis in serum (Al, Co, Cr, Cu, Li, Mg, Se, Tl, V, and Zn) and blood (As, Cd, Co, Cr, Hg, Mg, Mn, Pb, Se, Tl, and Zn).	1/2017–12/2017 (12 times per year, two serum and blood samples)

## PROFESSIONAL ACTIVITIES OF THE UNIT'S EMPLOYEES OUTSIDE THE INSTITUTE

### *I. Brčić Karačonji*

Member of the Working Group on the Early Warning System on New Psychoactive Substances in the Republic of Croatia at the Office for Combating Drug Abuse of the Government of the Republic of Croatia; member of the Working Group for drafting the Procedure for the implementation of measures for testing drugs of abuse and other substances of abuse at the workplace, at the Croatian Institute for Health Protection and Safety at Work; member of the Working Group of the Biocidal Products Committee of the European Chemicals Agency; member of the Presidency of the Croatian Society of Toxicology.

### *J. Jurasović*

Member of the Presidency of the Croatian Society of Toxicology.

### *M. Piasek*

Member of the international professional associations International Commission on Occupational Health – ICOH and MEDICHEM, also an ICOH scientific committee for occupational health in chemical industry; member of the Presidency of the Croatian Society of Toxicology.

### *I. Vinković Vrček*

Member of the Working Group of the Ministry of Health of the Republic of Croatia for the development of the Position of RC in the area of novel food; member of the Editorial Board of the journal *Diacovensia*; member of the Scientific Council for the Education and School of the Croatian Academy of Science and Art.

## SCIENTIFIC ADVANCEMENT OF EMPLOYEES

Scientific degree of scientific advisor were gained by N. Brajenović and I. Vinković Vrček.



## 2.2. Biochemistry and Organic Analytical Chemistry Unit

### EMPLOYEES

#### HEAD

Snježana Herceg Romanić, PhD, scientific advisor

#### RESEARCHERS

Zrinka Kovarik, PhD, permanent scientific advisor

Goran Šinko, PhD, scientific advisor

Sanja Fingler Nuskern, PhD, senior scientific associate

Gordana Mendaš Starčević, PhD, senior scientific associate

Anita Bosak, PhD, scientific associate

Maja Katalinić, PhD, scientific associate

Darija Klinčić, PhD, scientific associate (substitute: Tena Čadež, MSc from 6 Nov 2017)

Sanja Stipičević, PhD, scientific associate

Marija Dvorščak, PhD, postdoctoral researcher

Nikolina Maček Hrvat, PhD, postdoctoral researcher

Nikola Maraković, PhD, postdoctoral researcher

Antonio Zandona, MSc, doctoral researcher, assistant

Tamara Zorbaz, MSc, doctoral researcher, assistant

#### TECHNICAL STAFF

Maja Meštrović, technician

Kristina Vajković, technician

#### PARTICIPATING RETIRED RESEARCHERS

Prof Vlasta Drevenkar, PhD, permanent scientific advisor

Želimira Vasilić, PhD, scientific advisor

### RESEARCH

#### A. RESEARCH ACTIVITIES WITH INSTITUTIONAL FINANCING

##### A.1. Scientific collaborations

Collaboration with Dr Dejan Opsenica from the Institute of Chemistry, Technology and Metallurgics (IHTM), Belgrade, Serbia continued. New series of quinoline derivatives were analysed as human acetylcholinesterase inhibitors and butyrylcholinesterase inhibitors; dissociation constants were defined to define the inhibitory potential of these compounds. The kinetic results were also analysed by molecular modelling.

In collaboration with the Laboratory for Molecular Ecotoxicology of the Division for Marine and Environmental Research, Ruđer Bošković Institute we continued research on the structural and functional characterization of organic cation transporters (OCTs), which serve as uptake transporters of numerous endo- and xenobiotics, by focusing on the structural characteristics of zebrafish (dr) and human (h) OCT1 (41). Through extensive use of homology modelling and molecular docking methods, we predicted three-dimensional structures of drOCT1 and hOCT1 and identified crucial amino acid residues inside the active site cleft by modelling interactions between OCT1 and fluorescent substrates (ASP+, Rh123, berberine, DAPI, and EtBr).

Cooperation with scientific institutions in Serbia, the Institute of Physics Belgrade and the Faculty of Chemistry of the University of Belgrade, was achieved with the purpose of complementary analysis of persistent compounds in environmental samples and the application of advanced statistical methods. The first publication within the framework of the co-operation was written and accepted by an international journal (78). The topic of the publication is the processing of air contamination data by organochlorine compounds using so-called SOM Methods (Self-organizing maps). This cooperation is linked to the long-term cooperation of the Unit and the Department of Ecology, Agronomy and Aquaculture and the Department of Health Studies of the University of Zadar. Examination of the level of organochlorine pesticides and polychlorinated biphenyls in lake and sea fish samples representing water contamination indicators. Results of research were given in two publications, one sent to an international journal, and another in the process of preparation. Results of the study of the dependence of organochlorine pesticides and polychlorinated biphenyls in mother's milk on maternal age and number of births were presented in the publication in preparation.

*MONitoring NETwork for determination of POPs in ambient air using the polyurethane foam passive sampler* (MONET project under auspices of the RECETOX, Regional Center for Environmental Chemistry and Toxicology, Masaryk University, Brno, Czech Republic). Sampling continued as normal, since the initiation of the project in 2009.

#### **A.2. In-house scientific projects (Chapter 16.1.A.2.)**

- *Design, synthesis and evaluation of selective inhibitors of butyrylcholinesterase*

#### **B. RESEARCH PROJECTS FUNDED BY EXTERNAL SOURCES**

- *Design, synthesis and evaluation of new antidotes in nerve agents and pesticides poisoning* (CHOLINESTERASE, Chapter 16.1.A.1.)
- *Organic pollutants in environment – markers and biomarkers of toxicity* (OPENTOX, Chapter 16.1.A.1.)
- *Interaction of metallic nanoparticles with sulphur-containing biomolecules – implications for nano-bio interface* (NanoFaceS, Chapter 16.1.A.1.)
- *Activity and in silico guided design of bioactive small molecules* (Adesire, Chapter 16.1.B.1.)
- *Development of bioassay method for detection of herbicide residues in soil* (Chapter 16.1.B.4.)
- *Kinetic evaluation of PON1 interactions with pharmacologically active carbamates* (Chapter 16.2.A.10.)

#### **PROFESSIONAL SERVICES**

The analyses of PCB congeners in samples of used motor oil were performed for external clients (Inspekt RGH d.o.o., Sarajevo, Bosna and Hercegovina).

Professional project "Spatial and temporal distribution of pollutants (nitrates, phosphates, pesticides, heavy metals) from agriculture in different agroecological conditions" was started in collaboration with the Faculty of Agriculture, Zagreb and Hrvatske vode (subcontractor: S. Stipičević). Project period: 10/2017 - 12/2018. The aim of the project is to determine key parameters of the pollutant molecule in the soil under laboratory and field conditions, and to set up an empirical model to simulate various scenarios (precipitation intensity, concentrations and pollutant type, soil type) to estimate potential risk of water resources contamination in different agroecological conditions. At the beginning of the project, two wine-growing locations were selected: Jazbina, Zagreb (continental region) and Baštica, Zadar (Mediterranean region). Lysimeters were installed at both locations for sampling spring water and soil samples were collected for pedological characterization and for sorption experiments. Within our Unit, we investigated the sorption behaviour of  $\alpha$ -cypermethrin

insecticide in samples of dried, sieved soil (2 mm) from Jazbina. Soil was sampled in triplicates at soil depths: 0-25, 25-50, and 50-70 cm.

## PROFESSIONAL ACTIVITIES OF THE UNIT'S EMPLOYEES OUTSIDE THE INSTITUTE

### *A. Bosak*

Member of the Executive Board of the Croatian Society of Natural Sciences; shop steward representing scientific staff at the Independent Trade Union of Science and Higher Education for the branch IMROH.

### *S. Fingler Nuskern*

Member of the Technical Board of CSI/TO 147 Water Quality at the Croatian Standards Institute (CSI).

### *S. Herceg Romanić and G. Mendaš Starčević*

Members of the Working Group for monitoring and meeting the requirements of the Second National Plan for the Implementation of the Stockholm Convention on Persistent Organic Pollutants.

### *M. Katalinić*

Secretary (since 13 Jun 2017), member of the Executive Board and member of the Society and Science Committee of the Croatian Society of Biochemistry and Molecular Biology.

### *Z. Kovarik*

President (until 13 Jun 2017) and member of the Executive Board of the Croatian Society of Biochemistry and Molecular Biology; president (since 22 Feb 2017), the vice-president (until 22 Feb 2017) and member of the Executive Board of the Croatian Society of Natural Sciences; member of the Panel Biology of the Croatian Science Foundation; member of the Board for Natural Sciences, Chemistry, National Scientific Committee, member of the Scientific Advisory Board of the Organisation for the Prohibition of Chemical Weapons (SAB OPCW); member of the *International Advisory Board on Cholinesterases* and *International Advisory Board on Cholinergic Mechanisms*; member of the *FEBS Advance Course Committee* and NATO's Working Group Medical Chemical Defence against Chemical Warfare Agent Threats; member of the Editorial Board of *International Scholarly Research Notices–Toxicology* journal, organizer of the OPCW Workshop on Trends in Chemical Industry, 2-5 Oct 2017, Zagreb; member of the Organizing Committee, 45<sup>th</sup> Congress of the Federation of European Biochemical Societies – FEBS2020, 4-9 Jul 2020, Ljubljana, Slovenia.

### *M. Meštrović*

Delegate representing the non-scientific staff at the Independent Trade Union of Science and Higher Education for the branch IMROH.

## SCIENTIFIC ADVANCEMENT OF EMPLOYEES

Scientific degree of scientific associate was gained by M. Dvorščak and N. Maček Hrvat.

Scientific degree of permanent scientific advisor was gained by Z. Kovarik.

N. Maraković earned a PhD degree and was awarded a postdoctoral research associate appointment.



## 2.3. Radiation Dosimetry and Radiobiology Unit

### EMPLOYEES

#### HEAD

Ivica Prlić, PhD, professional advisor in science

#### RESEARCHERS

Ivan Pavičić, PhD, senior scientific associate

Marija Surić Mihić, PhD, scientific associate

Ana Marija Marjanović Čermak, PhD, postdoctoral researcher

Krunoslav Ilić, MSc, assistant (from 15 Dec 2017)

Luka Pavelić, MSc, assistant (from 1 Jun 2017)

Tomislav Meštrović, MSc, senior professional associate in science

Paula Čović, MSc, professional associate in science, funded by IMROH (from 15 Dec 2017)

Mihaela Justić, MSc, professional associate in science, funded by IMROH

Domagoj Kosmina, MSc, professional associate in science, funded by IMROH (until 31 Jan 2017)

Jerko Šiško, MSc, professional associate in science

#### TECHNICAL STAFF

Selvije Sefić, BSc, senior technician (substitute: Helena Jauk, MSc, from 1 Dec 2017)

Silvija Kobeščak, BSc, technician

Ljudevit Orešić, BSc, technician (until 15 Sep 2017)

### RESEARCH

#### A. RESEARCH ACTIVITIES WITH INSTITUTIONAL FINANCING

##### A.1. In-house scientific projects (Chapter 16.1.A.2.)

- *TTSem2: Thermometry, Thermography and sensory evaluation of electromagnetic radiation in medicine*

Research planned in WP1 *Thermographic characteristics of fracture thumb joint healing in adulthood* has finished and has been partly published within the doctoral thesis of Damir Halužan, MD, Faculty of Medicine, University of Zagreb. A research using experimental IR thermography methods, which are performed at KBC Zagreb (WP5), *Thermographic characteristics of breasts in women with invasive ductal cancer* is in progress. A proposed theme for the PhD thesis of a plastic surgery intern, Marko Mance, MD was accepted by the Doctoral Program Committee, School of Medicine, University of Zagreb. Ongoing research (WP4) within *Thermographic characteristics of fractured clavicle and humerus bone in children* (IMROH, KBC Zagreb and Children's Hospital Zagreb) has also carried out with the experimental collaboration of IMI associates. Preliminary results are expected during 2018. The continuation of clinical trials is under preparation (WP2) within *Mapping temperature symmetry of the skin region, in children and adults in both sexes*. Our plan is to take measurements during ambulatory examinations at the surgery polyclinic, KBC Zagreb. The purpose of this research is to attempt the standardization of physiological deviations in healthy population and measurement of standard deviation for individual regions. So far, similar measurements have been made, but there is no proper study regarding age-related differences. The preparation of the clinical part of the research (WP3) is ongoing under the title *Skin thermometry under the splint in fracture of thumb bone*. After Multiple test trials, special thermometers designed by IMROH external associates are ready for

contact measurement and storage of temperature information of predetermined portions of the skin/tissue throughout the time of carrying immobilization (cast) on the hand. The plan is to take measurements in patients at the Clinic for Surgery, KBC Zagreb, within the standard procedure of treating fractures (254).

- *SUVIndex: Development of UV radiation sensors*

The Unit together with its external associates from ALARA uređaji d.o.o., Haj-Kom d.o.o., and KBC Zagreb developed ultraviolet radiation sensors that together with a computer processor will enable continuous individual monitoring of exposure to the sun radiation of persons working in the open and are extensively exposed to UV radiation (workers in agriculture, sailors, fishermen, etc.), which will be the basis for the design of the work protocol on the implementation of preventive protection against excessive exposure to UV radiation.

## A.2. Other research activities

In research funded from own funds, we investigated the effects of RF radiation of 1800 MHz frequency and induction of oxidative stress by fibroblasts. The effect of irradiation on induction and induction of ROS, the amount of reduced GSH, the activity of GSH-peroxidase, and the level of malondialdehyde were determined. Compared to control, viability after radiation has not changed as well as the level of malondialdehyde. Level of GSH & activity of GSH peroxidase was increased. (38)

## B. RESEARCH PROJECTS FUNDED BY EXTERNAL SOURCES

- *Interaction of metallic nanoparticles with sulphur-containing biomolecules – implications for nano-bio interface* (NanoFaceS, Chapter 16.1.A.1.)
- *Quantum-chemical design, preparation and biological properties of organometallic nucleobase derivatives* (OrDeN, Chapter 16.1.B.1.)
- *Electromagnetic Radiation Dosimetry for the e-School Project Implementation: establishment of a system of digitally mature schools (pilot project)* (Chapter 16.2.A.1.)
- *HORIZON 2020 European Concerted Program on Radiation Protection Research, (CONCERT, Chapter 16.2.A.2.)*
- *NORM for building materials* (NORM4Building, Chapter 16.2.A.5.)

## PROFESSIONAL SERVICES

The **IMI\_FondNEK Study** was created in three volumes, of which the final bears the title "Radiological monitoring program for extended location of Radioactive Waste Storage Facility in the Republic of Croatia" - rev.4.0 - Extract after review procedure: Environmental Monitoring Program - The PSO program, i. e. the implementation of systematic testing and monitoring of ionizing radiation on Dvor Municipality in Sisak-Moslavina County - IMI - Class: 07-75 / 16- 00/5, Ur.br.:100-08 / 16-3 from November 2017. PSO involves the implementation of radiological monitoring using modern "trace method" developed on IMI, measurement  $H^*(10)$  using environmental dosimeters, testing radioactivity of soil, plants, water and human food, non-radiological parameters, and the possible characterization of stored materials in the area of Dvor municipality in Sisak-Moslavina county. The contractor was the Fund for Financing the Decommissioning of the Krško Nuclear Power Plant and the Disposal of NEK Radioactive Waste and Spent Nuclear Fuel (idea and project leader: I. Prlič (2016-2017) based on archival scientific and professional IMROH data). The study was reviewed and published in three volumes and will be conducted in the field when the Government of the Republic of Croatia approves and passes the National Strategy Implementation Program for the Disposal of Radioactive Waste, Spent Sources and Spent Nuclear Fuel (The National Program).

For the needs of the **INA Group** (Petrol Industry), in order to align their business operations and

development of protocols for the implementation of business activities involving manipulation of natural radioactive materials (NORM) and planning action in case of an extraordinary event that contains radiological risks to determine the need for specialized vocational training and the implementation of ionizing-related safety measures in radiation, several studies were contracted, two of which during 2017 for STSI d.o.o. Pipeline Service Workshop, member of INA Group:

- Initial risk assessment of exposure of workers to ionizing radiation from NORM during the regular production process of pipe cleaning at STSI d.o.o. Workshops for the Service of pipe tubing Stružec as defined by the Law on radiological and nuclear safety act (OG 141/13, 39/15, 130/17) and the accompanying Regulations.

Excerpt from the Study of employee radiation estimates and reference population groups in implementation production activities that may increase the irradiation of workers and residents of natural sources of ionizing radiation at INA group workshops in the Republic of Croatia - initial risk assessment, Class: 07-75 / 17- 00/6, Reg. no.: IMI 100-08 / 17- 4.

- An expert opinion that workers STSI d.o.o. - Stružec tubing service workshops are not / are exposed workers as defined by the Radiological and Nuclear Safety Act (OG 141/13, 39/15, 130/17) and the accompanying Regulations of the Worker's and referent population groups in Radiation Assessment Study in the implementation of the production activities they may come across increase in irradiation of workers and residents from natural sources of ionizing radiation at workshops of the INA Group in the Republic of Croatia - initial risk assessment: Class: 07-75 / 17- 00/6, Reg. no.: IMI 100-08 / 17- 3.

Head and chief of both study outcomes: I. Prlić; IMI associates: L. Pavelić, S. Kobeščak, M. Justić, M. Surić Mihić, M. Šoštaric, M. Avdić, J. Senčar, and External Associates of the Unit (IMI) Z. Cerovac, M. Hajdinjak).

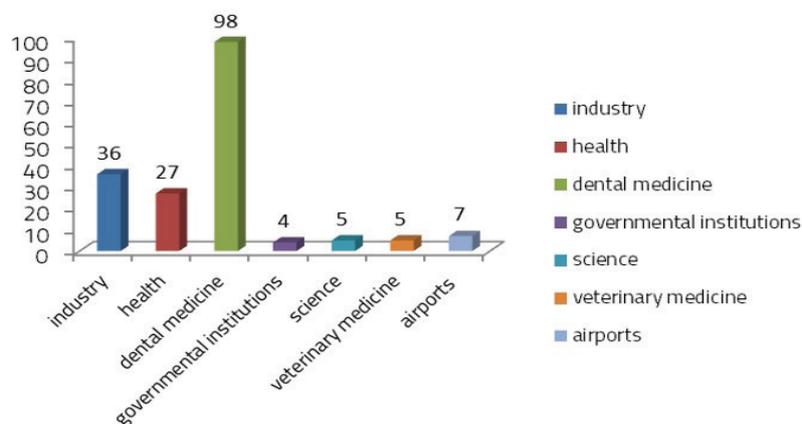
A total of 38 risk assessment studies were performed focusing on the use of ionising radiation sources in medicine, dental medicine, research, and industry with different contractors.

#### *List of contractors*

CONTRACTOR	REPORT AUTHOR
Andrija Štampar Teaching Institute of Public Health	M. Surić Mihić
Clinical Hospital Centre Zagreb	M. Surić Mihić
Community Health Centre Županja	M. Surić Mihić
Croatian Institute of Geology	J. Šiško
Dental centar EDENT d.o.o.	J. Šiško
Dental estetic studio d.o.o.	M. Surić Mihić
Dental Polyclinic Zagreb	M. Surić Mihić
Dental practice Alda Žagar	M. Surić Mihić
Dental practice Andrea Cattonaro	M. Surić Mihić
Dental practice Bojan Plantak	J. Šiško
Dental practice Daria Dragica	J. Šiško
Dental practice Darko Krnić	M. Surić Mihić
Dental practice Ivana Friganović Petrica	M. Surić Mihić
Dental practice Ivana Lijić	M. Justić
Dental practice Milan Arnautović	M. Surić Mihić
Dental practice mr.sc. Ines Jončić	M. Surić Mihić
Dental practice Snježana Friščić-Kastel	J. Šiško
Dental practice Zlatko Stanec	J. Šiško
Dentex d.o.o.	M. Justić
Digital smile d.o.o.	J. Šiško

Đuro Đaković – termoenergetska postrojenja d.o.o.	M. Surić Mihić
General hospital Gospić	M. Justić
HEP – Proizvodnja d.o.o.	J. Šiško
Kontrol product d.o.o.	J. Šiško
M.T.F. d.o.o.	J. Šiško
Osram d.o.o.	M. Surić Mihić
Penitentiary in Lepoglava	M. Justić
Polyclinic for dental pathology and endodontics with paradontology and orthodontics dr. Blašković	M. Surić Mihić
Polyclinic for dental prosthetics, orthodontics, and paradontology Ksaver	M. Surić Mihić
Polyclinic for oral surgery, dental prosthetics, orthodontics, panoramic X-ray, and dental laboratory Identalia	M. Surić Mihić
Polyclinic IDENT	J. Šiško
Polyclinic K-centar	M. Surić Mihić
Premium dent d.o.o.	J. Šiško
Private dental practice Maja Ana Pleslić Zagoda	J. Šiško
Private dental practice Mladen Novaković	M. Justić
Saint Jean Industries d.o.o.	M. Justić
VIK – dental d.o.o.	M. Surić Mihić
Zadar Harbour	M. Justić

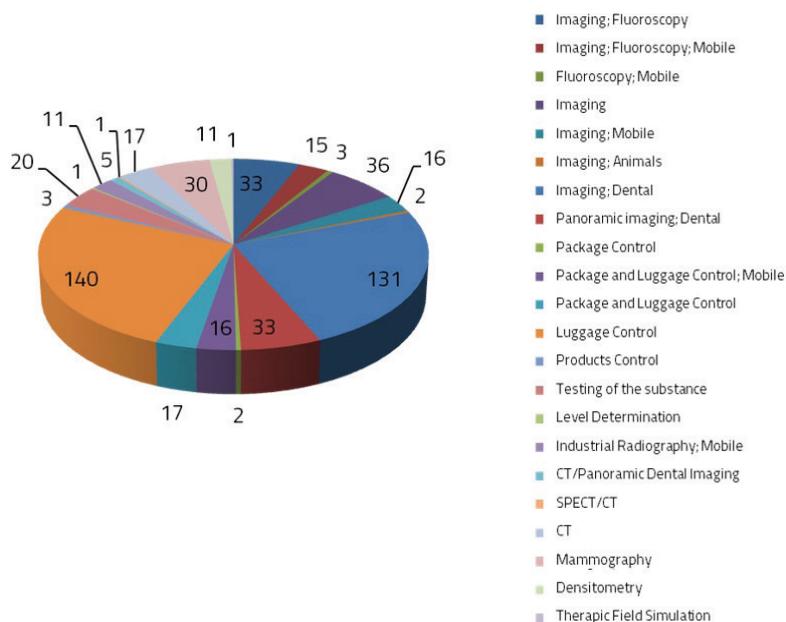
In 2017, the Unit performed personal dosimetry monitoring and quality control procedures of radiation sources for 181 contracting customers from different areas of work.



*Number of contracting customers of the Unit arranged by field of work*

More than 27,000 dosimetric measurements were performed during 2017 based on which over 3,750 dosimetry reports for customers of the dosimetry service were issued. The process of upgrading computational software for dosimetry data processing was started. The upgrade shall enable ring, wrist, and environmental dosimetry data processing. The Unit has taken preliminary actions enabling on-line delivery of dosimetry reports to customers of the authorized dosimetry service which would modernise management and customer relations. Documentation for accreditation of methods for measurement  $H_p(0,07)$  and  $H^*(10)$  using termoluminescent dosimeters (TLD) was also prepared during the past year.

More than 600 quality control tests and tests of radiation protection parameters for over 550 units of electrical equipment emitting ionising radiation and about 50 radioactive sources used in medicine, industry, and science was performed in 2017. Based on these tests, more than 1,200 expert reports and more than 1,300 expert opinions were issued.



*Number of units of electrical equipment emitting ionising radiation inspected by employees of the Unit, arranged by type*

In our laboratory, we conducted testing of human serum immune response to specific allergens of 7 individuals.

The identification of all types of asbestos in solid materials was also performed according to the International Organization for Standardization (General requirements for the competence of testing and calibration laboratories International Standards Organisation (ISO), Geneva: 1999). We completed eight analyses of solid materials sent from commercial companies to determine the presence and type of asbestos. Analysis of the material was performed by a standardized method for stereo and polarized microscopy MDHS 77-HSE Document "Method for the Determination of Hazardous Substances; series 77 - Asbestos in bulk materials "[in: HSG 248 Asbestos: The analysts' guide for sampling, analysis and clearance procedures. Appendix 2: Asbestos in bulk materials: sampling and identification by polarized light microscopy (PLM)].

#### *International laboratory intercomparisons in 2017*

ORGANISER	TEST	AREA	PLACE AND DATE
"Jožef Stefan" Institute, Ljubljana, Slovenia	"PRIMER 2017"	dH*(10)/dt / Identification of radioactive sources	Ljubljana, Slovenia, 13 Sep 2017

#### *List of accredited methods*

TEST METHOD	TYPE OF TEST, RANGE
ME-608-001 (In-house method)	Personal dosimetry of the photon radiation using TL dosimeters in the range 85 µSv - 100 mSv and energy range 33 keV - 1.3 MeV
ME-608-002 (In-house method)	Determination of ambient equivalent dose rate; H*(10)/t data dose range 100 nSv/h - 100 mSv/h and energy range 36 keV - 1.3 MeV

The Unit's quality manager: T. Meštrovič.

## PROFESSIONAL ACTIVITIES OF THE UNIT'S EMPLOYEES OUTSIDE THE INSTITUTE

*T. Meštrović*

Member of the Technical Committee TO-45 Nuclear instrumentation at the Croatian Standards Institute.

*L. Pavelić and J. Šiško*

Associate members of the European Radiation Dosimetry Group (EURADOS) Working Group WG3-S2.

*I. Pavičić*

Member of the Working Group in charge of drafting the Position of Croatia in the area of protection against electromagnetic fields.

*I. Prlić*

Appointed member of the Committee in charge of producing a Draft of the Amendments to the Act on Radiological and Nuclear Safety; member of the Working Group formed by the State Office for Standardisation and the Ministry of Health for legal metrology in the field of medical equipment (especially the one producing radiation); member of the Executive Board of the Croatian Biomedical Engineering and Medical Physics Society (CROMBES); member of the Education and Training Committee of the European Federation of Organisations for Medical Physics (EFOMP); member of Technical committees TO Non-destructive testing, TO-45 Nuclear instrumentation, and TO-62 Electronical equipment in medical practice; head of the section TO-62B Imaging in medicine at the Croatian Standards Institute; member of the Working Group for drafting and applying a Country Frame Programme (CFP) of the Republic of Croatia; member of the International Atomic Energy Agency (IAEA); member of the Working group of the European Commission Environmental Radiation-Effect: International Perspectives – part of the project related to Croatia; the Croatian representative in the International Organization for Medical Physics and International Union for Physical and Engineering Sciences in Medicine; member of the Ministry of Healthcare's Committee for the revision and evaluation of studies in the field of use of nonionizing radiation sources; member and expert of the European ALARA Network for Naturally Occurring Radioactive Materials (EAN NORM) group; international expert for the International Road Transport Union (IRU) and International Labour Organization (ILO); member of the Management Committee of MELODI (Multidisciplinary European Low Dose Initiative); member of the Management Committee of the international project COST4BUILDING Materials, Transport and Urban Development COST Action TU1301. Croatian member of the Task Group on Radioactive Source Security, ICRP (International Committee for Radiation Protection). Member of the reference group appointed by the Ministry of Science and Education for cooperation with EU EURATOM.

*M. Surić Mihić*

Associate member of the European Radiation Dosimetry Group (EURADOS); member of Working Groups WG2 and WG3-S2; member of Technical Committee TO-62 Electrical Equipment in Medical Practice at the Croatian Standards Institute. During 2017, engaged by the State office for radiological and nuclear security as an expert advisor for drafting ordinances following the Radiological and Nuclear Safety Act.



## 2.4. Environmental Hygiene Unit

### EMPLOYEES

#### HEAD:

Gordana Pehnac, PhD, senior scientific associate

#### RESEARCHERS

Ivan Bešlić, PhD, senior scientific associate

Mirjana Čačković, PhD, senior scientific associate (until 31 Jul 2017)

Ranka Godec, PhD, scientific associate

Silva Žužul, PhD, scientific associate

Silvije Davila, PhD, postdoctoral researcher

Jasmina Rinkovec, PhD, junior researcher, (assistant until 30 Jun 2017), postdoctoral researcher (from 1 Nov 2017)

Ivana Jakovljević, PhD, junior researcher, assistant

Valentina Gluščić, BSc, professional associate in science

Zdravka Sever Štrukil, Bsc, professional associate in science

Iva Šimić, Msc, professional associate in science, funded by IMROH

#### TECHNICAL STAFF

Ana Filipec, statistician, senior technician

Zvonimir Frković, senior technician

Samuel Ljevar, senior technician

Ana Mihaljević, senior technician

Marija Antolak, technician

Vjeran Dasović, technician (until 24 Sep 2017)

Karmenka Leš Gruborović, technician

Martin Mihaljević, technician

Martina Šilović Hujić, technician

#### PARTICIPATING RETIRED RESEARCHERS

Vladimira Vađić, PhD, permanent scientific advisor

Krešimir Šega, PhD, permanent scientific advisor

### RESEARCH

#### A. RESEARCH ACTIVITIES WITH INSTITUTIONAL FINANCING

##### A.1. Long term research activities

Measurements of metals in particulate matter and total deposited matter by inductively coupled plasma mass spectrometry (ICP-MS) continued at different locations with different pollution sources (253). The method for determination of platinum, palladium, and rhodium developed in the Unit in previous years was applied for the analysis of the PM<sub>10</sub> particle fraction samples collected at three locations and PM<sub>2.5</sub> particle fraction samples collected at one location in Zagreb (53, 242).

Measurements of polycyclic aromatic hydrocarbons (PAHs) at locations with different pollution sources were continued. PAH concentrations were determined in PM<sub>10</sub> particle fraction at three

locations in Zagreb with a different influence of pollution sources (traffic, industry, households) (245). Results obtained over ten years at one location in Zagreb were analysed in order to determine long-term trends and behaviour. Annual BaP concentrations showed an increasing trend as well as the sum of other seven measured PAHs. Contrary to PAH mass concentrations, the contribution of BaP in their sum did not show an increasing trend. Comparing PAH levels in  $PM_{10}$  and  $PM_{2.5}$  particle fractions, it was found that during winter more than 80 % of PAHs were present in the  $PM_{2.5}$  fraction (240). Comparing PAHs bonded on  $PM_{2.5}$  and  $PM_1$  particle fraction, it was found that most of the PAHs were contained in the smaller ( $PM_1$ ) fraction, especially during winter (more than 90 %) (230).

Measurements of elemental and organic carbon in  $PM_{2.5}$  particle fraction continued at monitoring sites with different characteristics (urban background and rural background). The spatial distribution of carbon was studied as well as the influence of traffic density on carbon concentrations in the air (146, 228, 247, 248, 281).

Measurements of ozone and its precursors nitrogen dioxide and carbon monoxide were continued and the relationship with other air pollutants was studied (154).

Measurements of anion ( $Cl^-$ ,  $NO_3^-$ ,  $SO_4^{2-}$ ) and cation ( $Na^+$ ,  $NH_4^+$ ,  $K^+$ ,  $Mg^{2+}$ ,  $Ca^{2+}$ ) content in  $PM_{2.5}$  particle fraction continued. This investigation focuses on the influence of the sampling site and season of the year on mass concentrations and relative contribution of measured water-soluble anion and cation species to the  $PM_{2.5}$  particle fraction. Daily  $PM_{2.5}$  samples were taken at the urban background sampling site (UBS) and rural background sampling site (RBS) in Croatia over three years 2014–2016. The  $PM_{2.5}$  mass concentration was significantly influenced by the season, reaching high values in the winter. The annual average  $PM_{2.5}$  mass concentration measured at the UBS and RBS ranged from  $19.6 \mu g m^{-3}$  to  $22.7 \mu g m^{-3}$  and from  $8.0 \mu g m^{-3}$  to  $9.4 \mu g m^{-3}$ , respectively. The annual average ion mass concentrations at UBS and RBS followed the order:  $SO_4^{2-} > NO_3^- > NH_4^+ > K^+ > Ca^{2+} > Cl^- > Na^+ > Mg^{2+}$  and  $SO_4^{2-} > NH_4^+ > NO_3^- > K^+ > Ca^{2+} > Mg^{2+} > Na^+ > Cl^-$ , respectively. Annual average mass ratios of  $(NO_3^-)/(SO_4^{2-})$  obtained in  $PM_{2.5}$  at UBS and RBS were indicating that mobile source emission was an important contributor to particle mass at UBS (221, 276).

#### **A.2. In-house scientific projects (Chapter 16.1.A.2.)**

- *Levels of platinum group elements (PGE) near roads*

Sampling of  $PM_{10}$  particle fraction has started at three locations in Zagreb. The project will provide a continuation of PGE monitoring in airborne particulate matter in order to determine concentration trends. The project will also include measurements of these elements in other environmental samples (vegetation, soil).

- *Organic content of  $PM_1$  particle fraction*

Sampling of  $PM_1$  particle fractions has started at one location in Zagreb. The elemental and organic carbon, as well as polycyclic aromatic hydrocarbons, will be measured in collected samples.

#### **B. RESEARCH PROJECTS FUNDED BY EXTERNAL SOURCES**

- *AIRQ – Project of extension and modernisation of the national network for continuous air quality monitoring* (Chapter 16.2.A.1.)
- *Apportioning air pollution sources on a regional scale* (Chapter 16.2.A.6.)

#### **PROFESSIONAL SERVICES**

The monitoring of air pollution continued in Zagreb at the measuring stations of the local measuring network. At Zagreb stations, the Institute measured different pollutants in the air: sulphur dioxide, black carbon,  $PM_{10}$  particle fraction, metals arsenic (As), cadmium (Cd), nickel (Ni), lead (Pb), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), polycyclic aromatic hydrocarbons (PAH) in  $PM_{10}$  particle fraction,  $PM_{2.5}$  particle fraction, nitrogen dioxide ( $NO_2$ ), ozone ( $O_3$ ), carbon monoxide

(CO), benzene, total deposited matter, and metals As, Cd, Ni, Pb, and Mn in the total deposited matter. Three stations located in Zagreb have become part of the worldwide system for monitoring the quality of the environment (GEMS), coordinated by the World Health Organization within the framework of the activities of the United Nations Environment Programme (UNEP).

The monitoring of air, water, soil, agricultural, and forest ecosystems and control of wild animals in the vicinity of the Central Gas Station (CGS) Molve continued. In 2017, in cooperation with the Institute of Public Health of the Koprivnica-Križevci County, the Institute performed measurements of hydrogen sulphide, mercaptans, and sulphur dioxide at five locations in the proximity of the CGS Molve.

The monitoring of air quality within the zone of influence of the Waste Water Treatment Plant in Zagreb was continued. The monitoring of levels of hydrogen sulphide, ammonium, and total mercaptans and meteorological parameters was carried out at five measuring stations.

Cooperation with the Croatian Agency for the Environment and Nature in the data processing of air conditions in the Republic of Croatia continued with the aim of establishing an environmental information system for the Republic of Croatia.

Pursuant to contracts with the Ministry of Environment and Energy and Meteorological and Hydrological Service of Croatia and the Air Protection Act (OG 130/11, 47/14, 61/17), the Environmental Hygiene Unit as a reference laboratory performs the sampling of particulate matter (PM<sub>10</sub> and PM<sub>2.5</sub>) and its physical and chemical analysis at measuring sites within the Croatian State Network for Air Quality Monitoring. The Unit also carries out equivalency of non-reference methods for the determination of particulate matter mass concentration (PM<sub>10</sub> and PM<sub>2.5</sub>) in the air.

In 2017, air pollutants were measured at the monitoring sites Zagreb-1, Zagreb-3, Sisak-1, Slavonski Brod-1, Slavonski Brod-2, Nacional Park Plitvice Lakes, Ksaverska cesta, Velika Gorica, Kutina, and Rijeka-2. Also, pursuant to the same contract, equivalence studies were performed for non-reference measuring methods of particulate matter fractions (PM<sub>10</sub> and PM<sub>2.5</sub>) at measuring sites Parg and Vela Straža (Dugi Otok) of the Croatian State Network for Air Quality.

In line with the contract with the Meteorological and Hydrological Service of Croatia, metals in total deposited matter were analysed at one monitoring station located on the military training polygon of Slunj.

In the vicinity of the Jakuševac waste site, the levels of PM<sub>10</sub> and mercaptans are continuously measured. During different seasons, levels of metals Pb, As, Ni, Cd and PAHs in PM<sub>10</sub> fraction were also measured as well.

Within the EL-TO Zagreb zone of influence, the levels of particle fraction PM<sub>10</sub> were also measured. Measurements of particle fraction PM<sub>10</sub> and PAHs in PM<sub>10</sub> fraction were carried out at a measuring site Pleso within Zagreb International Airport.

Cooperation on the project *Ecological map of the City of Zagreb* started pursuant to the contract with the City of Zagreb and Agreement with the Dr Andrija Štampar Teaching Institute of Public Health. The Unit provides quality control of air pollution measurements comparing the results obtained by sensors and by reference methods. The Unit also provides a service of 24-hour accessibility in case of accidental situations.

*List of intercomparisons*

ORGANISER	TEST	AREA	DATE
LGC	AIR PT Workplace Air, Ambient Air and Stack Emissions, Round 19 Group; Ambient Air (AR019); 13 – Metals	Determination of mass concentrations of metals Pb, Ni, As, Cd in particles	4/2017 5/2017
LGC	AIR PT Workplace Air, Ambient Air and Stack Emissions, Round: 19 Group: Stack Emissions (AR019); 33 – Metals	Determination of metals Tl, Mn, Cu, Cd, Co, Sb in deposited matter and sample volume of deposited matter	4/2017 5/2017
LGC	AIR PT Workplace Air, Ambient Air and Stack Emissions, Round: 19 Group: Stack Emissions (AR019); 39 – Dust analysis (solution)	Determination of total deposited matter	4/2017 5/2017
INERIS	INTERLABORATORY COMPARISONS 17/164060 2017 PROGRAM „Analysis of samples from stationary source emissions“. Order No. Program 9b : PAHs	Determination of polycyclic aromatic hydrocarbons: benzo(a)anthracene, benzo(b)fluoranthene, fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene, benzo(ghi)perylene, benzo(a)pyrene	5/2017
Lab Service Analytica S.r.l.	InterCinD 2017SE "INDUSTRIAL Fly Ash Organic PAH"	Determination of polycyclic aromatic hydrocarbons: benzo(a)anthracene, benzo(b)fluoranthene, fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene, benzo(ghi)perylene, benzo(a)pyrene, chrysene	6/2017

*List of accredited methods*

METHOD	TYPE OF TEST, RANGE
HRN EN 14625:2012 (EN 14625:2012)	Determination of the concentration of ozone in the ambient air
HRN EN 14626:2012 (EN 14626:2012)	Determination of the concentration of carbon monoxide in the ambient air
HRN EN 14902:2007 (EN 14902:2005), HRN EN 14902/AC:2007 (EN 14902:2005/AC:2006)	Determination of the concentration of Pb, Cd, As and Ni in the PM <sub>10</sub> fraction of suspended particulate matter
SIS-TP CENT/TR 16243:2011 (CEN/TR 16243:2011)	Determination of the mass concentration of elemental and organic carbon in the suspended particulate matter in the ambient air
HRN EN 15549:2008 (EN 15549:2008)	Determination of the concentration of benzo(a)pyrene in the ambient air
HRN EN 14211:2012 (EN 14211:2012)	Determination of the concentration of nitrogen oxide in the ambient air
HRN EN 12341:2014 (EN 12341:2014)	Determination of mass concentration of PM <sub>10</sub> and PM <sub>2.5</sub> particle fraction
HRN EN 14212:2012 (EN 14212:2012), HRN EN 14212:2012/Ispr. 1:2014 (EN 14212:2012/AC:2014)	Determination of the concentration of sulphur dioxide in the ambient air
HRI CENT/TR 16269:2017 (CEN/TR 16269:2011)	Determination of the mass concentration of anions and cations in the suspended particulate matter

VDI 4320 Part 2: 2012 (VDI 4320 Part 2:2012)	Determination of the dust deposition according to the Bergerhoff method
HRS CEN/TS 16645:2016 (CEN/TS 16645:2014)	Determination of the concentrations of benz(a)anthracene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, dibenz(a,h)anthracene, indeno(1,2,3-cd)pyrene and benzo(ghi)perylene in ambient air

Until 27 Aug 2017, the Unit's quality manager was M. Čačković. As of 28 Aug 2017, the Unit's quality manager is R. Godec.

## PROFESSIONAL ACTIVITIES OF THE UNIT'S EMPLOYEES OUTSIDE THE INSTITUTE

### *I. Bešlić*

Member of the Croatian Air Pollution Prevention Association's Presidency; member of the Working Group in charge of monitoring the activity plan for the national network for permanent air quality monitoring at the Ministry of Environmental Protection and Energy of the Republic of Croatia; member of the Commission for Reference Laboratory Work Monitoring at the Ministry of Environmental Protection and Energy of the Republic of Croatia; member of the Commission for the Selection of the Measuring Stations in the National Air Quality Monitoring Network; member of the Working Group for Air of the Croatian Accreditation Agency; member of the TO-146 Air Quality Committee of the Croatian Standards Institute; member of the Executive Editorial Board of the scientific journal *Archives of Industrial Hygiene and Toxicology*; member of the "Air Protection '17" conference Scientific Committee.

### *M. Čačković*

Member of the TO-146 Air Quality Committee of the Croatian Standards Institute; vice-president of the Working Group for Air of the Croatian Accreditation Agency; member of the "Air Protection '17" conference Scientific Committee.

### *S. Davila*

Member of the "Air Protection '17" conference Organizing Committee.

### *G. Pehnec*

President and International coordinator of the Croatian Air Pollution Prevention Association; member of the Working Group in charge of monitoring the activity plan in the national network for permanent air quality monitoring at the Ministry of Environmental Protection and Energy of the Republic of Croatia; member of the Commission for Air Quality Improvement Monitoring in the area of Slavonski Brod; president of the "Air Protection '17" conference Organizing Committee.

### *J. Rinkovec*

Member of the "Air Protection '17" conference Organizing Committee.

### *S. Žužul*

Treasurer and member of the Croatian Air Pollution Prevention Association's Presidency; member of the "Air Protection '17" conference Scientific Committee.

## SCIENTIFIC ADVANCEMENT OF EMPLOYEES

J. Rinkovec and I. Jakovljević earned a PhD degree.

J. Rinkovec was awarded a postdoctoral research associate appointment.



## 2.5. Occupational Health and Environmental Medicine Unit

### EMPLOYEES

#### HEAD

Prim Jelena Macan, PhD, MD, permanent scientific advisor (90 % of working hours and 10 % in the Institute's company)

#### RESEARCHERS

Jasminka Bobić, PhD, permanent scientific advisor

Prof Selma Cvijetić Avdagić, PhD, MD, permanent scientific advisor

Veda Maria Varnai, PhD, MD, permanent scientific advisor

Ljerka Prester, PhD, scientific advisor

Doc Adrijana Bjelajac, PhD, scientific associate (from 1 Sep 2017)

Željka Babić, PhD, assistant (until 11 Jul 2017), postdoctoral researcher (from 1 Sep 2017)

Jelena Kovačić, PhD, assistant (until 30 Jun 2017), postdoctoral researcher (from 1 Sep 2017)

Zrinka Franić, MD, junior researcher, assistant

Rajka Turk, MSc, professional advisor in science

#### TECHNICAL STAFF

Marija Kujundžić Brkulj, senior technician

Marija Lieberth, senior technician

Rajka Luzar, senior technician

Franka Šakić, senior technician (90 % of working hours in the Unit and 10 % in the Institute's company)

Mirela Deranja, technician

#### PARTICIPATING RETIRED RESEARCHERS

Božica Kanceljak-Macan, PhD, MD, permanent scientific advisor

Doc Biserka Ross (Radošević Vidaček), PhD, scientific advisor

### RESEARCH

#### A. RESEARCH ACTIVITIES WITH INSTITUTIONAL FINANCING

##### A.1. In-house scientific projects (Chapter 16.1.A.2.)

- *Employment status of patients with diagnosed occupational disease*

Project was finished according to plan in June 2017. All patients ( $n = 147$ ) who were diagnosed with an occupational disease in the period from 2005 to 2014 in the outpatient clinic for occupational and sports medicine of the Institute for Medical Research and Occupational Health were singled out for the study. The survey ultimately included 95 respondents who signed an informed consent. The survey included an overview of archived data and a telephone survey that compiled data on the employment status of patients after diagnosing the occupational disease. Results showed that carpal tunnel syndrome was the most common occupational disease, and the most common workplace where it occurs, besides the position of textile workers and tailors, is office work that includes work with a computer. Employment status after the recognition of occupational diseases changed in 59 respondents (62 %) of. After recognition of the occupational disease, 36 respondents (38 %) remained employed, 12 respondents (13 %) became unemployed, and 47 respondents (49 %)

were retired. Twenty-eight employed respondents (78 %) remained working for the same employer, and 7 changed their workplace. Eight respondents (22 %) found jobs with another employer, and 4 were retrained. The causes of unemployment were contract termination (10 respondents, i.e. 83 %), and closing down the business (2 respondents, i.e. 17 %). Professional rehabilitation procedures included 5 respondents (5 %). The highest number of retired respondents (35, i.e. 74 %) was granted a disability pension. The share of unemployed respondents after diagnosing occupational diseases was significantly higher in the period 2010-2014, compared to 2005-2009 (21 % vs. 6 %,  $p = 0.033$ ; Fisher exact test). Our results pointed out the inadequacy of care for workers with occupational diseases in Croatia, with a noticeable trend of more frequent dismissal of workers with a diagnosed occupational disease in Croatia from 2000 to present, without the management of diseased workers by employers and competent institutions through professional rehabilitation procedures. This project was the basis for a postgraduate degree thesis of Renata Ecimović Nemarnik, MD, under the mentorship of J. Macan (205).

- *Interaction of constitutional and occupational risk factors on the incidence of occupational contact dermatitis in hairdressing apprentices during vocational training*

The implementation started in Jun 2017. After preparatory activities, in the period between September and December 2017, 352 first-year hairdressing apprentices were included in the study and evaluated according to protocol. The recruitment of the apprentices was performed in 24 schools from four Croatian regions, i.e. 15 counties (central Croatia, Slavonija and Baranja, north Croatian coast, and Dalmatia). The database of the collected data is under construction and materials have been prepared for the genotyping of buccal mucosa samples that will be performed in collaboration with the Mutagenesis Unit. The first wave of follow-up is planned for May 2018.

- *Bone mineral density and fracture risk in institutionalized older people*

Around 300 subjects from four nursing homes in Zagreb who underwent ultrasound bone densitometry were included. Data on calcium intake and physical activity were obtained by questionnaire. Preliminary results show a high prevalence of osteopenia and osteoporosis (>80 %) as well as a mean calcium intake lower than recommended. A large number of subjects (>50 %) had a very low physical activity frequency and a significantly higher prevalence of osteoporosis regardless of calcium intake. The association between pulmonary function determined by spirometry and bone density determined by ultrasound densitometry was analysed in a cross-sectional study on 370 university students. The most important determinant of bone density and lung function parameters was body mass index, with no direct association between them (14). A study on the relationship between vitamin D and bone density in a sample of 400 middle-aged women was completed. The prevalence of vitamin D deficiency was 39.2 % and no significant association of vitamin D deficiency with osteopenia or osteoporosis was found. The results of the research were sent to a journal for publication.

- *Contact hand dermatitis in dentists and medical doctors: prevalence and risk factors*

The project started in Nov 2017. The preparation for the start of the project in Feb 2018 is in progress. The main hypothesis of this research is that the prevalence of hand contact dermatitis is much higher in dentists and medical doctors with surgical professions in comparison to non-surgical professions and persons who are not occupationally exposed to skin irritants/allergens, which depends on the interaction between exposure to skin irritants/allergens and individual characteristics of the skin barrier. The aims of the projects are: 1) to determine the prevalence of irritant and allergic contact dermatitis in dentists and medical doctors with surgical and non-surgical professions in relation to persons who are not occupationally exposed to skin irritants/allergens, 2) to establish environmental and constitutional factors that impact the incidence and course of irritant and allergic contact dermatitis and their interaction.

## B. RESEARCH PROJECTS FUNDED BY EXTERNAL SOURCES

- *Biopsychosocial predictors of quality of life in older persons in different living arrangements* (Chapter 16.1.B.3.)
- *Good practice case study on dangerous substances* (Chapter 16.2.A.3.)
- *Joint scientific implementation and evaluation of the Healthy Skin@Work Campaign* (Chapter 16.2.A.4.)
- *Development and Implementation of European Standards on Prevention of Occupational Skin Diseases* (StanDerm, Chapter 16.2.A.5.)
- *Diagnosis, Monitoring and Prevention of Exposure-Related Noncommunicable Diseases* (DiMoPEX, Chapter 16.2.A.5.)
- *Network on the Coordination and Harmonisation of European Occupational Cohorts* (OMEGA-NET, Chapter 16.2.A.5.)

## ● PROFESSIONAL SERVICES

Professional activities of the Unit included the organisation and implementation of teaching modules for medical doctors, residents in occupational and sport medicine, and clinical pharmacology and toxicology. A one-month training course for eight residents in occupational and sport medicine was held in the field of "Occupational diseases, work-related diseases, and occupational toxicology". Training in "Clinical Toxicology" across one week was conducted for one resident. J. Macan was appointed as main supervisor by the Croatian Ministry of Health for eight residents in occupational and sports medicine.

In collaboration with the Poison Control Centre and Unit of Analytical Toxicology and Mineral Metabolism, two waves of metal concentration measurements in biological samples for residences in Slavonski Brod, and one mercury determination in exposed workers samples were carried out. This was followed by a health risk assessment for the determined metal levels (315, 316, 333).

## ● PROFESSIONAL ACTIVITIES OF THE UNIT'S EMPLOYEES OUTSIDE THE INSTITUTE

*Ž. Babić*

Member of the Committee for Safe Use of Medicines of the Agency for Medicinal Products and Medical Devices of Croatia.

*J. Bobić*

Member of the Committee for Acknowledgement of Clinical Psychologists at the Croatian Psychological Chamber.

*J. Macan*

Member of the Croatian Society for Allergology and Clinical Immunology Management Committee; member of the Croatian Society of Occupational Health Management Committee and president of the Society's Zagreb branch; member of the European Initiative for Prevention of Occupational Skin Diseases at the European Academy for Dermatology and Venereology; member of the Committee for Medical Ecology and the Working Group for developing national positions in the field of protection from electromagnetic fields at the Ministry of Healthcare, Republic of Croatia; member of the Board for Ethics in Science and Higher Education at the Croatian Agency for Science and Higher Education; chair of the Ethics Committee at the Institute for Medical Research and Occupational Health; court expert witness in occupational medicine; reviewer for projects within the EU COST programme.

*R. Turk*

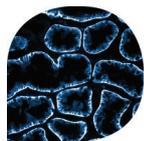
Member of the Biocidal Products Committee of the Ministry of Health and a substitute member of the Biocidal Products Committee of the European Chemicals Agency; member of the Committee for Safe Use of Medicines of the Agency for Medicinal Products and Medical Devices of Croatia.

*V. M. Varnai*

Member of the Committee for Risk Assessment (RAC) at the European Chemicals Agency (ECHA).

#### **SCIENTIFIC ADVANCEMENT OF EMPLOYEES**

Ž. Babić and J. Kovačić earned a PhD degree and were awarded a postdoctoral research associate appointment.



## 2.6. Molecular Toxicology Unit

### EMPLOYEES

#### HEAD:

Davorka Breljak, PhD, scientific advisor

#### RESEARCHERS

Marija Ljubojević, PhD, senior scientific associate

Ivana Vrhovac Madunić, PhD, scientific associate

Dean Karaica, PhD, junior researcher, assistant

#### TECHNICAL STAFF

Ljiljana Babić, technician

#### PARTICIPATING RETIRED RESEARCHERS

Ivan Sabolić, PhD, MD, permanent scientific advisor

### RESEARCH

#### A. RESEARCH ACTIVITIES WITH INSTITUTIONAL FINANCING

Within the framework of the expired national research project funded by the Ministry of Science and Education (Grant No. 022-0222148-2146) and current national research project AGEMETAR (Chapter 16.1.A.1.), we published three scientific articles (45, 68, 95) and one popular article (123).

In co-operation with two research teams from Germany led by Prof F. Lang (University of Tübingen, Tübingen, Germany) and Prof H. Koepsell (University of Würzburg, Würzburg, Germany), we published two scientific articles (56, 57). In co-operation with a research group led by prof. V. Vallon (University of California, San Diego, USA), results were presented at an international scientific meeting (256). These results will be published in 2018 within an article under preparation. Within the frame of scientific cooperation at the Institute, one book chapter was published (180), whereas some results were published in a Book of Abstracts (294). As part of scientific cooperation with Croatian scientist within/outside of Institute, one review article was published (86).

In the paper related to I. Vrhovac Madunić's PhD thesis "Expression of Sodium-Glucose Cotransporters Sglt1 (Slc5a1) in murine organs" under the mentorship of D. Breljak (68), we investigated the mRNA/protein expression and cell localization of sodium-glucose cotransporter 1 (Sglt1/Slc5A1) in various organs/tissues of mice. We also investigated species differences in Sglt1 localization between mice and rats. Since Sglt1 participates in glucose absorption in the intestine and glucose reabsorption in the kidneys, thus regulating glucose homeostasis, the pharmaceutical industry is intensively developing SGLT1 inhibitors for diabetes therapy. However, the expression level and cellular localization of Sglt1 protein in organs/tissues of mice, as frequently used experimental animals, has not been thoroughly investigated due to a lack of the specific anti-Sglt1 antibodies. Using the *Sglt1*-knockout mouse model, we undoubtedly excluded the crossreactivity of the used anti-Sglt1 antibody and confirmed its specificity as a basic tool for immunochemical analysis. Immunolocalization studies showed that cellular localization of Sglt1 protein in mice and rats is similar in many organs including the small intestine, liver, and kidneys. However, we observed species differences between rats and mice. In the rat, protein Sglt1 is localized in the lungs, heart and brain, while in mice it was detected in eyes, tongue epithelial cells, pancreatic ducts, prostate,

and periurethral glands. Using classical and quantitative RT-PCR, different expression patterns of Sglt1 mRNAs were confirmed in various organs/tissues of rats and mice.

An article related to D. Karaica's PhD thesis "Effect of sex hormones on the expression of chloride/formate exchanger (Cfex, Slc26a6) in rat organs" under the mentorship of D. Breljak was largely revised and will be prepared for submission in 2018.

In an article by Salker et al. (56), we investigated the expression of sodium-glucose cotransporter 1 (SGLT1/SLC5A1) in the uterus of humans and mice since rice in endometrial glycogen during periimplantation and early pregnancy precedes cellular glucose uptake. The results of these studies have shown that endometrial epithelial cells in both human and mice express a significant level of SGLT1. Using functional studies, electrogenic glucose transport was detected in the uterus of wild-type (*Slc5a1<sup>+/+</sup>*), but not in *Sglt1*-knock out (*Slc5a1<sup>-/-</sup>*) mice. The results of these studies have also shown that endometrial glycogen content, litter size, and weight of offspring at birth were significantly lower in (*Slc5a1<sup>-/-</sup>*) mice as compared to wild type (*Slc5a1<sup>+/+</sup>*) mice. Furthermore, it was found that SLC5A1 expression was upregulated upon decidualization of primary endometrial stromal cells in humans, which is a key point for embryo implantation. Also, the endometrial expression of SLC5A1 protein was attenuated in women with recurrent pregnancy loss when compared to healthy women. The results of these studies reveal a novel mechanism establishing adequate endometrial glycogen stores for pregnancy as a key factor for embryo implantation during periimplantation and early pregnancy.

In an article by Sharma et al. (57), it was demonstrated for the first time that sodium-glucose cotransporter SGLT1 participates in the orchestration of the host defence against *Listeria monocytogenes* infection, which causes listeriosis. SGLT1-deficient mice and wild type littermates were infected with  $1 \times 10^4$  CFU *L. monocytogenes* intravenously. By qRT-PCR, transcript levels of SGLT1, TNF- $\alpha$ , IL-6, and IL-12a were determined, as well as SGLT1 protein abundance and localization by immunohistochemistry. Results demonstrated that genetic knockout of SGLT1 significantly compromised bacterial clearance following *Listeria monocytogenes* infection with significantly enhanced bacterial load in liver, spleen, kidney, and lung, and significantly augmented hepatic expression of TNF- $\alpha$  and IL-12a. While all wild type mice survived, all SGLT1 deficient mice died from the infection. The study showed that SGLT1 is required for bacterial clearance and host survival following murine *Listeria monocytogenes* infection.

In an article by Madunić et al. (86), we reviewed the role of apigenin in anticancer research, as well as several cancer signalling pathways, including MAPK, PI3K/Akt, and NF- $\kappa$ B pathways and their specific role in different cancer types. Apigenin is a natural flavonoid found in several dietary plant foods such as vegetables and fruits. A large number of studies conducted over the past years have shown that this particular natural compound has potential antioxidant, anti-inflammatory, and anticancer properties. Therefore, apigenin has generated a great deal of interest as a possible chemotherapeutic modality due to its low intrinsic toxicity and remarkable effects on normal versus cancerous cells, compared with other structurally related flavonoids. Based on the available literature, the beneficial effects of apigenin as a future anticancer modality are promising but they require further *in vitro* and *in vivo* studies to enable its translation from bench to bedside.

In a popular article by Karaica et al. (123), we described a short historical overview of the incrementally gathered knowledge on urinary tract stone disease (urolithiasis) in humans as well as the development of medical procedures to treat them.

In a book chapter by Ljubojević et al. (180), we presented the most common sources of nanosilver from spent sources, the properties of silver nanoparticles that can influence bioaccumulation, bioavailability, and environmental risk and demonstrated the impact that still unexplored nanowaste can have on humans and the living environment in general. The multi-purpose use of nanosilver and nanosilver-functionalized materials creates a pool of different sources of nanosilver waste

in the environment. The usual sources come from biocidal activities, incineration, release in the environment or in living organisms (household, medical treatment/equipment with nanosilver or drinking water treatment) while silver nanomaterials are in use or during their disposal. Silver nanoparticles may adhere to soils or undergo transport in ground, surface, and drinking waters in completely different way as compared to bulk materials. There is still no general strategy for safe management of nanowaste. Although with a lower production volume than other nanomaterials and decreasing sewage sludge content, its intentional and unintentional environmental release, with still unknown toxicological impacts due to chronic and/or low-dose exposure, higher bioavailability in the water, air, soil, and its transformation to silver ions, makes nanosilver even more dangerous.

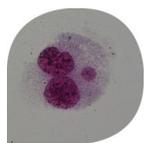
#### **B. RESEARCH PROJECTS FUNDED BY EXTERNAL SOURCES**

- *Aging-related expression of membrane transporters in rats* (AGEMETAR, Chapter 16.1.A.1.)
- *Adverse effects of single and combined mycotoxins produced by Aspergilli* (MycotoxA, Chapter 16.1.B.1.)

#### **PROFESSIONAL ACTIVITIES OF THE UNIT'S EMPLOYEES OUTSIDE THE INSTITUTE**

##### *I. Vrhovac Madunić*

Member of the Commission for Science and Society of the Croatian Society of Biochemistry and Molecular Biology (HDBMB); member of the Scientific Committee in the International Society for Ethnopharmacology (ISE).



## 2.7. Mutagenesis Unit

### EMPLOYEES

#### HEAD

Nevenka Kopjar, PhD, permanent scientific advisor

#### RESEARCHERS

Prof Vera Garaj-Vrhovac, PhD, permanent scientific advisor

Prof Davor Želježić, PhD, permanent scientific advisor

Vilena Kašuba, PhD, permanent scientific advisor

Mirta Milić, PhD, scientific associate

Goran Gajski, PhD, scientific associate

Marko Gerić, PhD, postdoctoral researcher

Vedran Mužinić, MSc, doctoral researcher, assistant

#### TECHNICAL STAFF

Maja Nikolić, senior technician

### RESEARCH

#### A. RESEARCH ACTIVITIES WITH INSTITUTIONAL FINANCING

##### A.1. *In vitro* studies

Several physical and/or chemical agents (cytostatic drugs, wastewater, heavy metals, and natural products) either alone or in mixtures were evaluated *in vitro* for their cyto/genotoxic effects using cells of human and animal origin. These results were published in a series of scientific papers, proceedings, and abstracts indicating either their harmful (23, 44, 278) or beneficial effects (61, 162, 257). Cyto/genotoxicity of inclusion complexes of diazepam with 2-hydroxypropyl- $\beta$ -cyclodextrin was investigated on human lymphocytes *in vitro*. At given concentrations, the tested compounds did not show genotoxicity. However, the investigated inclusion of diazepam complexes induced an accelerated proliferation of human peripheral blood lymphocytes *in vitro*, therefore possibly shortening the duration and dynamics of the cell cycle (24). On the same cell model *in vitro*, toxicity of hydroquinone was investigated. At 140  $\mu\text{g mL}^{-1}$  and 280  $\mu\text{g mL}^{-1}$ , hydroquinone inhibited lymphocyte division and lead to crosslinking in DNA. At 8  $\mu\text{g mL}^{-1}$  using the alkaline comet assay, increased DNA damage along with an increased number of nuclear buds was observed, but no significant micronuclei frequency was noted (26). Potential toxicity of dental bulk-fill resin composites was investigated *in vitro*. Bulk-fill resin composites did not induce relevant genotoxic effects in human leukocytes (63). We investigated the impact of newly synthesized TiO<sub>2</sub>NMs in the shape of nanowires, nanotubes, and nanoplates at 10 - 100  $\mu\text{g mL}^{-1}$  on the level of primary DNA damage in immortalized human keratinocytes (HaCaT) cell line immediately and 24 hours after treatment together with a UVB medial erythema dose (2 kJ m<sup>-2</sup>, 312 nm). Although particles immediately after radiation demonstrated a protective effect only at the highest concentrations, the protective effect was seen 24 hours after and the highest protective effect was for titanium nanoplates (152).

## A.2. Human biomonitoring studies

Cytogenetic status and oxidative stress parameters were analysed in patients with thyroid disease. Patients had worse oxidative status and higher genome damage compared to control subjects (258). Cytogenetic status was also evaluated in vegetarians compared to omnivores, indicating higher genome damage in the vegetarian group (279). In a retrospective study, seasonal variations as predictive factors of the comet assay parameters were analysed suggesting that seasonal variation is another variable that needs to be accounted for when conducting a cohort study (145). Moreover, an overview of the generally forgotten impacts that the increasing number of cancer cases, workers who handle antineoplastic drugs, and health services can have on the environment was also presented (66).

As a part of the already finished collaboration with Italian researchers funded by the Italian Cancer Research Society (IARC-Italian Association for Cancer Research, project number: IG 2010 No. 10491- "Micronucleus assay in buccal exfoliated cells to measure DNA damage and predict cancer in healthy subjects") related to the use of micronucleus cytome assay on buccal cells as a biomarker of genomic instability in alcohol-consuming people, we published a paper that showed higher frequencies of karyolytic and pycnotic cells in regular smokers who are also regular consumers of higher amounts of strong alcohols when compared to moderate users and non-smokers and that also polymorphisms of genes involved in the ethanol metabolism do not have any effect in sensitivity between such individuals (68). As a part of the same project, together with another project of the Italian Workers Protection Association project (INAIL- Istituto nazionale Assicurazione Infortuni sul Lavoro – "Evaluation of biomarkers of genetic instability, oxidative damage and premature death in blood, mucous membrane cells and bronchial exhalation from workers with the risk of asbestos exposure"), we published an article about interlaboratory comparison in which a Croatian and an Italian laboratory represented by M. Milić demonstrated comparable results (6).

Also as a part of both INAIL and IARC project, we published a work on the use of 3-(2-deoxy- $\beta$ -D-erythro-pentafuranosyl)pyrimido[1,2- $\alpha$ ]purin-10(3H)-1-deoxyguanosine (M1dG) adducts as an indicator of oxidative stress and lipid peroxidation measured in blood as a potential auxiliary indicator of current and past exposure to asbestos in people who have been exposed to asbestos for at least 12 years. In 327 examined workers, the frequency of this type of adduct on 108 nucleotides was 4 and 2.3 in control samples. The results provided a solid basis for a theory of oxidative stress as the cause of toxicity from asbestos exposure (7). Possible DNA damage to oral epithelial cells of human volunteers exposed to whitening kinds of toothpaste was evaluated. Oral epithelial cells were sampled prior to and 30, 60, 90, and 120 days after the beginning of the use of the tested kinds of toothpaste. Chromosomal damages were analysed by micronucleus assay. The use of certain whitening kinds of toothpaste may cause a limited and biologically insignificant genotoxic effect on buccal epithelial cells (99). We investigated the levels of DNA damage induced after 2 Gy and 4 Gy of  $^{60}\text{Co}$  *ex vivo* gamma radiation in peripheral blood lymphocytes of medical workers exposed to anaesthetics and in control subjects. There was no significant difference in the mean levels of tail DNA between control and exposed samples irradiated with the same dose. The effect of smoking was not observed in the control group. We found differences in repair capability which were associated with polymorphisms in genes hOGG1 XRCC3 as well as their homozygous state (148).

Our Unit was also involved in a Global Interlaboratory Comparison on Cytogenetic and Genomic Assays, in the frame of the European Network of Biodosimetry, RENEB (311).

## A.3. Studies on animal models

We investigated the effects of 14-day and 28-day exposure of male and female Wistar rats to strawberry tree water leaf extract, arbutin, and hydroquinone on haematological parameters and

primary DNA damage in their leukocytes. Strawberry tree water leaf extract did not significantly impair DNA damage in leukocytes and haematological parameters. However, in male rats exposed to hydroquinone we observed increased leukocyte counts compared to controls (79). Radioprotective effects of propolis and its polyphenolic compounds (quercetin, caffeic acid, chrysin, and naringin) on the level of genome damage were evaluated in Swiss albino mice. The mice were treated for three days with test substances ( $100 \text{ mg kg}^{-1}$ , *i.p.*) and then irradiated at a dose of 4 Gy. Groups of mice pre-treated with propolis and flavonoids had a smaller number of MN compared to the irradiated mice. A particularly effective radioprotector was quercetin. Test substances did not cause significant damage to the genome of non-irradiated mice (140).

#### A.4. In-house projects (Chapter 16.1.A.2.)

- Interaction of constitutional and occupational risk factors on the incidence of occupational contact dermatitis in hairdressing apprentices during vocational training
- Investigation of interactions between irinotecan and tetrahydrocannabinols on rat experimental model using integrated biochemical, molecular biology, pathohystologic and analytical methods

#### B. RESEARCH PROJECTS FUNDED BY EXTERNAL SOURCES

- *Aging-related expression of membrane transporters in rat* (AGEMETAR, Chapter 16.1.A.1.)
- *Organic Pollutants in Environment – Markers and Biomarkers of Toxicity* (OPENTOX, Chapter 16.1.A.1.)
- *Interaction of metallic nanoparticles with sulphur-containing biomolecules – implications for nano-bio interface* (NanoFaceS, Chapter 16.1.A.1.)
- *Adverse effects of single and combined mycotoxins produced by Aspergilli* (MycotoxA, Chapter 16.1.B.1.)
- *Biophysical Design of Antimicrobial peptides and Innovative Molecular Descriptors* (BioAmpMode, Chapter 16.1.B.1.)
- *The comet assay as a human biomonitoring tool* (hCOMET, Chapter 16.2.A.5.)
- *“Good biomarker practice” to increase the number of clinically validated biomarkers* (CliniMARK, Chapter 16.2.A.5.)
- *Personalized Nutrition in Aging Society: Redox Control of Major Age-related Diseases* (NutRedOx, Chapter 16.2.A.5.)
- *El proyecto general es evaluación del riesgo genotóxico por exposición a contaminantes ambientales* (Chapter 16.2.A.7.)
- *Occupational Exposure to Cytotoxic Agents in Veterinary Hospitals and Clinics* (CytoVet, Chapter 16.2.A.8.)
- *A Sustainable Future for the Danube River Basin as a Challenge for the Interdisciplinary Humanities* (Danube:Future, Chapter 16.2.A.9.)
- *Potential new non-invasive biomarkers of chronic exposure to arsenic* (Chapter 16.2.A.10.)

#### PROFESSIONAL SERVICES

The Mutagenesis Unit performs five different analyses: analysis of chromosomal aberrations, analysis of sister chromatid exchanges (SCE), micronucleus assay, comet test, and cell viability assay.

The professional services included collaboration with occupational health specialists and occupational medicine clinics involved in medical examinations of workers occupationally exposed to physical mutagens (ionising and non-ionising radiation) and/or chemical mutagens (cytotoxic drugs and other genotoxic agents). During 2017, 4 analyses using the micronucleus assay were performed for the purpose of health surveillance of medical workers employed in the General Hospital of the

Šibenik-Knin County occupationally exposed to cytotoxic drugs. One analysis of chromosomal aberrations was performed within the health surveillance of medical worker employed at the Hospital Centre Sestre Milosrdnice, Zagreb occupationally exposed to ionising radiation.

#### ■ PROFESSIONAL ACTIVITIES OF THE UNIT'S EMPLOYEES OUTSIDE THE INSTITUTE

##### *G. Gajski*

Member of the Supervisory Board of the Croatian Association for Cancer Research (HDIR); member of the Editorial board of *Medicine*<sup>®</sup> (Wolters Kluwer Health, Inc.); member of the Editorial board *International Journal of Current Toxins Research* (Revotech Press).

##### *V. Garaj-Vrhovac*

Member of the Supervisory Board of HDZZ; member of the Scientific Field Committee for Natural Sciences – field of biology at the Agency for Science and Higher Education; member of the Standing committee for natural sciences at the Croatian Science Foundation.

##### *N. Kopjar*

Member of the Expert Commission for Postgraduate Studies in the Department of Biology of the Faculty of Science of the University of Zagreb (until 30 Sep 2017); Editor in Chief of the journal *Arhiv za higijenu rada i toksikologiju - Archives of Industrial Hygiene and Toxicology*; member of the Presidency of the Croatian Society of Toxicology.

##### *M. Milić*

Member of the Court of Honour of the Croatian Society of Toxicology.

##### *D. Želježić*

Member of the Editorial board of *BioMed Research International*; member of the Panel for the Evaluation of Project Proposals in the Field of Biomedicine and Health, Fundamental Medical Sciences field – Croatian Science Foundation; expert in biological methods of testing in the Member State Committee of the European Chemicals Agency (ECHA); expert in genotoxicity of the Working Group for Food Enzymes of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) – European Food Safety Authority (EFSA).



## 2.8. Toxicology Unit

### EMPLOYEES

#### HEAD

Maja Peraica, PhD, MD, permanent scientific advisor

#### RESEARCHERS

Prof Radovan Fuchs, PhD, DVM, permanent scientific advisor (Deputy Director)

Prof Ana Lucić Vrdoljak, PhD, permanent scientific advisor (Director)

Ivana Novak Jovanović, PhD, senior scientific associate

Dubravka Rašić, PhD, scientific associate

Suzana Žunec, PhD, scientific associate

#### TECHNICAL STAFF

Jasna Mileković, senior technician

Lea Stančin, technician

### RESEARCH

#### A. RESEARCH ACTIVITIES WITH INSTITUTIONAL FINANCING

##### A.1. In-house projects (Chapter 16.1.A.2.)

- *Biomonitoring of contaminants using the biomarkers in European brown bear (Ursus arctos)*

This project was completed during 2017. It is estimated that 95 % of the brown bear's diet is of plant origin. Maize is an important part of its diet because of its availability at supplemental feeding sites. This maize is usually of poor quality and is exposed to weather conditions favourable for mould and mycotoxin production. In the final year of the project, the concentration of mycotoxin ochratoxin A (OTA) was measured in plasma of eight brown bears (*Ursus arctos*) (269). One was from the Zagreb Zoo, one from the bear shelter in Kuterevo, and six of them were wild bears caught for telemetry research. OTA was determined using immunoaffinity columns and HPLC with a fluorescence detector. The shelter bear and one wild bear had much higher plasma OTA levels (18.7 and 32.61 ng mL<sup>-1</sup>) than the rest of the bears (2.05 - 6.62 ng mL<sup>-1</sup>). This is the first report on OTA concentrations in European bears and its concentration may be compared only to other wild animals, such as wild boars. The average OTA concentration (9.89 ng mL<sup>-1</sup>) is comparable to wild boars in Poland. The OTA concentration was also measured in 19 bear kidney samples collected during 2013 and 36 during 2015. A higher OTA concentration was found in bear kidneys collected during 2015. Wild bear kidney and liver samples (27 and 10, respectively) were also analysed for OTA presence.

- *Investigation of interactions between irinotecan and tetrahydrocannabinols on rat experimental model using integrated biochemical, molecular biology, pathohystologic and analytical methods*

The electrochemical properties of irinotecan on a static mercury drop electrode (SMDE) were investigated and the mechanism of electrochemical reduction was elucidated. Voltammetric measurements showed that irinotecan undergoes quasireversible one electron one proton electrochemical reduction. Voltammetric properties of irinotecan depended on the analyte concentration, pH, and the scan rate. Based on the adsorptive character of irinotecan, a new adsorptive stripping square-wave voltammetric method for its electroanalytical determination was proposed. The voltammetric response could be used to

determine irinotecan in the concentration range from  $1.0 \times 10^{-7}$  to  $1.5 \times 10^{-6}$  mol L<sup>-1</sup> and from  $5.0 \times 10^{-9}$  to  $1.2 \times 10^{-7}$  mol L<sup>-1</sup>, depending on the accumulation time. The calculated limits of detection and quantification for irinotecan were found to be  $2.6 \times 10^{-9}$  and  $8.7 \times 10^{-9}$  mol L<sup>-1</sup>. A manuscript covering the results of this study has been accepted for publication (89). Within a pilot study, male rats (strain Wistar HsdBrlHan) were exposed to irinotecan (100 mg kg<sup>-1</sup>, administered once, *i. p.*), tetrahydrocannabinol (THC, 7 mg kg<sup>-1</sup>, administered once for 3 and 7 days, *p. o.*), and their combinations. Rats were killed 24h after application of the last THC dose. We collected blood and tissue samples for different biochemical and molecular biology analyses. The study focused on: (I) haematological methods, (II) biochemical markers of liver function, (III) markers of oxidative stress in plasma, liver and brain tissue, and (IV) assessment of primary DNA damage in leukocytes, hepatocytes and brain cells. We also collected samples of urine and faeces from exposed and control rats aimed to optimise analytical methods for the detection of THC metabolites. Parameters of oxidative stress including lipid peroxidation, total antioxidant capacity, and activity of antioxidative enzymes superoxide dismutase and catalase were measured at the Toxicology Unit. The obtained results are now interpreted, and will be used for the preparation of scientific papers.

- *Investigation of electrochemical and antioxidant properties of polyphenols and their complexes with essential metals*

In this project, we investigated the oxidation potentials of a number of polyphenolic compounds using square-wave voltammetry and we developed a theoretical model that enables their prediction. These studies resulted in the publication of two manuscripts (42, 43) and were presented at the 29<sup>th</sup> International Course and Conference on the Interfaces among Mathematics, Chemistry and Computer Sciences (237).

The first manuscript (43) describes models for the estimation of the first ( $E_{p1}$ ) and second ( $E_{p2}$ ) oxidation potentials for seven polyphenolic compounds measured at different pH values (pH 2, 3, 5, 6, and 7). As descriptors, we used the number of vicinal and non-vicinal OH groups, the number of OH neighbouring pairs, and the total number of OH groups. The separate models for the different pH's yielded an S.E. ranging from 0.001 to 0.013, and the common models for pH 2, 3, 5, 6, and 7 (by inclusion of pH as a variable) gave S.E. = 0.016 and 0.013 for  $E_{p1}$  and  $E_{p2}$ , respectively. An overall model for  $E_{p1}$  and  $E_{p2}$  was also developed, by the inclusion of an indicator variable ( $ln = 0$  and 1 for  $E_{p1}$  and  $E_{p2}$ , respectively) along with the pH variable. The model yielded S.E. = 0.036.

In our second manuscript (42), we presented a new and simpler regression model for the estimation of the first oxidation potentials ( $E_{p1}$ ) of flavonoids based on the number of phenolic, alcoholic, and carboxylic OH groups. In the regression we included the  $E_{p1}$  of 12 polyphenols (mostly flavonols and catechins) measured in our laboratory at pH 3. The model yielded  $r = 0.986$  and S.E. = 0.040. Later successive inclusions of previously reported  $E_p$  values into the regression model, 7 at pH 3, the model ( $n = 19$ ) yielded  $r = 0.980$ , S.E. = 0.046 and 19 at pH 7 the model ( $n = 38$ ), yielded  $r = 0.985$ , S.E. = 0.044. We began studies on complexation reactions between flavonoids and some essential metals (Zn, Cu) using electrochemical (square-wave voltammetry) and spectrophotometric (UV/Vis spectroscopy) methods. The aim of these studies was to elucidate how complexation with metal ions affects the antioxidant potential of flavonoids.

## A.2. Other activities

Research regarding simultaneous exposure to platinum and cadmium on the activity of antioxidant enzymes and protective effects of selenium and zinc was performed on whole blood and isolated erythrocytes (61). This was a collaborative research with Analytical Toxicology Unit.

**B. RESEARCH PROJECTS FUNDED BY EXTERNAL SOURCES**

- *Aging-related expression of membrane transporters in rat* (AGEMETAR, Chapter 16.1.A.1.)
- *Design, synthesis and evaluation of new antidotes in nerve agent and pesticide poisoning* (CHOLINESTERASE, Chapter 16.1.A.1.)
- *Organic pollutants in environment – markers and biomarkers of toxicity* (OPENTOX, Chapter 16.1.A.1.)
- *Adverse effects of single and combined mycotoxins produced by Aspergilli* (MycotoxA, Chapter 16.1.B.1.)
- *Development of Voltammetric Methods for the Characterisation of Natural Antioxidants* (Chapter 16.1.B.1.)

**■ PROFESSIONAL ACTIVITIES OF THE UNIT'S EMPLOYEES OUTSIDE THE INSTITUTE***A. Lucić Vrdoljak*

Member of the Working Group in charge of monitoring the activity plan for the National Network for Permanent Air Quality Monitoring of the Meteorological and Hydrological Service and the Institute for Medical Research and Occupational Health at the Ministry of Environmental Protection and Energy of the Republic of Croatia.

*M. Peraica*

President of the Croatian Society of Toxicology.

*D. Rašić*

Secretary and member of the Croatian Society of Toxicology's Presidency.



## 2.9. Radiation Protection Unit

### EMPLOYEES

#### HEAD

Gordana Marović, PhD, permanent scientific advisor

#### RESEARCHERS

Dinko Babić, PhD, scientific advisor

Zdenko Franić, PhD, scientific advisor

Gina Branica Jurković, PhD, senior scientific associate

Assist Prof Branko Petrinec, PhD, scientific associate

Tomislav Bituh, PhD, scientific associate

Božena Skoko, PhD, assistant

Marko Šoštarić, PhD, junior researcher, assistant

Iva Franulović, BSc, professional associate in science

Milica Kovačić, BSc, professional associate in science

#### TECHNICAL STAFF

Mak Avdić, senior technician

Jasminka Senčar, senior technician

Ljerka Petroci, technician

### RESEARCH

#### A. RESEARCH ACTIVITIES WITH INSTITUTIONAL FINANCING

##### A.1. Long term research activities

Radioecological studies of contamination of environment in the Republic of Croatia by naturally occurring and fission radionuclides continued. A long-term investigation of distribution of  $^{134}\text{Cs}$  and  $^{137}\text{Cs}$  in environmental samples in Republic of Croatia was performed with special attention to  $^{134}\text{Cs}/^{137}\text{Cs}$  activity ratio and its transfer from air and fallout to other environmental samples after the Chernobyl and Fukushima-Daiichi nuclear accidents (226).

The use of moss is a good example of determining the radiological contamination of the ecosystem by fission radionuclides. The research results presented in the last few years (139) are a continuation of long-term investigations of the presence of radiocesium in mosses carried out in the Unit as part of an extended program for monitoring the radioactivity of environmental samples in the Republic of Croatia.

In cooperation with the Faculty of Veterinary Medicine of the University of Zagreb, research has extended on wildlife species, especially bears and wolves. The results of the determination of caesium in bear meat as a source of dose burden in Croatia, especially for the critical population of hunters and their families were presented (151).

The results of long-term investigations of radioactive contamination of the cistern waters collected on twenty locations along the Adriatic coast were presented and used as the basis for estimation of effective doses received by critical population still using cistern waters (20).

Long-term research of radioactive contamination of human foodstuffs, especially milk, was presented in a journal for the popularization of science (126). The radioactivity of milk in Slavonia

served for measuring the dose resulting from  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  ingested by milk consumption and differences in dose estimates for the respective radionuclides were discussed (144).

The potential radiological impact of an oil refinery near the city of Slavonski Brod was investigated. The absorbed dose rates were measured at several locations in the city. Also, the activity concentrations of natural radionuclides were measured in several soil samples. The measured values did not show deviations from the expected values, indicating that the oil refinery have not caused any radiological impact on the city of Slavonski Brod area (47, 159).

Special attention was paid to problematics of field measurements of radioactivity in order to achieve faster and more efficient data acquisition in the case of emergency or nuclear/radiological accidents and the role of the mobile radioecological laboratory has been demonstrated (155).

The differences in lipid composition of fatty acids between species of the order Anguilliformes, fish representing three families [Mediterranean moray *Muraena Helena* (Linnaeus 1758); European conger, *Conger conger* (Linnaeus, 1758); and European eel *Anguilla Anguilla* (Linnaeus, 1758)] were determined. These were the first results on fatty acids for morays (18). Both moray and conger were less fatty than eel but had higher  $\omega$  FA content, especially  $\omega$ -3 and had higher content of proteins compared to lipids.

We continued research on natural radioactivity in various environmental samples as well as of radionuclides originating from technological processes for which raw materials that contain natural radioactivity are used. The activity concentrations of naturally occurring radionuclides in groundwater from piezometers in the vicinity of Plomin thermal power plant (141) were determined.

The results of research of radionuclide transfer of  $^{238}\text{U}$ ,  $^{235}\text{U}$ ,  $^{232}\text{Th}$ ,  $^{226}\text{Ra}$ ,  $^{210}\text{Pb}$ , and  $^{40}\text{K}$  to plants growing on landfills of slag and ash, resulting from burning coal. The results were compared with transfer factors for control samples of same plant species that grow on untreated and unpolluted soil. Significant increases in activity concentrations in plants were observed only for  $^{226}\text{Ra}$  (58).

In co-operation with the Faculty of Agriculture of the University of Zagreb, results of the research of the radioactive contamination of selected soils that differ in physical, chemical, and other properties were presented. Activity concentrations of naturally occurring radionuclides ( $^{40}\text{K}$ ,  $^{238}\text{U}$ ,  $^{226}\text{Ra}$ ,  $^{232}\text{Th}$ ,  $^{235}\text{U}$ ) and  $^{137}\text{Cs}$  were measured in soil samples. It has been showed that the geological properties and properties associated with the sampling sites have a significant impact on measured concentrations of natural radionuclides, while the activity concentrations of  $^{137}\text{Cs}$  affects were affected by the chemical and physical properties of the soil. Based on the measured activity concentrations, the absorbed dose rates at sampling sites were also determined (60).

The experience of the Institute's accreditation in the field of radioecology and ionizing radiation dosimetry was discussed (143).

Radiochemical and metrological methods of radioactivity monitoring are being developed for environmental matrices. Using new findings in the field of radiation science and radiation protection, as well as in metrology and sampling, help to standardize procedures and harmonize methods and perform quality assurance. The Unit continued to develop radiation protection measures in the case of nuclear/radiological accidents, with emphasis on the role of mobile radiological measurement laboratories.

Studies related to the history of beekeeping on Banovina region, as well as methods for determining the quality and counterfeiting of honey were presented. The relationship between beekeeping and genetically modified organisms was presented, with a particular emphasis on European regulation concerning the labelling of genetically modified organisms in honey (115-117, 172).

The results of measurements and analyses of electrical conductivity and magnetic susceptibility of polyaniline doped by dodecylbenzensulfonic acid have been published. Typical behaviour was demonstrated for Mott-Anderson insulators, and the presence of a soft Coulomb and minor Hubbard energy gap around Fermie's energy was found. The effective closure of Hubbard's gap

coincides with crossover from a variable range to crossover between the nearest neighbours in electronic transport (3).

### A.2. In-house projects (Chapter 16.1.A.2.)

- *Radiological characterization of Kopački rit*

Activity concentrations of  $^{137}\text{Cs}$  in soil samples from the Kopački rit Nature Park were studied and published (123, 241).

## PROFESSIONAL SERVICES

- *Monitoring the environmental radioactivity in the Republic of Croatia* (collaboration with the State Office for Radiological and Nuclear Safety, Zagreb)

The Radiation Protection Unit continued to monitor the state of environmental radioactivity in the Republic of Croatia, based on its collaboration with the State Office for Radiological and Nuclear Safety. Locations and regions of measurement and samplings sites, radiochemical and other methods as well as the used instruments used are harmonized with recommendations of the European Committee from 2000 regarding the monitoring of environmental radioactivity.

- *Monitoring of the radioactivity in the vicinity of coal fired power plant Plomin* (collaboration with the HEP Generation d.o.o., Sector for thermal power plants, Thermal power plant Plomin I, Plomin)

Radiological analyses of imported energy coal for the operation of the Plomin thermal power plant (TE Plomin) were conducted and several dozens of expert opinions on the suitability of coal for incineration in a thermal power plant as well as on the possible use of waste ash slag and filter dust in construction or cement industry were issued. As part of the monitoring programme, we also carried out investigations of radioactive contamination of groundwater samples from the piezometers and the determination of the ambient dose equivalent rate in the zone of possible impact of the ash landfill. About dozen professional reports related to this topic were issued.

- *Monitoring of the radioactivity of natural gas field Molve* (collaboration with Koprivnica-Križevci County, Koprivnica)

In the area of the natural gas field Molve, on the central gas station as well as on location of selected gas wells M-9 and M-10, measurements of ambient dose equivalent were carried out, the necessary samples were collected, and analyses were carried out for the purpose of reports on the state of radioactivity of the gas field Molve, according to a contract with the Koprivnica-Križevci County, all in cooperation with the Environmental Hygiene Unit, IMROH.

- *Monitoring of the radioactivity in speleological object Barač caves* (Public Institute for Protected Area Management for the Area of Rakovica Municipality, Rakovica)

The Public Institute for Protected Area Management for the Area of Rakovica Municipality ordered preliminary research on the radiological condition of the speleological object within the excursion site of Barač cave. The activity concentration of radon gas and the ambient dose equivalent rate were measured. Equivalent doses were estimated for employees as well as for visitors to the excursion site. Further co-operation has been agreed on the basis of preliminary research.

- *Detection of ionizing radiation pathways during the production of mineral fertilizers* (Petrokemija d.d., Kutina)

Monitoring of the state of natural radioactivity during the production of mineral fertilizers was continued. Field measurements were carried out. Input raw materials, deposited phosphogypsum, surrounding soil and vegetation, groundwater from the piezometers at the phosphogypsum landfill as well as the well water from nearby area were sampled. At the selected production site locations, as well as on selected locations of the phosphogypsum landfill, ambient dose equivalent rates were measured.

- *Other professional activities*

Monitoring the state of environmental radioactivity in the "Šumbar" research area continued with emphasis on the radioactivity of selected species of flora and fauna. This was supplemented by continuous measurements of the ambient dose equivalent rate.

In cooperation with the Faculty of Veterinary Medicine at the University of Zagreb, research on radioactive contamination of game meat, especially bears and wolves was extended.

About one hundred analyses of radioactive contamination of various export-oriented goods were carried out, in accordance with the European Union provisions on safe flow of goods.

A dozen samples of water for human consumption were analysed according to the Plan for monitoring of radioactive substances in water intended for human consumption upon the approval of the Minister of Healthcare and at the proposal of the State Institute for Radiological and Nuclear Safety (requirements according to the *Regulation on Compliance Parameters and Water Analysis Methods human consumption*, OG 125/13, 141/13, 128/15). Appropriate opinions regarding radioactivity were issued.

Development of radiochemical and other metrological methods for monitoring radioactive contamination in various matrices continued as well as their standardization and harmonization through quality assurance. Also, the Unit continued to develop radiation protection measures in case of nuclear/radiological accidents, with emphasis on the role of mobile radiological measurement laboratories.

### International comparisons

IMROH is, according to the notification act issued by the State Office for Radiological and Nuclear Safety authorized as an expert technical service for conducting expert work on ionizing radiation protection. The Unit's associates organize and conduct interlaboratory comparisons between related laboratories and institutions. Quality assurance is carried out with constant improvements regarding sampling, preparation, and processing of samples, development of radiochemical analyses and metrological methods. Continued activities are carried out to implement the requirements of related standards in the practice of the Unit.

Intercomparison measurements of dose rate and field gammaspectrometry measurements (radionuclide identification) were performed in the exercise entitled "PRIMER 2017" held on 13 Sep 2017 at the "Jožef Stefan" Institute, Slovenia, IJS Reactor Center, Brinje, Dol in Ljubljana. During the exercise, a vehicle and measuring equipment were used procured through the European Union project "Purchasing vehicle with mobile radiation detection equipment for the purpose of improving the system for non-network monitoring of radioactivity in the environment in the Republic of Croatia in common and emergency situations" for the purposes of the Unit's work in radiation protection.

International Atomic Energy Agency (IAEA), Joint Research Centre and Jožef Štefan Institute organized several intercomparison tests on various matrices in which the Unit participated.

#### List of intercomparisons

ORGANISER	TEST	AREA	DATE
IAEA	IAEA-TEL-2017-03 World-wide proficiency test on determination of anthropogenic and natural radionuclides in water, milk powder, Ca-carbonate	Determination of radionuclides by high-resolution gammaspectrometry in energy range 40 - 2000 keV	6 Mar– 17 Oct 2017
JRC EC	JRC EC proficiency test on artificial radionuclides in maize powder	Determination of radionuclides by high-resolution gammaspectrometry in energy range 40 - 2000 keV	31 May– 24 Oct 2017

IAEA	IAEA-RML-2017-01 Mediterranean seawater spiked with H-3, Co-60, Sr-90, Cs-134 and Cs-137	Determination of radionuclides by high-resolution gamma spectrometry in energy range 40–2000 keV; Determination of <sup>90</sup> Sr activity concentration	9 Sep 2017– 22 Jan 2018
Jožef Stefan Institute, Ljubljana, Slovenia	“PRIMER 2017” Intercomparison of dose rate measurements and identification of radionuclides by in-situ gamma spectrometric measurements	Preparedness of the field vehicle team in cases of radiological/ nuclear accidents	13 Sep 2017

#### Accredited methods

TEST METHOD	TYPE OF TEST, RANGE
RU-602-5.4-1 (In-house method)	Determination of radionuclides by high-resolution gamma spectrometry in energy range 40 - 2000 keV
RU-602-5.4-4 (In-house method)	Determination of <sup>90</sup> Sr activity concentration
RU-602-5.4-5 (In-house method)	Determination of <sup>226</sup> Ra activity concentration

The Unit's quality manager: T. Bituh.

### PROFESSIONAL ACTIVITIES OF THE UNIT'S EMPLOYEES OUTSIDE THE INSTITUTE

#### D. Babić

Member of the Scientific Committee of the 11<sup>th</sup> Symposium of the Croatian Radiation Protection Association with international participation.

#### T. Bituh

Member of the Scientific Committee of the 11<sup>th</sup> Symposium of the Croatian Radiation Protection Association with international participation; member of the WG3 international project COST TU1301 NORM4Building; technical auditor for the projects within the Operational programme competitiveness and cohesion 2014–2020 (Strengthening the economy by applying research and innovation).

#### Z. Franić

Member of the Editorial Board of *Journal of Radiation Industry*; member of the Advisory Editorial Board of the *Archive of Industrial Hygiene and Toxicology*; member of the Management Board of the Croatian Radiation Protection Association; member of the Management Board of the Croatian Systems Society; member of the Technical Committee TO E45 (Nuclear Instrumentation) of the Croatian Standards Institute; member of the Advisory Board of the Croatian Standards Institute; member of the Management Board of the National Foundation for Civil Society Development; member of the European Commission Board of Governors of Joint Research Centre (JRC EC); member of the Programme Committee HORIZON 2020 for SC5 (Climate Activities, Environment, Resource Efficiency and Raw Materials); member of the Ethics Committee of the Dental Polyclinic Zagreb; member of the Scientific Committee of the 11<sup>th</sup> Symposium of the Croatian Radiation Protection Association with international participation; member of the Management Board of the Beekeepers Association “Pčelinjak”; President of the NGO “Zrinska gora”.

#### G. Marović

Member of the Supervisory Board of the Croatian Radiation Protection Association; member of the Management Board of the Croatian Nuclear Society; member of the Public Committee of the Croatian

Nuclear Society. member of Scientific Committee of the 11th Symposium of the Croatian Radiation Protection Association with international participation; member of the Programme Committee of the 12<sup>th</sup> International Conference on the Nuclear Option for CO<sub>2</sub> Free Energy Generation (Former Nuclear Option in Countries with Small and Medium Electricity Grids”) to be held in Zadar, 3 – 6 Jun 2018.

*B. Petrinec*

Vice-president of the City Council of the Town of Ivanić-Grad; member of the Management Board of the Croatian Radiation Protection Association; member of Technical Committee TO E45 (Nuclear Instrumentation) of the Croatian Standards Institute; member of the Editorial Board of the *Archive of Industrial Hygiene and Toxicology*; member of the Editorial Board of the *Vatrogastvo i upravljanje požarima*; quality manager for the Firefighters Community of the Town of Ivanić-Grad; firefighter judge; senior firefighting officer 1<sup>st</sup> class; firefighter with special authorisations and responsibilities; head of IMROH’s defence preparation; member of the Scientific Committee of the 11<sup>th</sup> Symposium of the Croatian Radiation Protection Association with international participation.

*J. Senčar*

Member of the Management Board of the Croatian Radiation Protection Association; member of Organization Committee of the 11<sup>th</sup> Symposium of the Croatian Radiation Protection Association with international participation.

*M. Šoštarić*

Member of the Management Board of the astronomical society “Perzeidi” in the city of Križevci.



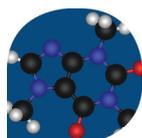
## 2.10. Independent researchers

Aleksandra Fučić, PhD  
permanent scientific advisor

### RESEARCH

#### RESEARCH PROJECTS FUNDED BY EXTERNAL SOURCES

- *The role of oestrogen and androgen receptor activation in the stroma of oral cancer and their impact on the survival of patients* (ACTIVESTROMORALCANCER, Chapter 16.1.B.1.)
- *Scientific Centre of Excellence for Reproductive and Regenerative Medicine: Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials* (CERRM, Chapter 16.1.B.2.)
- *European Human Biomonitoring Initiative* (HBM4EU, Chapter 16.2.A.2.)



## 2.11. Independent researchers

Ante Miličević, PhD  
scientific advisor

### RESEARCH

#### A. PROJECTS AND COLLABORATION WITH INSTITUTIONAL FINANCING

##### A.1. In-house projects (Chapter 16.1.A.2.)

- *Investigation of electrochemical properties and antioxidant activity of polyphenolic compounds and their complexes with essential elements*

Within the project, we measured the oxidation potentials of a set of about 15 flavonoids and developed computational models for the estimation of first oxidation potentials,  $E_{p1}$ , and published two papers (42, 43) and one abstract from the international meeting (237).

- *Design, synthesis and evaluation of selective inhibitors of butyrylcholinesterase*

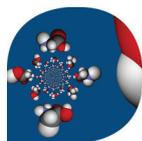
#### B. RESEARCH PROJECTS FUNDED BY EXTERNAL SOURCES

- *Interaction of metallic nanoparticles with sulphur-containing biomolecules – implications for nano-bio interface* (NanoFaceS, Chapter 16.1.A.1.)
- *Investigation of chemistry and antioxidant activity of polyphenolic compounds with essential elements* (Chapter 16.2.A.10.)

### PROFESSIONAL ACTIVITIES OUTSIDE THE INSTITUTE

#### A. Miličević

The chief shop steward at the Independent Trade Union of Science and Higher Education for the branch IMROH.



## 2.12. Independent researchers

Jasmina Sabolović, PhD  
senior scientific associate

### RESEARCHERS

Jelena Budimčić, MSc, doctoral researcher, assistant

Marijana Marković, PhD, postdoctoral researcher (until 1 Jan 2017)

### RESEARCH

#### A. PROJECTS AND COLLABORATION WITH INSTITUTIONAL FINANCING

A poster abstract on the theoretical conformational analysis of the ternary (L-cisteinato)(L-asparaginato)copper(II) complex, which was presented at "The Thirteenth International Symposium on Neurobiology and Neuroendocrinology of Aging" (Bregenz, Austria) last year, was published in a special issue of a journal indexed in WoS (262). The  $^{13}\text{C}$  and  $^1\text{H}$  Fermi contact chemical shifts were calculated for *cis*-isomer and *trans*-isomer of anhydrous and hydrate bis(glycinato)copper(II) complexes using the density functional theory method to assist the assignment of  $^2\text{H}$  and  $^{13}\text{C}$  signals and paramagnetic shifts in experimental spectra measured by the magical angle spinning solid-state NMR spectroscopy (98).

#### B. RESEARCH PROJECTS FUNDED BY EXTERNAL SOURCES

- *Combined molecular modelling and experimental studies of physiologically and stereochemically important copper(II) amino acid complexes* (CopperAminoAcidates, Chapter 16.1.A.1.)

## 3. PROJECTS

### 3.1. NATIONAL PROJECTS

#### A. RESEARCH PROJECTS LED BY IMROH'S RESEARCHERS

(according to source of funding)

##### A.1. CROATIAN SCIENCE FOUNDATION (6 projects)



LEADER	PROJECT	DURATION
<b>Davorka Breljak, PhD (IMROH)</b>	<b>Aging-related expression of membrane transporters in rat (AGEMETAR, IP-2013-11-1481)</b>	<b>1 Oct 2014– 31 Mar 2019</b>

#### ASSOCIATES

IMROH: G. Gajski, M. Gerić, J. Jurasović, D. Karaica, M. Ljubojević, V. Micek, I. Novak Jovanović, T. Orct, M. Peraica, D. Rašić, I. Sabolić, I. Vrhovac Madunić  
External associates: L. Nanić and Rubelj (Ruđer Bošković Institute)

#### SUMMARY

In accordance with the project's work plan, urine, blood, and tissues (liver/kidney/brain) were collected from males/females following 21-month melatonin and resveratrol treatment in a rat experimental model. For the immunofluorescence analysis, organs were fixed by perfusion *in vivo*, whereas for the monitoring of renal/hepatic endocytosis, animals were perfused with FITC-dextran *in vivo*. Furthermore, total cell membranes/cytosolic fractions/RNA/DNA were isolated from renal/hepatic tissues.

We investigated the 21-month effect of melatonin and resveratrol in tissues of all experimental groups; various parameters were measured in urine, blood serum, kidneys, liver and brain using various techniques such as DNA/RNA isolation, reverse transcription, RT-PCR (end-point/quantitative), SDS-PAGE/western analysis, immunocytochemistry/fluorescence microscopy, ELISA, comet assay, ICP-MS, HPLC, and telomere length determination. Four project seminars were held where the results of scientific research were presented (<https://www.imi.hr/en/projekt/aging-related-expression-of-membrane-transporters-in-rat-agemetar/>). Also, a novel statistical approach has been introduced and will be applied for further data analysis. Results were also presented at international scientific conference (280) and published in journal (266). Other results will be prepared for publication.

In the scientific article Orct et al. (45), we investigated the sex differences of macroelement and microelement concentrations in various rat tissues/organs of both sexes as well the impact of residual blood in organs on the concentration of these elements. Concentrations of six macroelements (Na, Mg, P, S, K, Ca) and 14 microelements (Fe, Mn, Co, Cu, Zn, Se, I, As, Cd, Hg, Pb, Li, B, Sr) were determined by ICP-MS in whole blood, blood plasma, liver, kidneys, and brain from control (non-perfused) animals and animals whose blood was removed by perfusion *in vivo*. Results of ICP-MS analysis have shown that concentrations of numerous macro- and micro-elements are significantly different in intact (non-perfused) organs in comparison to blood-free (perfused) organs. The concentrations of many macroelements and microelements in the liver, kidneys, and brain were sex-dependent; concentrations of some macroelements and microelements showed the same pattern of sex differences in intact (non-perfused) and blood-free (perfused) organs. However, the sex-dependent concentration of some macroelements and microelements was determined only in blood-free (perfused) organs. Results of this study indicate that residual blood in organs can significantly influence the concentration of various elements and their sex-dependency in a rat experimental model.

In the scientific paper Sabolić et al. (95), we investigated three isoforms of metallothionein (MT): MT1, MT2, and MT3, whose localization along the rodent nephron has not been investigated in detail. Using classic RT-PCR and immunochemical methods, MT expression was investigated at mRNA/protein level along the rat nephron including cortex (CO), outer stripe (OS), inner stripe (IS) and inner medulla (IM). Using the RT-PCR, we observed various expression patterns for MT mRNA along the nephron: a) MT1: CO > OS = IS = IM, b) MT2: IM > CO > IS = OS, and c) MT3: IM > CO = OS = IM. Results of immunocytochemical analysis have shown

that antibody anti-MT1/2 heterogeneously stained the cell cytoplasm and nuclei in proximal tubules and thin ascending limb, whereas anti-MT3 antibody weakly stained the cell cytoplasm of various cortical tubules and strongly the nuclei in all nephron segments. However, in isolated nuclei, MT1/2 protein expression was not detected, whereas MT3 expression was. In MT1/2-positive cells of proximal tubules, intracellular staining was either diffuse or bipolar, but the MT1/2 protein was not detected in the isolated brush-border/basolateral/endosomal membranes. In the lumen of some proximal tubules, heterogeneously sized MT1/2 -rich vesicles were observed and were poorly positive for NHE3, but negative for V-ATPase, CAIV, and megalin, whereas their interior was positive for CAII and negative for the cytoskeleton. They seemed to be pinched off from the luminal membrane of MT1/2 rich cells as confirmed by transmission electron microscopy. We have shown that MT is heterogeneously expressed in the cell cytoplasm and/or nuclei along the rat nephron.



LEADER	PROJECT	DURATION
Zrinka Kovarik, PhD (IMROH)	<b>Design, Synthesis and Evaluation of New Antidotes in Nerve Agents and Pesticides Poisoning (CHOLINESTERASE, IP-2013-11-4307)</b>	<b>1 Sep 2014– 30 Aug 2018</b>

#### ASSOCIATES

IMROH: A. Bosak, M. Katalinić, A. Lucić Vrdoljak, N. Maček Hrvat, N. Maraković, G. Mendaš Starčević, G. Šinko, T. Zorbaz, S. Žunec  
External associates: Z. Radić (University of California at San Diego, La Jolla, SAD); V. Vinković (Ruđer Bošković Institute)

#### SUMMARY

Organophosphates (OPs) such as nerve agents (VX, soman, tabun, sarin, and cyclosarin) and pesticides (paraoxon) inhibit both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) by phosphorylating their catalytic serine thereby terminating cholinergic transmission, which leads to life-threatening consequences. Phosphorylated cholinesterase (ChE) can be reactivated by oximes; however, the efficacy depends on the structure of both the OP and oxime. Therefore, it is unlikely to find a universal reactivator that would provide adequate therapy in the case of exposure to any OP. An alternative approach to reduce the *in vivo* toxicity of OPs focuses on the use of bioscavengers. Exogenous enzymes like human BChE or AChE mutants that degrade OP with a turnover when combined with an oxime can be used as a pretreatment or therapy after OP exposure. We reported the catalytic scavenging of tabun by JAR-288C oxime and AChE mutant Y337A *ex vivo* in whole human blood (hWB) (259). Moreover, efficient detoxification of soman and VX by oxime assisted reactivation of AChE mutant-conjugates was shown both *ex vivo* and *in vivo* in mice (232, 234, 259, 260, 286).

Oximes mostly synthesized using [3+2] cycloaddition between alkyne and azide building blocks were tested in the reactivation of the tabun-AChE conjugate. Several oximes with significantly improved *in vitro* reactivating efficacy were identified and their antidotal efficacy was confirmed in tabun-exposed mice (232, 259). Furthermore, we selected twenty efficient reactivators for tabun, and screened them for reactivation of sarin-, cyclosarin-, VX-, and paraoxon-inhibited AChE. Several oximes possessed potential to reactivate some of the tested OPs but not a single compound efficient for all tested OPs was detected (259).

Unlike OPs, oximes approved for use in therapy do not cross the blood-brain barrier (BBB) in therapeutically significant doses due to their permanent positive charge. Therefore, we tested novel uncharged and possibly centrally active reactivators that showed promising *in vitro* reactivation profiles for VX-, sarin-, and cyclosarin-inhibited AChE/BChE (273, 308, 309). Molecular docking enabled the detection of possible interactions of these oximes with amino acids in the enzyme active site gorge (309). *In silico*-determined physicochemical parameters predicted the amount of non-ionic species of antidotes at physiological pH, the cytotoxicity profile showed that oximes do not induce significant cell death and induction of ROS production in cells was also tested to evaluate oxidative stress induction (252, 272, 273, 308, 309).

A library of new chiral n-substituted 2-hydroxyiminoacetamide oximes was tested in both racemic and enantiomerically pure form for AChE and BChE reactivation and some of the tested compounds were shown to be promising reactivators of sarin, VX or cyclosarin (202, 287). A part of our research was directed on the evaluation of the oxime's efficiency since the parameters that describe it are often incomparable among laboratories. We concluded that the estimation of reactivation efficiency depends on experimental design and data analysis; moreover, monitoring the side reactions – oximolysis, reversible inhibition, and adequate dilution in Ellman reaction to effectively quench the reactivation reaction, should be included (85, 310).

The selectivity of reversible interactions of ChE with the oxime derivatives of cinchonine was explored. Compounds reversibly inhibited human ChE, showing selectivity towards BChE (29). Therefore, the studied alkaloids have the potential for use in treatment of neurodegenerative diseases. Cinchona oximes were also tested in reactivation of various OP-inhibited ChE and were more efficient in the reactivation of BChE (29). The cytotoxicity tests supported studies of these compounds as long as their biological activity was targeted in the lower micromolar range (23).

Due to BChE being of interest in treating neurodegenerative diseases, a part of our research was focused on the evaluation of the inhibition of atypical and fluoride-resistant BChE variants with the aim of relating the BChE polymorphism by two groups of compounds that differ in the mode of binding to BChE (219). Our results emphasize that in the case of carbamates and  $\beta$  2-agonists, carriers of atypical BChE variants will be less affected compared to the usual variant (219). This is especially important for drugs for which BChE is a key enzyme in the metabolism. We investigated the influence of bronchodilating  $\beta$  2-agonists on the activity of AChE and usual, atypical and fluoride-resistant BChE (8). The inhibition potency of racemate and enantiomers of fenoterol as a resorcinol derivative, isoetharine and epinephrine as catechol derivatives and salbutamol and salmeterol as saligenin derivatives was determined and the tested compounds reversibly inhibited ChE with the highest inhibition potency towards the usual BChE variant, but none of the ChE displayed any stereoselectivity (8).

A review article was published dedicated to the identification of lead compounds for the development of new drugs using methods of dynamic combinatorial chemistry, where a targeted biological macromolecule serves as template for the synthesis of new potential drugs (37).



LEADER	PROJECT	DURATION
<b>Martina Piasek, PhD (IMROH)</b>	<b>Assessment of daily exposure to metals and maternal individual susceptibility as factors of developmental origins of health and disease (METALORIGINS, IP-2016-06-2017)</b>	<b>1 Jun 2017–31 May 2021</b>

#### ASSOCIATES

IMROH: J. Jurasović (deputy leader), T. Orct, A. Pizent, M. Lazarus, I. Brčić Karačonji, N. Brajenović, A. Mikolić, B. Tariba Lovaković, A. Sekovanić, A. Sulimanec Grgec, T. Živković Semren, Z. Kljaković-Gašpić, J. Kovačić

External associates: D. Pašalić, S. Stasenko, K. Branović Čakančić

#### SUMMARY

The maternal womb is the first environment of a developing organism. Prenatal insults in this physiologic microenvironment, such as maternal nutrient deficiencies, toxic metal exposure and cigarette smoking may disrupt foetal growth not only directly, but also have impact on postnatal health across the life course increasing the risk of various diseases in adulthood. We started the project by assessing selected health benefits and risks of unavoidable, daily environmental exposure and intake of nutritionally essential and main toxic metals/metalloids in a vulnerable population group of women during child-bearing period that, along with maternal individual (non-genetic and genetic) susceptibility, may alter intrauterine foetal epigenetic regulation and thus be factors of the developmental origins of health and disease (the DOAHD paradigm). Exposures *via* food and tobacco smoking (based on self-reporting and smoking quantified by maternal urine cotinine) and health risks will be assessed in a cross-sectional epidemiological study using methods of human biological monitoring in 150 postpartum woman-infant pairs after term vaginal deliveries in a maternity hospital (in addition to previously collected data and similar samples in >200 participants). Biomarkers of exposure/intake will be toxic (Cd, Pb, Hg, As) and essential (Ca, Fe, Zn, Cu, Se) metal/metalloid levels in maternal blood/serum and urine, umbilical cord blood/serum and placental tissue. Biomarkers of effects will be antioxidant enzymes (SOD, GPx), metallothionein (MT), and placental steroid hormones (progesterone, estradiol). Associations of maternal metal/metalloid and MT levels with *MT2A* genetic polymorphisms will be assessed. Potential postnatal health risks due to developmental programming changes will be evaluated by epigenetic markers, DNA methylation and microRNA expression levels. Sensitive and sophisticated methods and equipment for human sample preparations and analyses will be used. The unique contribution of this project to biomedical research will be to provide novel data in toxicogenomics – environmental epigenetics of metals, first of a kind

in Croatia.

During first year of the project are planned comprehensive preparatory activities including pertinent literature search along with internal consulting and experience exchange within the research team related to the planned methods and new procedures; supplying with the appropriate equipment, kits and reagent sets to start the research; preparation of the documentation to provide the required ethics committee reports of all of the collaborating institutions for the research on healthy postpartum women (mother-infant pairs) with collection of biological samples of human origin and their analyses; design of the required forms (informed consent to be signed prior to the inclusion in the study and the questionnaire form); design of the protocols used during the investigation (procedures during participant recruitment and sample collection in maternity clinics in Zagreb, protocol for sampling in home laboratory, protocols for sample preparation and storage and protocols for the planned analyses). Recruitment of research participants (mother-infant pairs) will be started with the questionnaire data and the biological samples collection along with the creation of the project web page and data basis accompanied by the planned internal seminar meetings.



LEADER	PROJECT	DURATION
<b>Jasmina Sabolović, PhD (IMROH)</b>	<b>Combined molecular modelling and experimental studies of physiologically and stereochemically important copper(II) amino acid complexes (CopperAminoAcidates, IP-2014-09-3500)</b>	<b>1 Sep 2015–31 Aug 2019</b>

#### ASSOCIATES

IMROH: J. Budimčić

External associates: D. Mrvoš-Sermek (Faculty of Science, Zagreb); M. Ramek and M. Marković (Technische Universität Graz, Graz, Austria); G. Szalontai (NMR laboratórium, Pannon Egyetem, Veszprém, Hungary)

#### SUMMARY

According to the project work plan, we combined the experimental and computational studies of copper(II) complexes with amino acids. Solubility of copper(II) complexes with L-isoleucine and D-*allo*,L-isoleucine, which are poorly soluble complexes, was examined in different solvents in a systematic way with the aim of finding appropriate solvent combinations with the concentrations of the complexes suitable for the solution NMR measurements. The solution NMR measurements were performed in Pannon Egyetem, Veszprém, Hungary, during a work visit of J. Sabolović and J. Budimčić to project associate G. Szalontai. The solubility investigations resulted in several new single crystals and an extremely interesting case of a *cis-trans* isomerization even at room temperature. The *cis-trans* isomerization was additionally studied by associates D. Matković-Čalogović and D. Vušak (Faculty of Science, University of Zagreb) using mechanochemical and aging methods. The new polycrystalline samples of bis(isoleucinato)copper(II) were characterized by the single-crystal and powder X-ray diffraction and by the  $^{13}\text{C}$  and  $^2\text{H}$  magic angle spinning (MAS) solid-state NMR (ssNMR) measurements. The  $^{13}\text{C}$  and  $^1\text{H}$  Fermi contact shifts were calculated using the density functional theory (DFT) method for a number of complexes (i. e., for a cluster consisting from 25 up to 33 complexes) extracted from the new X-ray crystal and molecular structures of *trans* and *cis* bis(isoleucinato)copper(II) complexes. Apart from that, conformational analyses were computed in the gas phase and aqueous solution for the copper(II) complex with D-*allo*,L-isoleucine using DFT and molecular dynamics simulations (220). Combining the results obtained by the solution based and mechanochemical syntheses, X-ray diffraction, MAS ssNMR, solution NMR, and DFT are described in a scientific paper which is expected to be published in 2018.

In collaboration with M. Ramek, M. Marković and Iliana Mutapčić (Technische Universität Graz, Graz, Austria) we performed complete and systematic conformational analyses in the gas phase and aqueous solution of the physiological bis(L-asparaginato)copper(II) (289) and ternary (L-histidinato)(L-asparaginato)copper(II) (275) complexes using DFT. The aqueous solution was modelled implicitly using a polarisable continuum model (PCM). For the physiological ternary complex (L-histidinato)(L-threoninato)copper(II), the DFT conformational analyses in the gas phase and aqueous solutions and the study of intramolecular and intermolecular noncovalent interactions (due to a large number of hydrogen-bond donor and acceptor atoms in both amino acid ligands) on the structural and energetic properties revealed an interesting property of conformational flexibility of the complex in aqueous solution (50, 301). The conformations in different

coordination modes have very similar thermodynamical stability in aqueous solution. The flexibility explains previous experimental findings on the lack of inter-residual strain and the abundance of the ternary over the parent bis(L-threoninato)copper(II) complex determined in aqueous solutions at physiological pH values (50, 301). A paper reporting quantum-chemical calculations using different DFT functionals and bases set to predict equilibrium geometries of anhydrous and aqueous bis(amino acidato)copper(II) complexes, bis(L-histidinato)copper(II) in particular, was published (55). Reliable DFT calculations can provide insight into the influence of intermolecular interactions on the molecular geometry in the crystal lattice or solution when compared with a DFT gas-phase minimum. The DFT results assessed with different density functionals for isolated bis(L-histidinato)copper(II) were benchmarked against those from high-level second-order perturbation Møller–Plesset (MP2) calculations (55). M. Marković held an oral presentation at a congress in Opolo (Poland) about our DFT results on the influence of noncovalent interactions on the stability of bis(L-treoninato)copper(II) and bis(L-*allo*-treoninato)copper(II) (275).



LEADER	PROJECT	DURATION
<b>Assist Prof Ivana Vinković Vrček (IMROH)</b>	<b>Interaction of metallic nanoparticles with sulphur-containing biomolecules – implications for nano-bio interface (NanoFaceS, IP-2016-06-2436)</b>	<b>15 Mar 2017– 14 Mar 2021</b>
<b>ASSOCIATES</b>		
IMROH: M. Milić, G. Šinko, I. Pavičić, A. Miličević External associates: I. Capjak, M. Milić, B. Vuković, S. Šupraha Gopreta, V. Šerić, W. Goessler, D. Horak, E. Omanović-Miklićanin		
<b>SUMMARY</b>		
<p>Nanomedicine, the application of nanotechnology to healthcare, has great impact on innovation in medical treatments and therapies. Nanoparticles (NPs) have enormous potential in the medical arena as drug and gene delivery vehicles, fluorescent labels and contrast agents. However, extensive <i>in vivo</i> applications of NPs require a more exhaustive exploration of the physicochemical and physiological processes coupled with introduction of NPs to biological environments. The dynamic physicochemical interactions, kinetics, and thermodynamic exchanges between NP surfaces and the surfaces of biological components give rise to the ‘nano-bio’ interface. It is impossible to unequivocally describe all of the events at this interface, but additional information on the more specific interplay of NPs with bioactive components of living cells and tissues are of the highest relevance for the prospective evolution of nanomedicine.</p>		
<p>The NanoFaceS project aims to provide a body of new information and new knowledge to the nanomedical endeavour in addressing the scientific uncertainties related to the beyond-state-of-the-art interaction of engineered metal-based NPs, used in theranostics, and sulphur-containing biomolecules (S-biomols), important in living systems due to their complex functional roles. NanoFaceS will use a model system comprising (i) a set of silver, gold, and iron oxide NPs with varying physicochemical properties, i.e. size, surface charge and chemical composition and (ii) six representative S-biomols: cysteine, glutathione, metallothionein, albumin and insulin. The underlying concept of NanoFaceS will be based on understanding interactions between NPs and S-biomols by implementing a multimethodological and multidisciplinary approach that would yield competence on biological consequences of NP interaction with S-biomols. As a major outcome, the project will provide substantial knowledge to the nanomedical landscape.</p>		
<p>During the first year, project activities include testing, optimizing, and validating synthesis protocols for metallic nanoparticles (NPs), characterisation of prepared and purified NPs in terms of size, shape, mass, surface area, chemical composition, physical, optical properties, stability evaluation of NPs by means of aggregation and dissolution in ultrapure water, phosphate buffer pH 7.4, and cell culture media (DMEM).</p>		
<p>Dissemination activities resulted in the publication of 2 research (11, 91) and 2 review (1, 13) papers, one book chapter (180), one abstract presented at international conference (305) and one MSc thesis (210).</p>		



LEADER	PROJECT	DURATION
<b>Prof Davor Želježić (IMROH)</b>	<b>Organic Pollutants in Environment – Markers and Biomarkers of Toxicity (OPENTOX, IP-2013-11-8366)</b>	<b>1 Sep 2014– 31 Aug 2018</b>
<b>ASSOCIATES</b>		

IMROH: N. Brajenović, I. Brčić Karačonji, V. Drevenkar, M. Dvorščak, S. Fingler Nuskern, R. Fuchs, S. Herceg Romanić, V. Kašuba, D. Klinčić, Z. Kljaković Gašpić, N. Kopjar, A. Lucić Vrdoljak, A. M. Marjanović Čermak, G. Mendaš Starčević, V. Miček, A. Mikošević, M. Milić, V. Mužinić, I. Pavičić, A. Pizent, R. Rozgaj, S. Stipičević, B. Tariba Lovaković, I. Trošić, Ž. Vasilić, T. Živković Semren, S. Žunec

#### SUMMARY

The genotoxic potential of glyphosate was evaluated in *in vitro* conditions in HepG2 cells, which exhibit endogenous ability of metabolically changing of exogenous substances. Neither 4-hour nor 24-hour exposure induced primary damage to DNA. However, concentrations that reflect realistic exposure scenarios did induce a significant increase in micronuclei formation, which are considered as secondary biomarkers of exposure to genotoxins. Furthermore, it was detected that the tested levels of glyphosate exhibited a significant effect on the induction of oxidative stress in terms of increased lipid peroxidation, activity of glutathione peroxidase and catalase, as well as decrease in total antioxidative capacity (28). By applying the comet-FISH technique, we showed that neither realistic scenario nor relevant concentration of the insecticides chlorpyrifos, imidaclopride, and  $\alpha$ -cypermethrin affected the structural or numerical integrity of the genes *TP 53* or *c-Myc*, which play a crucial role in cell-cycle regulation and, consequently, may be involved in the process of carcinogenicity (70). The effect of  $\alpha$ -cypermethrin at low doses on the missegregation of chromosomes 9, 18, X, and Y and the aneuploidy induction was also evaluated. We proved that even at low concentrations the insecticide  $\alpha$ -cypermethrin significantly affects the regularity of chromosome segregation in aneuploidy of cell division and leads to an uneven distribution of them between newly formed daughter cells (265).

We showed that chlorpyrifos, imidaclopride and  $\alpha$ -cypermethrin at low doses relevant to realistic exposure scenarios do not adversely affect microtubules and actin itself in HepG2 and 132N1 cells. After treatment, the structure of microtubules remained intact. Exposure of SH-SY5Y neuroblastoma and 132N1 astrocytoma cells to the tested insecticides did not result in increased levels of reactive oxidative species (ROS), or any changes in the redox potential of the cells. In line with these results, we failed to detect an increase in malondialdehyde or carboxylate formation.

In *in vivo* conditions, we showed that congeners of polychlorinated biphenyls (PCB) and organochlorine pesticides extracted from maternal placentas did not induce primary damage to DNA in exposed human leukocytes. It was determined that the most frequent PCB congener in placentas was PCB-28 (104).

The results of a 28-day exposure of rats to terbuthylazine at doses relevant to environmental exposure showed a potential to disturb an oxidant/antioxidant status reflected by changes of the total antioxidant capacity and activities of antioxidant enzymes. The results also indicated that such low doses of terbuthylazine had the potential to produce relatively low-level DNA instability in leukocytes. Measured concentrations of urinary and plasma terbuthylazine and its metabolites showed that terbuthylazine was completely metabolized and rapidly excreted from the rats' body. The observed effects could be due to the ability of animals to adapt to the repeated treatments with low doses of terbuthylazine as well as due to its rapid elimination (62, 292).

The treatment of Wistar rats with doses of chlorpyrifos corresponding to realistic exposure scenarios for 28 consecutive days did not significantly inhibit acetylcholinesterase activity in plasma or brain neurons; although, in these tissues an increase of lipid peroxidation was detected. Nevertheless, no effects on lipid peroxidation, ROS level, complete antioxidant activity or the level of reduced glutathione activity were detected. However, a significant increase of activity of glutathione peroxidase in blood, and superoxidase dismutase in erythrocytes was detected. The treatment of rats with chlorpyrifos at low doses increased the formation of primary damage to DNA measured by comet assay, though these increases were not statistically relevant (81, 283).

Analysis of organochlorine contaminants in human milk samples using validated method was presented in a congress abstract (255) and an original scientific article in preparation. In a review article, phthalate properties, monitoring, their use, toxic effects on human health, permitted daily intake for humans as well as the legal framework for maximum permissible concentrations of phthalates in different matrices and products were described (9).

The concentration of 23 elements, total phenols and antioxidant capacity in the samples of strawberry tree

honey (*Arbutus unedo* L.) from south Dalmatia showed richer nutrition values than the majority of unifloral honeys collected in Croatia. Toxic element levels in the analysed honey samples indicated pristine area origin (100).

We evaluated transplacental, translactational genotoxicity, and endocrine disruption effect of the herbicide tembotrione in *in vivo* conditions. Endocrine disruption was assessed by measuring the levels of estradiol and testosterone in serum of both, female, and male newborn, at weaning age and prepubertal rats (264). Mother rats were treated with herbicide since day 16 of gestation until weaning. By applying the alkaline comet assay on leukocytes and liver cells at each sampling point, no effect on the level of primary damage to DNA was detected in offsprings.

## A.2. IN-HOUSE SCIENTIFIC PROJECTS (14 projects)



LEADER	PROJECT
<b>Anita Bosak, PhD</b>	<b>Design, synthesis and evaluation of selective inhibitors of butyrylcholinesterase</b>
ASSOCIATES: M. Katalinić, G. Šinko, Z. Kovarik, A. Miličević, A. Zandona External associate: I. Primožić and A. Ramić (Faculty of Science, Zagreb)	
<b>Prof Selma Cvijetić Avdagić, PhD, MD</b>	<b>Bone mineral density and fracture risk in institutionalized older people</b>
ASSOCIATES: J. Macan, V. M. Varnai, J. Bobić, Ž. Babić, J. Kovačić, M. Deranja, M. Kujundžić Brkulj, R. Luzar	
<b>Ranka Godec, PhD</b>	<b>Organic content of PM<sub>1</sub> particle fraction</b>
ASSOCIATES: G. Pehnc, I. Bešlić, I. Jakovljević, Z. Sever Štrukil, I. Šimić	
<b>Maja Lazarus, PhD</b>	<b>Biomonitoring of contaminants using the biomarkers in European brown bear (<i>Ursus arctos</i>)</b>
ASSOCIATES: J. Aladrović, M. Avdić, M. Erk, V. Filipović Marijić, Đ. Huber, J. Jurasović, S. Mataušić, T. Orct, M. Peraica, D. Rašić, S. Reljić, A. Sekovanić, N. Selva, A. Sergiel, L. Vranković Partners: Ruđer Bošković Institute (Laboratory for Biological Effects of Metals); Institute of Nature Conservation of Polish Academy of Sciences, Krakow, Poland (Department of Wildlife Conservation); Faculty of Veterinary Medicine, Zagreb (Department of Biology and Department of Physiology and Radiobiology)	
<b>Prof Ana Lucić Vrdoljak</b>	<b>Investigation of interactions between irinotecan and tetrahydrocannabinols on rat experimental model using integrated biochemical, molecular biology, pathohistologic and analytical methods</b>
ASSOCIATES: Ž. Babić, N. Brajenović, I. Brčić Karačonji, M. Dvorščak, R. Fuchs, A. Jurić, N. Kopjar, V. Micek, A. Mikolić, I. Novak Jovanović, Lj. Prester, S. Žunec Partner: University North, Koprivnica	
<b>Prim Jelena Macan, PhD, MD</b>	<b>Employment status of patients with a diagnosed occupational disease</b>
ASSOCIATES: M. Deranja, M. Lieberth, F. Šakić External associate: R. Ecimović Nemarnik, resident in occupational and sport medicine	
<b>Prim Jelena Macan, PhD, MD</b>	<b>Interaction of constitutional and occupational risk factors on the incidence of occupational contact dermatitis in hairdressing apprentices during vocational training</b>
ASSOCIATES: S. Cvijetić Avdagić, V. M. Varnai, J. Bobić, Zr. Franić, Ž. Babić, J. Kovačić, A. Bjelajac, M. Deranja, M. Kujundžić Brkulj, F. Šakić, M. Milić	
<b>Prim Jelena Macan, PhD, MD</b>	<b>Contact hand dermatitis in dentists and medical doctors: prevalence and risk factors</b>
ASSOCIATES: A. Bjelajac, Ž. Babić, Zr. Franić, F. Šakić External associates: L. Lugović Mihić and I. Japundžić (School of Dental Medicine, Zagreb)	

<b>Ante Miličević, PhD</b>	<b>Investigation of electrochemical properties and antioxidant activity of polyphenolic compounds and their complexes with essential elements</b>
ASSOCIATES: I. Novak Jovanović, I. Pavičić External associate: N. Bregović (Faculty of Science, Zagreb)	
<b>Assist Prof Branko Petrinec, PhD</b>	<b>Radiological characterization of Kopački rit</b>
ASSOCIATES: D. Babić, T. Meštrović, M. Šoštarić Partners: Physics Department of J. J. Strossmayer, Osijek	
<b>Martina Piasek, PhD, MD</b>	<b>Exposure to cadmium and its effects during gestation and postnatal period: investigations in laboratory rats</b>
ASSOCIATES: A. Mikolić, J. Jurasović, T. Orct, A. Sulimanec Grgec, Lj. Prester; technicians: S. Mataušić, M. Komesar	
<b>Ivica Prlić, PhD</b>	<b>Development of UV radiation sensors (SUVIndex)</b>
ASSOCIATES: J. Macan, Lj. Orešić (until 15 Sep 2017), M. Surić Mihić, L. Pavelić (since 1 Jun 2017) Partners: Haj-Kom d.o.o. (M. Hajdinjak), ALARA Uredaji d.o.o. (Z. Cerovac), KBC Zagreb, ACI Marina Vodice	
<b>Ivica Prlić, PhD</b>	<b>Thermometry, thermography and sensory evaluation of electromagnetic radiation in medicine</b>
ASSOCIATES: M. Surić Mihić, I. Bešlić, Lj. Orešić (until 15 Sep 2017), L. Pavelić (since 1 Jun 2017), J. Šiško, M. Justić, S. Kobeščak Partners: KBC Zagreb (Prof A. Antabak, PhD, head of the clinical part of the research and associates at KBC Zagreb, Zagreb Children's Disease Clinic), General Hospital Karlovac, Haj-Kom d.o.o. (M. Hajdinjak), ALARA Uredaji d.o.o. (Z. Cerovac)	
<b>Jasmina Rinkovec, PhD</b>	<b>Levels of platinum group elements (PGE) near roads</b>
ASSOCIATES: G. Pehnc, S. Žužul, I. Bešlić, S. Davila	

## B. COLLABORATION ON RESEARCH PROJECTS OUTSIDE THE INSTITUTE

(according to source of funding)

### B.1. CROATIAN SCIENCE FOUNDATION (7 projects)



LEADER	PROJECT	DURATION
<b>Assoc Prof Biljana Balen, PhD (Faculty of Science, Zagreb)</b>	<b>Nanosilver phytotoxicity: mechanisms of action and interaction in tobacco cells (NanoPhytoTox, IP-2014-09-6488)</b>	<b>1 May 2016– 30 Apr 2020</b>
<b>ASSOCIATES</b>		
IMROH: I. Vinković Vrček		
<b>SUMMARY</b>		
Project activities included the toxicity evaluation of differently coated silver nanoparticles (AgNPs) on tobacco seedlings and plants, stability evaluation of differently coated AgNPs in plant culture media, and a study on bioaccumulation and biodistribution of AgNPs in plants. The results published in one original research paper (74) were also presented at the 13 <sup>th</sup> Multinational Congress on Microscopy (271).		



LEADER	PROJECT	DURATION
<b>Prof Tomica Hrenar, PhD (Faculty of Science, Zagreb)</b>	<b>Activity and <i>in silico</i> guided design of bioactive small molecules (Adesire, IP-2016-06-3775)</b>	<b>1 Mar 2017– 28 Mar 2021</b>

#### ASSOCIATES

IMROH: A. Bosak

#### SUMMARY

An *in silico* analysis of the interactions of human acetylcholinesterase (AChE)/butyrylcholinesterase (BChE) and ten cinkonidine derivatives and their corresponding enzyme cinkonidine was performed. Interactions in the active site of both cholinesterases were responsive to the shown selectivity of the investigated compounds and the stereoselectivity of cholinesterase were determined by molecular modelling. A publication has been sent to a Q1 journal.



LEADER	PROJECT	DURATION
<b>Šebojka Komorsky-Lovrić, PhD (Ruđer Bošković Institute, Zagreb)</b>	<b>Development of Voltammetric Methods for the Characterisation of Natural Antioxidants (IP-2013-11-2072)</b>	<b>1 Jul 2014– 30 Jun 2017</b>

#### ASSOCIATES

IMROH: I. Novak Jovanović

#### SUMMARY

The aims of this project are the electrochemical characterization of natural antioxidants and the development of electroanalytical methods for the quantification of antioxidants in natural samples (such as fruits and vegetables).

We investigated the electrochemical properties of capsaicin and developed a new electroanalytical method for the quantification of capsaicinoids in dried hot pepper samples using microparticle voltammetry (16). Analysis was performed on a paraffin-impregnated graphite electrode (PIGE) using square-wave voltammetry (SWV) under optimal experimental conditions. Concentrations of capsaicinoids in hot pepper samples were determined from internal calibration curves constructed using a standard addition method and good correlation was obtained between our results and those reported in literature (correlation factor  $r = 0.976$ ).

We were mainly engaged in investigating the electrochemical properties of carotenoids. We developed a fast, simple and reliable method for the electroanalytical determination of  $\beta$ -carotene in extracts of fruits (grapefruit, mango) and vegetables (butternut, carrot, sweet potato, spinach, kale, and broccoli) by using differential pulse voltammetry on a glassy carbon electrode. Linear responses for  $\beta$ -carotene were obtained for concentrations from  $4.0 \times 10^{-6} \text{ mol L}^{-1}$  to  $1.1 \times 10^{-4} \text{ mol L}^{-1}$ , and the calculated LOD and LOQ were  $2.5 \times 10^{-6} \text{ mol L}^{-1}$  and  $8.2 \times 10^{-6} \text{ mol L}^{-1}$ , respectively. The results of this study were presented at the 6<sup>th</sup> Regional Symposium on Electrochemistry of South-East Europe (296).



LEADER	PROJECT	DURATION
<b>Assoc Prof Maja Šegvič Klarič, PhD (Faculty of Pharmacy and Biochemistry, Zagreb)</b>	<b>Adverse effects of single and combined mycotoxins produced by Aspergilli (Mycotox A, IP-09-2014-5982)</b>	<b>8 Feb 2016– 7 Feb 2020</b>
<b>ASSOCIATES</b>		

IMROH: D. Breljak, N. Kopjar, M. Peraica, D. Rašić, D. Želježić

#### SUMMARY

Mycotoxins ochratoxin A (OTA) and citrinin (CTN) are very frequent grains contaminants commonly found together. The frequency and the concentration of CTN is usually much higher than that of OTA (270). As the mechanisms of toxicity of both mycotoxins involve the increase of ROS production and oxidative stress, the mechanisms of their toxic effects when given together was studied on experimental animals. Adult male Wistar rats were orally treated either only with OTA (0.125 and 0.250 mg kg<sup>-1</sup> b. w.), only with CTN (2.0 mg kg<sup>-1</sup> b. w.), or with both (0.125 mg kg<sup>-1</sup> b. w. + 2.0 mg kg<sup>-1</sup> b. w.) for 21 days. A group of animals receiving OTA (0.250 mg kg<sup>-1</sup>) was treated with resveratrol (RSV, 2.0 mg kg<sup>-1</sup>), a well-known antioxidant compound. At the Toxicology Unit, the concentration of malondialdehyde (MDA) and glutathione (GSH) was measured in plasma, kidneys, and liver of experimental animals, as was the activity of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) in plasma. The concentration of MDA was significantly higher in kidneys of animals treated with OTA (34.21 ± 3.90 and 41.81 ± 5.51 ng g<sup>-1</sup> tissue), as well as in animals treated with OTA (0.125 mg kg<sup>-1</sup>) + CTN (45.07 ± 12.59 ng g<sup>-1</sup> tissue) than in control animals receiving water (18.19 ± 5.45 ng g<sup>-1</sup> tissue). RSV did not reverse these effects in kidneys (23.96 ± 1.83 ng g<sup>-1</sup> tissue). In plasma, both OTA doses and CTN increased MDA levels (0.68 ± 0.08, 0.65 ± 0.05, and 0.65 ± 0.09 ng mL<sup>-1</sup>, respectively). The combined treatment did not further increase the MDA concentration (0.69 ± 0.07) and RSV was effective in decreasing it (0.43 ± 0.03). RSV was also effective in the kidney having restored its GSH levels (303). OTA and CTN did not decrease the GSH concentration in kidneys and liver, while in plasma higher OTA dose significantly increased plasma GSH.

At the Mutagenesis Unit, primary DNA damage in liver and renal cortex by alkaline and oxidative DNA damage by hOGG1 modified comet assay were assessed. We detected a significant increase in alkaline comet assay parameters in both kidney and liver cells following treatment with citrinin, and combinatorial treatment with ochratoxine and citrinin. Furthermore, a significant increase in primary DNA damage was detected in kidney cells following simultaneous treatment with ochratoxine, citrinin, and resveratrol. A significant effect on the level of oxidative damage to DNA was not recorded in liver or kidney cells. The obtained results are now interpreted and will be used for future publications.

Previous studies found that OTA increases CTN accumulation in the kidneys and liver of experimental animals treated with both mycotoxins (267). At the Molecular Toxicology Unit liver and kidney tissue samples were collected from animals treated as previously described. For immunofluorescence analysis, organs were fixed with 4 % PFA, and for western analysis total cell membranes were isolated from the renal and hepatic tissue homogenates. In all experimental groups of animals, the effect of various doses of mycotoxins (CTN and OTA) and RSV on membrane transporter protein expression in kidneys and liver was investigated. Protein expression (western analysis) and cellular localization (immunofluorescence analysis) of two sodium and glucose cotransporters SglT1/Slc5a1 and SglT2/Slc5a2 was also analysed in all of the animal experimental groups.



LEADER	PROJECT	DURATION
<b>Prof Valerije Vrčec, PhD (Faculty of Pharmacy and Biochemistry, Zagreb)</b>	<b>Quantum-chemical design, preparation and biological properties of organometallic nucleobase derivatives (OrDeN, IP-2016-06-1137)</b>	<b>1 Mar 2017– 28 Feb 2021</b>

## ASSOCIATES

IMROH: A. M. Marjanović Čermak

## SUMMARY

Organometal nucleobase derivatives (OrDeNs) are a new generation of conjugates in which metallocenes are linked to the underlying superstructural elements of inheritance. Due to their electrophoretic and bioactive properties, OrDeNs are used in (bio) analytical and medical chemistry and are penetrating into the field of therapy, molecular diagnostics, and nanotechnology. The main purpose of this project is to design and synthesize new biologically active OrDeNs for which quantum-chemical calculations suggest desirable electrochemical and biological properties. With the assistance of quantum-chemical accounts, the reaction conditions for an efficient preparation of OrDeNs will be defined, with a high percentage of utilization and a high degree of regioselectivity. Electroactive and biological properties of newly-prepared compounds will be determined, which will be compared against the results of the obtained quantum-chemical calculations. Analogously published results that OrDeNs may be in the group of apoptosis inducers and tumor cell growth inhibitors will be subjected to biological testing on several different tumor cell lines within this project.



LEADER	PROJECT	DURATION
<b>Prof Vanja Vučićević Boras, PhD (School of Dental Medicine, Zagreb)</b>	<b>The role of oestrogen and androgen receptor activation in the stroma of oral cancer and their impact on the survival of patients (ACTIVESTROMORALCANCER, IP-2014-09-6985)</b>	<b>1 Oct 2015– 30 Sep 2019</b>

## ASSOCIATES

IMROH: A. Fučić

## SUMMARY

The results of the project showed significant interaction of androgenic receptors and Ki67 in stroma and epithelial cells of patients with oral cancer (251). A review article on the factors that activate the stroma of oral cancer, thereby facilitating neoplastic progression is in preparation. The work is based on currently available data on the interaction between metalloproteinases, cytokines, growth factors, hypoxia factor, and extracellular adhesion proteins in stroma and neoplastic cells. Their interaction is further illustrated by the use of the systems biology language Systems Biology Graphical Notation in order to sublimate accumulated knowledge and enable more efficient recognition of possible new biomarkers in the diagnosis and oral cancer screening and in finding new therapeutic goals (102).



LEADER	PROJECT	DURATION
<b>Prof Damir Vukičević, PhD (Faculty of Science, Split)</b>	<b>Biophysical Design of Antimicrobial peptides and Innovative Molecular Descriptors (BioAmpMode, no. IP-2013-11-8481)</b>	<b>15 Sep 2014– 14 Dec 2017</b>

#### ASSOCIATES

IMROH: G. Gajski

#### SUMMARY

The starting point of this interdisciplinary project is the collection of bioinformatics and chemoinformatics data that link molecular structure with physicochemical and biological properties. In the case of antimicrobial peptides, the measured biological activity and therapeutic index data will be collected from published papers with the goal of constructing robust and accurate predictive models. A large amount of data on 20 natural amino acid attributes will also be collected and analysed. The plan is to propose new scales of amino acid attributes that may act as a basis for a multitude of future studies. In our experience, the construction of good predictive models is not possible without biophysical insights aiming to propose appropriate molecular descriptors crucial for the data-mining procedure and model building. These models should be based on newly-developed molecular descriptors. The study of molecular descriptors will be an important part of this project, their extremal properties, their complexity of calculation and their interrelationships. Dedicated algorithms for *in silico* identification or proposal of structures with the desired properties will be built and verified through synthesis, characterisation, and testing of predicted antimicrobials. The activity, selectivity, conformation, and mechanism of action of lead compounds will be explored using biophysical, biochemical, and microbiological techniques. Initial drug development is within the scope of this project, with the aim of finding new classes of non-toxic peptide antibiotics that are active against multidrug resistant bacteria. New peptide antimicrobials will be searched for using several different algorithms and methods developed previously as well as by developing additional ones. The first peptides have already been evaluated and the results showed that these may serve as useful lead compounds for developing anti-infective agents against resistant Gram-negative and Gram-positive species (54, 243).

## B.2. MINISTRY OF SCIENCE AND EDUCATION OF THE REPUBLIC OF CROATIA

Scientific Centres of Excellence



LEADER	PROJECT	DURATION
<b>Prof Davor Ježek, PhD (School of Medicine, Zagreb)</b>	<b>Scientific Center of Excellence for Reproductive and Regenerative Medicine (CERRM); ERDF, OP Competitiveness and Cohesion, under grant agreement No. KK.01.1.1.01.0008, Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials</b>	<b>2014–2019</b>

#### ASSOCIATES

IMI: A. Fučić

#### SUMMARY

Within the framework of the project, a review of health risks of transplacental exposure to xenobiotics was prepared. This paper presents the mechanisms that lead to the risk of cancer development in children such as leukaemia, neuroblastoma/brain tumours, hepatoblastoma, and Willmov tumours including prenatal induced genomic, epigenetic and/or non-genomic damage (22). In order to investigate the effect of diabetes in mothers on their newborns, genome damage, cell free DNA and *N*-glycosylation was investigated in cord blood. The results have been related to the mother's lifestyle based on a detailed questionnaire and compared to the control group. The level of genome damage is significantly associated with residence (urban vs. rural). The level of glycosylation differs significantly between children of healthy mothers and those suffering from diabetes. There was a significant association between glycosylation levels and genome damage (21). The

methylation of the RASSF1A promoter in the peripheral blood of testicular cancer patients was investigated. The meta-analysis showed a methylation of the RASSF1A promoter as a risk factor for testicular cancer. Methylation level was higher in patients with testicular cancer before chemotherapy than after therapy. The role of RASSF1A hypermethylation in the metastasis of testicular cancer (39) was demonstrated.

### B.3. UNIVERSITY OF ZAGREB



LEADER	PROJECT	DURATION
<b>Prof Jasminka Despot Lučanin, PhD (Croatian Studies, Zagreb)</b>	<b>Biopsychosocial predictors of quality of life in older persons in different living arrangements</b>	<b>2017–2018</b>
<b>ASSOCIATES</b>		
IMROH: A. Bjelajac		
<b>SUMMARY</b>		

The aim of the study is to examine the biopsychosocial predictors of the quality of life of older persons living in different housing arrangements. The participants in our previous studies [2014, 2015, and 2016 (149)] were clients of retirement homes, which represent a specific form of housing arrangement and therefore the possibility of generalization was limited. For this reason we included community-dwelling older adults in this project. One hundred and seventy community-dwelling participants of older age (60 yrs+) were examined and their results will be compared to the results of 168 retirement home residents examined in the last wave in 2016. By means of individual structured interview, the following data is collected: sociodemographic characteristics (age, gender, education, marital status, family members), objective health (chronical illness), self-perceived health (general and compared to age peers), social support (emotional, instrumental, socialization), sleep quality, and life satisfaction. The results can be applied in all fields aimed at improving the quality of life of older persons, i.e. development of specific community services. Until now, the results have been presented at one scientific meeting in Croatia (233), two scientific meetings abroad (277, 284), and one professional meeting "Factors contributing to the quality of life of older persons" organized by the foundation "Zajednički put".

### B.4. CROATIAN ACADEMY OF SCIENCE AND ARTS FOUNDATION



LEADER	PROJECT	DURATION
<b>Assoc Prof K. Barić, PhD (Faculty of Agronomy, Zagreb)</b>	<b>Development of bioassay method for detection of herbicide residues in soil</b>	<b>1 Sep 2016–31 Aug 2017</b>
<b>ASSOCIATES</b>		
IMROH: S. Stipičević		
<b>SUMMARY</b>		

A bioassay method for detecting the susceptibility of mesotrione residues in sugar beet on two soil types was developed. Mesotrione is an herbicide that indirectly inhibits carotenoid production in susceptible plants. Although defined as non-persistent, it is known that mesotrione's persistence can vary depending on the soil's physicochemical properties. The phytotoxicity of mesotrione residues in sugar beet grown was determined on two soil types: hipogley and humofluvisol. Mesotrione was applied on both soils at doses of 0; 0.25; 0.5; 1; 2; 4; 6; and 8 µg a.i. per 200 g of soil. The sugar beet was cultivated in a growth chamber under controlled climate conditions for 3 weeks. Symptoms of phytotoxicity (leaf bleaching) were assessed at 7, 14, and 21 days after application (DAA) by using a 0 - 100 % scale (where 0 % = no effect and 100 % = plant death). The fresh weight of sugar beet was determined on the 21<sup>st</sup> day, after which total carotenoid content was determined by spectrophotometry. The highest visually evaluated phytotoxicity on both soils was determined on 21 DAA. On humofluvisol, damages were noticed already at the lowest dose (0.25 µg a. i.). The sugar beet plants were completely damaged at 4 µg a. i. on humofluvisol and at 6 µg a. i. on hipogley soil. A correlation in the reduction of fresh weight with the reduction of total carotenoid content was established on both soils. A significantly higher reduction of both parameters already at the lowest dose was established on humofluvisol. The conference abstract was prepared.

**C. PROFESSIONAL PROJECTS**

PROJECT	CONTRACTOR	LEADER
<b>Service provider: Environmental Hygiene Unit</b>		
Monitoring air pollution in the City of Zagreb (since 1963)	City of Zagreb, City Office for Energy, Environmental Protection and Sustainable Development	V. Vađić (1963–2014), G. Pehnec (since 2015)
Monitoring of the Total Effects of CPS Molve on the Ecosystem (since 1998)	INA-Naftaplin and Institute for Public Health of the Koprivnica-Križevci County	V. Vađić (1998–2014), G. Pehnec (since 2015)
Monitoring Air Quality at the CWWTP Construction Site in Zagreb (since 2003)	Zagrebačke otpadne vode d.o.o.	V. Vađić (2003–2014), G. Pehnec (since 2015)
Monitoring Air Pollution at National Network Stations for the Purpose of Continued Air Quality Monitoring (since 2015)	Ministry of Environment and Nature Protection and Meteorological and Hydrological Service of Croatia	G. Pehnec
Drafting Equivalency Studies at Measurement Stations of the National Network for Continued Air Pollution Monitoring (since 2015)	Ministry of Environment and Nature Protection and Meteorological and Hydrological Service of Croatia	I. Bešlić
Monitoring Air Pollution at a Station at Military Training Polygon in Slunj (since 2009)	Meteorological and Hydrological Service of Croatia	V. Vađić (2009–2014), G. Pehnec (since 2015)
The Ecological map of the City of Zagreb	City of Zagreb	G. Pehnec, S. Davila
<b>Service provider: Radiation Dosimetry and Radiobiology Unit</b>		
IMI_FondNEK "Radiological monitoring programme for extended location of Radioactive Waste Storage Facility in Republic of Croatia rev.4.0 – Environment monitoring programme	Fund for financing the decommissioning of the Krško Nuclear Power Plant and the disposal of NEK radioactive waste and spent nuclear fuel	I. Prlič
Estimation of radiation exposure of workers and members of the public due to activities which involve the presence of natural radiation sources that lead to a significant increase in the exposure of workers or members of the public at working areas of INA group in Republic of Croatia	STSI - Integrated technical services Ltd., a member of INA group	I. Prlič

**Service provider: Radiation Protection Unit**

Background Radioactivity Monitoring in the Republic of Croatia, IMI-CRZ-96 (since 1959)	State Office for Radiological and Nuclear Safety, Zagreb	G. Marović
Pathways of Ionising Radiation During NPK Fertilizers Production	Mineral Fertilizer Factory <i>Petrokemija</i> d.d., Kutina	T. Bituh
Results of Monitoring of Environmental Radioactivity in Vicinity of Plomin Coal-Fired Power Plant, IMI-P-383	HEP proizvodnja d.o.o., Thermal power plant Plomin I, Plomin	G. Marović
Results of Radioactivity Measurements at Gas Field Molve, IMI-P-384	Koprivnica-Križevci County, Koprivnica	G. Marović
Radioactivity Measurements and Monitoring in Barać Caves	Public institute for protected area management for the area of Rakovica Municipality, Rakovica	T. Bituh



## 3.2. INTERNATIONAL PROJECTS

### A.1. EUROPEAN REGIONAL DEVELOPMENT FUND (ERDF, 2 projects)



LEADER	PROJECT	DURATION
<b>Croatian Academic and Research Network (CARNet)</b>	<b>Electromagnetic radiation dosimetry for implementation of the e-Schools pilot project: establishing a system for developing digitally mature schools (3-16-MV-OP)</b>	<b>1 Mar 2015– 28 Feb 2018</b>

#### ASSOCIATES

IMROH: I. Prlić (coordinator), M. Justić, D. Kosmina, J. Macan, T. Meštrović, Lj. Orešić, M. Surić Mihić, J. Šiško, V. M. Varnai

External associates: M. Hajdinjak (HAJ-KOM d.o.o.); Z. Cerovac (ALARA Uređaji d.o.o.); H. Mesić (Prirodopolis)

#### SUMMARY

In recent years, the use of Wi-Fi radio transceivers has been growing rapidly. Using such technology, electronic devices are connected by radio to a computer network via microwave or radio frequency (RF) electromagnetic fields, thus eliminating or reducing the need to connect with network cables. The best example is a laptop that is connected to the Internet via a Wi-Fi router. Today, Wi-Fi access points can be found in many public and private areas, which means that people surrounded by Wi-Fi signals are occasionally exposed to low levels of electromagnetic fields when using the Internet for business or private purposes (such as a router as access points).

Wi-Fi radio connectivity is currently being measured as part of the “e-School project: Establishment of Development Digital Mature Schools System (pilot-project)” project implemented by the Croatian Academic and Research Network (CARNet) from 2015 to 2018. The Radiation Dosimetry and Radiobiology Unit are in charge of the continuous monitoring of electromagnetic field levels within school buildings that are part of the project. Their measurements have thus far shown that the level of exposure to the electromagnetic fields produced by Wi-Fi devices in the controlled spaces is far below the referent limit value for the general population set by the European Committee for Electrotechnical Standardization (CENELEC) and the Ministry of Healthcare of the Republic of Croatia. The e-School pilot project includes 10 % of schools (101 primary and 50 secondary schools) from all over Croatia and is part of the programme “e-School: Complete computerization of the school business processes and teaching processes for the purpose of creating digital mature schools for the 21st century”. In digitally mature schools, the active and everyday use of information and communication technology (ICT) in the teaching and business of the school contributes to: the development of digital competence of students, development of digital competence of teachers, and efficient and transparent school management. More information: [www.e-skole.hr/](http://www.e-skole.hr/).



LEADER	PROJECT	DURATION
<b>Cleo Kosanović, PhD, Meteorological and Hydrological Service of Croatia</b>	<b>AIRQ – Project of extension and modernisation of the national network for continuous air quality monitoring (KK.06.2.1.02.0001.)</b>	<b>2017–2021</b>

#### ASSOCIATES

IMI: G. Pehnc (leader), R. Godec, I. Bešlić, S. Žužul, S. Stankić Drobniak, B. Roić, S. Barbarić, M. Herman

#### SUMMARY

An EU grant contract for this project was concluded between the Meteorological and Hydrological Service of Croatia, Ministry of Environment and Energy, and the Environmental Protection and Energy Efficiency Fund on 14 Sep 2017. The main institution is the Meteorological and Hydrological Service of Croatia and the

Institute for Medical Research and Occupational Health is the partner. The project will receive a grant in the amount of 125,123,500 HRK (85 % funded by the ERDF OP Competitiveness and Cohesion and 15 % by the Environmental Protection and Energy Efficiency Fund). The purpose of the project is the improvement of air quality management and monitoring in order to achieve efficient control and air quality management in urban areas, zones, and agglomerations. The aim is to support the implementation of legislation in the field of air quality and environmental protection (Directive, Air Protection Act) including the development of sustainable strategies and projects that create preconditions for the adequate assessment, planning, and provision of measures by measurements of relevant parameters.

Through this project, the Environmental Hygiene Unit will acquire equipment worth 14,475,000 HRK for the purpose of air quality monitoring at measuring stations of the State Network in the part related to sampling and physical and chemical analysis of PM<sub>10</sub> and PM<sub>2.5</sub> particle fractions and equivalence testing of non-reference methods for the determination of PM<sub>10</sub> and PM<sub>2.5</sub> mass concentrations, in accordance with legal obligations.

## A.2. EUROPEAN RESEARCH AND INNOVATION PROGRAMME – HORIZON 2020 (2 projects)



LEADER	PROJECT	DURATION
<b>Dr Marike Gehring-Kolossa, German Environmental Agency</b>	<b>European Human Biomonitoring Initiative (HBM4EU, under grant agreement No 733032)</b>	<b>2017–2021</b>

### ASSOCIATES

IMROH: A. Fučić

### SUMMARY

Within the project, a paper on endocrine disruptors that are in the focus of the project's interest is in preparation and preparations for a meta-analysis were performed. (<https://www.hbm4eu.eu/about-hbm4eu/>).



LEADER	PROJECT	DURATION
<b>Thomas Jung, PhD (Bundesamt für Strahlenschutz, Salzgitter, Germany)</b>	<b>European Concerted Programme on Radiation Protection Research (CONCERT, 662287 COFOUND EJP-Topic: NFRP-2014-2015), within the framework of Euroatom Horizon 2020</b>	<b>2015–2020</b>

### ASSOCIATES

IMROH: I. Prlić (leader for Croatia and POM Contact point, Programme Manager since Dec 2014), T. Bituh, I. Brčić Karačonji, R. Fuchs, A. Lucić Vrdoljak, J. Macan, M. Surić Mihić, J. Tončić, D. Želježić  
Partners: 28 national managers and Programme Owners from 22 European Member States including Norway and Switzerland, and 4 radiation protection associations being MELODI, ALLIANCE, NERIS and EURADOS

### SUMMARY

The CONCERT (*European Joint Programme for the Integration of Radiation Protection Research* under Horizon 2020) operates as an umbrella structure for the research initiatives jointly launched by the radiation protection research platforms MELODI (the fields of low dose risk research), ALLIANCE (radioecology), NERIS (nuclear emergency preparedness), EURADOS (dosimetry), and medical radiation protection. CONCERT as a co-funded action (70 % EC and 30 % national funding) aims to integrate national and European research programmes in order to make better use of public R&D resources by enhancing the visibility of infrastructures and facilitating access to them in order to make radiation protection more effectively by joint research efforts in key areas. Programme is coordinated by the Federal Office of Radiation Protection (BfS) in Germany. To reach its goals, CONCERT has seven Work Packages: three Packages are mainly concerned with joint programming and organising as well as administering open research calls, another three Packages are dedicated to integrative activities such as access to research infrastructure, education and training and stakeholder involvement as

well dissemination, and finally one on coordination of CONCERT itself.

CONCERT guides radiation protection research in Europe. This joint effort is performed with a strategic perspective on supporting excellent science, on building and maintaining high competence in radiation and radiation protection science, as well as further promoting integrative and multidisciplinary research at a European level. CONCERT contributes to the sustainable integration of European and national research programmes in the field of radiation protection. A crucial step is, of course, to initiate and fund concerted joint research actions.

Based on the platform SRAs and joint programming, CONCERT will develop research priorities, align them with priorities from participating Member States and seek further input from society and stakeholders. It will reach out to engage the wider scientific community in its projects, aiming to answer the needs in radiation protection for the public, occupationally exposed people, patients in medicine, and the environment. CONCERT will support the implementation of the revised European Basic Safety Standards by giving the best possible advice based on evidence from research.

The aim of CONCERT is to set in motion the convergence of the three focusing forces – scientific community, national agencies and research institutions – and EURATOM policies in order to achieve new breakthroughs in radiation protection research. CONCERT strives for a better integration of the radiation protection scientific community at EU level, leading to a better coordination of research efforts and provision of more consolidated and robust science based policy recommendations to decision makers in this area. In the long-term, these efforts will translate into additional or improved practical measures in view of the effective protection of people and the environment.

CONCERT has the mission to further reduce uncertainties in the assessment and management of radiation risks to the environment and humans by targeted science. To achieve this, CONCERT will initiate an open exchange of knowledge and information between science, regulation and society. With this purpose, two major open RTD calls of approximately 10,000,000 € in spring 2016 and 7,000,000 € in spring 2017, respectively, have been launched. Universities and research institutes from all over Europe have the opportunity to join in research consortia and submit proposals.

CONCERT is open to new national Programme Owners and Programme Managers at all times.

<http://www.concert-h2020.eu/en/Publications>

### A.3. EUROPEAN AGENCY FOR SAFETY AND HEALTH AT WORK



LEADER	PROJECT	DURATION
<b>Ferenc Kudász, MD (National Public Health Institute, Budapest, Hungary)</b>	<b>Good practice case study on dangerous substances</b>	<b>2017–2018</b>

#### ASSOCIATES

IMROH: J. Macan, Ž. Babić, Zr. Franić, F. Šakić, M. Deranja

#### SUMMARY

Collaboration with the Hungarian National Public Health Institute was initiated within the project between the European Occupational Safety and Health Agency (EU OSHA) and its Hungarian counterpart. IMROH signed a subcontract with the Hungarian partner, and collaboration is executed through two case studies of good practice in safety at work with chemicals in Croatian companies. The implementation of the study is in progress.

**A.4. EUROPEAN ACADEMY OF DERMATOLOGY AND VENEROLOGY**European Academy of  
Dermatology and Venereology

LEADER	PROJECT	DURATION
<b>Prof Swen Malte John (Universität Osnabrück, Njemačka)</b>	<b>Joint scientific implementation and evaluation of the Healthy Skin@Work Campaign, subcampaign Skin Cancer: Safe Work Under the Sun</b>	<b>Jun 2017– Oct 2017</b>

**ASSOCIATES**

IMROH: J. Macan, Ž. Babić, Zr. Franić, F. Šakić, M. Deranja

**SUMMARY**

Cooperation with the European Academy for Dermatology and Venereology (EADV) was made through the participation in EADV project no. 18 "Joint scientific implementation and evaluation of the Healthy Skin@Work Campaign", subcampaign "Skin Cancer: Safe Work Under the Sun". Research was carried out with the German partners in the period from 1 Jun to 31 Oct 2017 in the form of solar UV irradiation personal dosimetry at the workplaces of masons. Five masons from the vicinity of Zagreb carried out measurements on each working day with equipment provided by the German partner. Data analysis and interpretation are in progress. This project involves partners from Germany, Croatia, Romania, Denmark, Italy, and Spain.

**A.5. EU COST ACTION PROGRAMME (9 projects)**EUROPEAN COOPERATION  
IN SCIENCE AND TECHNOLOGY

DiMoPEX



LEADER	PROJECT	DURATION
<b>Prof Lygia Therese Budnik, PhD (University Medical Center Hamburg – Eppendorf, Germany)</b>	<b>Diagnosis, Monitoring and Prevention of Exposure-Related Noncommunicable Diseases (DiMoPEX, CA15129)</b>	<b>2016–2019</b>

**ASSOCIATES**

IMROH: J. Macan (Management Committee member), Ž. Babić, Zr. Franić, J. Kovačić, R. Turk, V. M. Varnai

**SUMMARY**

DiMoPEX represents an opportunity for interdisciplinary collaboration between scientists in the field of chronic non-communicable diseases caused by environmental factors. Furthermore, the project is dedicated to increase the interest of young scientists for this research field, especially for the different aspects of exposition to environmental factors. During 2017, an associate of our Occupational and Environmental Medicine Unit took part in a course designed for doctoral students with the topic of meta-analysis methodology. A meeting of all project associates was held in Oct 2017, and the Unit's associates presented two posters covering the topic of risk communication based on the results of an epidemiology study. They also presented potential new research topics (285, 306).



LEADER	PROJECT	DURATION
<b>Prof Mustapha Cherkaoui Malki (Universite Dijon Bourgogne, Dijon, France)</b>	<b>Personalized nutrition in aging society: redox control of major age-related diseases (NutRedOx, CA16112)</b>	<b>2017–2021</b>

#### ASSOCIATES

IMI: M. Gerić, V. Garaj-Vrhovac (Management Committee members)  
Partners: 110 partners from 33 European countries and Armenia, Georgia, Algeria, Morocco, Ukraine, Albania

#### SUMMARY

The importance of a healthy ageing process becomes apparent when considering that: (a) the generation 50+ (G50+) already has a share in population of around one third across Europe, with obvious regional variations, (b) this share is likely to increase further in the future, and (c) vitality at older age is not only an important measure of quality of life but also key to participation and productivity. The theme “nutrition and ageing” has many different aspects and poses numerous challenges, which provide a fertile ground for many research themes and networks. Among them, the NutRedOx network will focus on the impact of redox active compounds in food on healthy ageing, chemoprevention, and redox control in the context of major age-related diseases. The main aim of the NutRedOx network is the gathering of experts from across Europe, including other Mediterranean countries, from different disciplines involved in the study of biological redox active food components and relevant to the ageing organism, its health, function, and vulnerability to disease. Together, these experts will form a major and sustainable EU-wide cluster in the form of the “NutRedOx Centre of Excellence” able to address the topic from different perspectives, with the long-term aim of providing a scientific basis for (improved) nutritional and lifestyle habits (279), to train the next generation of multidisciplinary studies in this field, to raise awareness of such habits among the wider population, and to engage with industry to develop age-adequate foods and medicines.



LEADER	PROJECT	DURATION
<b>Prof Andrew Collins (Institute of Basic Medical Sciences, University of Oslo, Norway)</b>	<b>The comet assay as a human biomonitoring tool (hCOMET, CA 15132)</b>	<b>2016–2020</b>

#### ASSOCIATES

IMROH: G. Gajski (Management Committee member), M. Milić (Management Committee member, Core Group member, WG 1 leader, responsible for the website of the network)  
Partners: 66 partners from 23 European countries, India and Cuba

#### SUMMARY

Many human biomonitoring studies have used the comet assay to measure DNA damage. In most cases, the assay is applied to peripheral blood mononuclear cells. Results from relatively small individual studies are often inconsistent and it is advantageous to carry out a pooled analysis of combined data from all available studies. hCOMET will be a network comprising researchers active in human biomonitoring with this assay. Results supplied by these researchers will be compiled as a single database representing a large number of individual DNA damage measurements. The pooled analysis will allow us to determine which factors affect DNA damage, and to what extent. In addition, hCOMET will address the issue of interlaboratory reproducibility of the assay by devising standard protocols so that in the future the comparison of results from different studies is facilitated (<http://www.hcomet.org>).

In 2017, the collection of database research results was completed and preliminary analyses were carried out. Two scientific papers were published, one co-authored by G. Gajski (77).

This project has also organized the International Congress/Workshop in Spain, Navarra, University of Navarre - ICAW 2017 (International Comet Assay Workshop) 29 – 31 Aug 2017 with 4 poster presentations

and 5 oral presentations of invited lecturers. One of the invited lectures was M. Milić (293). As part of ICAW 2017, 9 abstracts were published in the Book of Abstracts; one abstract by M. Milić (295). The project also held a workshop for those who wanted to learn methods in the alkaline comet assay – “The Basic Training Course for Comet Assay and DNA Repair”, Oslo, 8 - 11 Mar 2017, Norwegian Institute of Public Health, Oslo, Norway, with two different modules: DNA repair module (10 participants) and DNA damage module (7 participants). We also released a new STSM Contest and a Contest for a New Course of Statistic Analysis in Comet Tests that will be held in February of 2018 in Rome, Italy. As many as 24 participants were selected to be sponsored for the entire workshop, transportation, and accommodation and one of the lecturers is M. Milić. As part of Working Group 6, G. Gajski is preparing two papers that should be completed by the beginning of 2018.



LEADER	PROJECT	DURATION
<b>Francy Crijns, PhD (Zuyd University of Applied Sciences, Heerlen, Netherlands)</b>	<b>Anti-Microbial Coating Innovations to prevent infectious diseases (AMICI, CA15114)</b>	<b>2016–2020</b>

#### ASSOCIATES

IMROH: I. Vinković Vrček (substitute for a member of the Management Committee)

#### SUMMARY

A Management Committee meeting was held at the Satakunta University of Applied Sciences, Pori, Finland from 6 to 7 Jun 2017. During the meeting, the AMICI Opinion paper on antimicrobial coatings in healthcare systems (1) was presented. The Core Group and Working Group 5 meetings were held in Tallin, Estonia from 15 to 17 Nov 2017, when a decision was made regarding the annual AMICI conference in 2018, which will be held in Zagreb, Croatia.



LEADER	PROJECT	RAZDOBLJE
<b>Hans-Heiner Gorris, PhD (Universität Regensburg, Regensburg, Germany)</b>	<b>The European upconversion network – from the design of photon-upconverting nanomaterials to biomedical applications (UPCON, CM1403)</b>	<b>2014–2018</b>

#### ASSOCIATES

IMROH: I. Vinković Vrček (member of the Management Committee)

#### SUMMARY

A Management Committee meeting was held in Aveiro, Portugal from 29 Jun to 1 Jul 2017. During the meeting of Working group 5 *Toxicity*, a plan for the preparation of the UPCON Opinion paper on toxicity testing of upconverting nanomaterials was set up.



LEADER	PROJECT	DURATION
<b>Dr Theo M. Luijder (Erasmus Universitair Medisch Centrum Rotterdam, Rotterdam, the Netherlands)</b>	<b>“Good biomarker practice” to increase the number of clinically validated biomarkers (CliniMARK, CA16113)</b>	<b>2017–2021</b>

#### ASSOCIATES

IMROH: G. Gajski (Management Committee member)

Partners: 80 partners from 28 European countries

#### SUMMARY

Thousands of circulating proteins have been shown to be hallmarks of emerging disease, response to treatment, or a patients' prognosis. The identification of these small molecule biomarkers holds great promise for significant improvement of personalized medicine based on simple blood tests. For instance, diagnosis and prognosis with biomarkers [e.g., carcinoembryonic antigen (CEA)] has significantly improved patient survival and decreased healthcare costs in colorectal cancer patients. Unfortunately, despite significant investments to increase the number of biomarker studies, only ~150 out of thousands of identified biomarkers has currently been implemented in clinical practice. This is mainly caused by the time-consuming process of reliably detecting biomarkers, the irreproducibility of studies that determine a biomarker's clinical value, and by a mismatch in studies performed by academia and what is required for regulatory and market approval. To increase the number of clinically validated biomarkers, rather than further increasing the number of biomarker discovery studies, CliniMARK will improve the quality and reproducibility of studies and establish a coherent biomarker development pipeline from discovery to market introduction.



LEADER	PROJECT	DURATION
<b>Prof Swen Malte John (Universität Osnabrück, Germany)</b>	<b>Development and Implementation of European Standards on Prevention of Occupational Skin Diseases (StanDerm, TD1206)</b>	<b>2013–2017</b>

#### ASSOCIATES

IMROH: J. Macan (member of the Management Committee), Ž. Babić

#### SUMMARY

The project completed in May 2017. It gathered experts from 31 countries with the aim of developing and implementing European standards on the prevention of occupational skin diseases. Associates of the Unit actively collaborated in the project as members of the Management Committee, as well as members in work groups dedicated to the development of European standards. They also participated in the design of intervention studies linked to occupational contact dermatitis and occupational skin carcinoma. In 2017, a joint publication on the status of the recognition process of contact dermatitis as occupational disease in EU countries was published (35). As the main project result, a position paper was published on minimal standards in prevention, diagnosis, and treatment of occupational skin diseases in Europe, which was agreed on by all StanDerm project associates (2). With the project's support, a study on skin health and safety at work in beautician apprentices was conducted in a vocational school for personal services and a publication was published (34). More information: <http://www.standerem.eu/>.



LEADER	PROJECT	DURATION
<b>Dr Ingrid Sivesind Mehlum (National Institute of Occupational Health, Oslo, Norway)</b>	<b>Network on the coordination and harmonisation of european occupational cohorts (OMEGA-NET, CA16216)</b>	<b>2017–2021</b>

#### ASSOCIATES

IMROH: J. Macan, V. M. Varnai (Management Committee members)

#### SUMMARY

The main aim is to establish a network in order to optimize the usage of cohort from working and general population in Europe. The aims of the OMEGA-NET project are the promotion of collaboration between existing cohort studies, gathering information on employment and occupational exposure, coordination and harmonization studies on exposure assessment in the working population, and the promotion of integrative strategies for studies regarding workers' health in Europe. The promotion of evidence-based preventive strategies directed to health at work is expected. The first meeting of the Management Committee was held on 26 Oct 2017 at the COST Association, Brussels, Belgium.



LEADER	PROJECT	DURATION
<b>Prof Wouter Schroyers, PhD (UHasselt University Belgium, Diepenbeek, Belgium)</b>	<b>NORM for building materials (NORM4BUILDING, TU 1301)</b>	<b>2013–2017</b>

#### ASSOCIATES

IMROH: I. Prlić (national coordinator), T. Bituh, M. Surić Mihčić

Partners: 30 partners from 26 European countries

#### SUMMARY

Naturally occurring radionuclides are present in the earth's crust and the minerals and ores that are then used in industry. Throughout industrial processes, naturally occurring radionuclides can become concentrated in by-products, such as fly ash produced in large quantities from coal burning, slags from steelworks and metal recycling industries, phosphogypsum from the phosphate industry, and red mud from the aluminium processing industry. Depending on the activity concentration, some of these by-products can be considered naturally occurring radioactive materials (NORM).

When by-products are investigated for use in construction materials, then many factors have to be evaluated, taking into account the presence of trace elements such as metals and naturally occurring radionuclides. The study of the safe use of by-products in construction requires the involvement of experts from many different fields (construction and chemical engineering, environmental sciences, natural radioactivity, economics, etc.). The COST Action NORM4Building brings together a large variety of experts with different backgrounds in order to handle this type of multidisciplinary research. In total, the NORM4Building network currently (Jan 2017) comprises more than 120 researchers, covering very different fields, from 30 different European countries plus the United States. The main objective of the COST Action TU1301 is the exchange of multidisciplinary knowledge and experience (radiological, technical, economical, legislative, and ecological) in order to investigate and evaluate the use of by-products from industries that encounter NORM in their industrial processes. An important focus is the radiological evaluation of the use of by-products in new types of construction materials that are currently under research. The NORM4Building network develops strategies for the use of NORM residues in ceramics, concrete, and cement with a focus on the use of NORM residues in emerging building materials such as alkali-activated materials (i.e., inorganic polymers). The COST Action NORM4Building has prompted a lot of research in a field where information was lacking to assure that aspects regarding natural radioactivity are taking into consideration for new residue-based construction materials before they are introduced in the market.

The COST network consists of four Working groups and their specific actions:

- Working group 1: developed of a NORM4Building database with radiological information on raw materials, by-products, and construction materials
- Working group 2: provided a more in-depth discussion on the properties of the by-products that can enable or hinder the use in construction materials
- Working group 3: investigated industrially useful measurement methodologies and protocols for the determination of the activity concentration of naturally occurring radionuclides in construction materials; also organized intercomparisons
- Working group 4: developed new research on computational methodologies – room models to evaluate and predict indoor gamma dose rates and indoor radon concentrations on the basis of the activity concentration, considering the leaching aspects of naturally occurring radionuclides from construction materials to evaluate the end of life of NORM containing construction materials. The basis for the radiological evaluation of building materials is the new Council Directive 2013/59/EURATOM, laying down basic safety standards for protection against the dangers arising from exposure to ionizing radiation.

The results of these activities are published in the book: “Naturally Occurring Radioactive Materials in Construction”, Integrating Radiation Protection in Reuse (COST Action Tu1301 NORM4BUILDING); ISBN: 9780081020081 (e-version), 9780081020098 (hard copy) (173).

The collaboration of the research community on COST NORM4Building project resulted in the establishment of two new research platforms: EAN-NORM and EU-NORM (<http://ean-norm.eu/ena/>). More information regarding the project: <http://norm4building.org/>.

## A.6. INTERNATIONAL ATOMIC ENERGY AGENCY



LEADER	PROJECT	DURATION
<b>Roman Padilla Alvarez, PhD</b> <b>(Department of Nuclear Sciences and Applications, IAEA Laboratories Seibersdorf, Austria)</b>	<b>Apportioning air pollution sources on a regional scale (RER/1/015)</b>	<b>2016–2017</b>

### ASSOCIATES

IMROH: I. Bešlić, S. Davila, R. Godec

### SUMMARY

Collection by referent method HREN 12341:2014 of PM<sub>2.5</sub> particle fraction samples at one urban background site, every three days, continued and has finally finished as planned. During the period 4 Mar 2016 – 20 Mar 2017, 133 daily samples were collected. PTFE filters with a pore size of 3 μm and reference materials for sample analysis delivered by IAEA were used. Samples were analysed for elemental composition by ED-XRF method, as well for total, elemental, and organic carbon content by TOT method. After the samples were analysed, source apportionment procedure started and is still in progress. A data file in proposed format was prepared for upload to the project database in Sofia, Bulgaria. For the purpose of the project, 113 samples collected in Tirana, Albania were analysed at the Environmental Hygiene Unit for their reflection in order to calculate black carbon content. S. Davila participated at the: Regional Training Course on Advanced Methods in Positive Matrix Factorization (PMF) and Potential Source Contribution Functions (PSCF) under the framework of TC Project RER/1/015 – “Apportioning Air Pollution Sources on a Regional Scale”, held in Lisbon, Portugal from 13 to 17 Nov 2017.

## A.7. MINISTRY OF ENVIRONMENTAL PROTECTION OF MEXICO



LEADER	PROJECT	DURATION
<b>Rafael Valencia, PhD (Universidad Autónoma de Tlaxcala, Universidad Nacional Autónoma de México)</b>	<b>El proyecto general es evaluación del riesgo genotóxico por exposición a contaminantes ambientales</b>	<b>2016–2020</b>

### ASSOCIATES

IMROH: M Milić

### SUMMARY

The Mexican colleagues within the project introduced new methods in their laboratory throughout 2017, including a comet assay on lymphocytes and buccal cells, a micronucleus cytome test on lymphocytes, and analysis of cholinesterase activity, all in preparation for 2018 when we should start including people exposed to pesticides into studies.

## A.8. POLYTECHNIC INSTITUTE OF LISBON, PORTUGAL



LEADER	PROJECT	DURATION
<b>Dr Susana Viegas (Lisbon School of Health Technology, Polytechnic Institute of Lisbon, Lisbon, Portugal)</b>	<b>Occupational exposure to cytotoxic agents in veterinary hospitals and clinics (CytoVet)</b>	<b>2017–2019</b>

### ASSOCIATES

IMI: G. Gajski  
Izvan Instituta: C. Ladeira

### SUMMARY

The CytoVet project will provide experimental data that should enable the prediction of adverse effects and risk assessment for exposed workers in veterinary hospitals and clinics. The project will answer whether exposure to cytotoxic agents might pose a risk to human health in occupational settings (66).

## A.9. EU STRATEGY FOR THE DANUBE REGION



LEADER	PROJECT	DURATION
<b>Prof Verena Winiwarter, PhD (Institut für Soziale Ökologie, Alpen-Adria-Universität Klagenfurt, Austria)</b>	<b>Danube: Future Project – A Sustainable Future for the Danube River Basin as a Challenge for the Interdisciplinary Humanities</b>	<b>2013–2020</b>

### ASSOCIATES

IMROH: G. Gajski

### SUMMARY

Danube: Future aims at developing interdisciplinary research and education in the Danube River Basin (DRB) simultaneously as a basis for the solution of pressing environmental issues and a sustainable future of the region. Danube: Future is a multi-year program that consists of three modules: core, capacity building, and sustainability related research with a long-term socio-ecological component. Danube: Future is a unique combination of regional, national, and supra-national initiatives in interdisciplinary sustainability research with training and capacity building. It contributes to the sustainable development of the DRB with a particular focus on the contribution of humanities.

## A.10. SCIENTIFIC AND RESEARCH BILATERAL COOPERATION IN SCIENCE AND TECHNOLOGY (3 projects)



LEADERS	PROJECT	DURATION
<b>Mirta Milić, PhD (IMROH)</b> <b>Prof Walter Gössler, PhD (Institut für Chemie, Karl-Franzens-Universität Graz, Austria)</b>	<b>Potential new non-invasive biomarkers of chronic exposure to arsenic</b>	<b>2016–2017</b>

### ASSOCIATES

IMROH: A. M. Marjanović Čermak, I. Pavičić, I. Vinković Vrček  
 External associates: S. Bräuer

### SUMMARY

This year, we completed a two-year bilateral project. A summary of the work was published at the European Toxicologist Congress (EUROTOX) held in Bratislava in a Book of abstracts (263) and two other summaries were applied for the World Congress of Toxicologists to be held in Belgrade, Serbia in April 2018. It has been shown that the concentration of arsenic can be measured in buccal cells, which has not been published or done so far. Results on the exposed individuals compared to control samples showed higher concentrations of arsenic and its metabolites in urine, which exceeded  $10 \mu\text{g L}^{-1}$  and in some individuals  $100 \mu\text{g L}^{-1}$ . There were higher frequencies of micronucleus, cells with nuclear buds, and cells with so-called broken egg structures. Binuclear, basal, and apoptotic buccal cells in the exposed individuals, as well as drinking water samples showed arsenic values at concentrations ranging from  $34.9 - 41.2 \mu\text{g L}^{-1}$  in the exposed areas. Scientific papers with research results are under preparation.



LEADERS	PROJECT	DURATION
<b>Anita Bosak, PhD (IMROH)</b> <b>Prof Aljoša Bavec, PhD (Inštitut za biokemijo, Medicinska fakulteta, Univerza v Ljubljani)</b>	<b>Kinetic evaluation of PON1 interactions with pharmacologically active carbamates</b>	<b>2016–2017</b>

### ASSOCIATES

IMROH: M. Katalinić, Z. Kovarik, N. Maraković, G. Šinko, T. Zorbaz  
 External associates: M. Goličnik, T. Marš, K. Miš, S. Pirkmajer, J. Stojan

### SUMMARY

Kinetic constants describing the effect of a number of pharmacologically important carbamates on arylesterase activity PON1 and an *in silico* analysis of PON1 and carbamate interactions by molecular modelling was performed (274).



MINISTARSTVO ZNANOSTI  
I OBRAZOVANJA  
REPUBLIKE HRVATSKE



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LEADERS	PROJECT	DURATION
<b>Ante Miličević, PhD (IMROH)</b> <b>Prof Svetlana Marković, PhD (Faculty of Science, University of Kragujevac, Serbia)</b>	<b>Investigation of chemistry and antioxidant activity of polyphenolic compounds with essential elements</b>	<b>2016–2017</b>
<b>ASSOCIATES</b>		
IMROH: N. Raos External associates: B. Lučić, S. Roca and D. Vikić-Topić (Ruđer Bošković Institute); N. Bregović and V. Tomišić (Faculty of Science, Zagreb)		
<b>SUMMARY</b>		
<p>In the second year of the project, we proceeded with work on the previously synthesized complexes. The obtained UV-Vis and IR spectra and stoichiometry and stability constants of complexes (investigated by UV-Vis and NMR titrations) were used for modelling the structures of complexes by DFT computational method. The results will be published within scientific papers. In relation to the theme of the project, we published two scientific papers (42, 43) and two abstracts from international scientific meetings (237, 302).</p>		

**B. PROFESSIONAL PROJECTS**

PROJECT	CONTRACTOR	LEADER
<b>Environmental Hygiene Unit</b>		
GEMS/AIR – Global Environment Monitoring System (WHO/UNEP) Programme, City Air Quality Trends, Coordination for Croatia (since 1973)	World Health Organization (WHO)/ United Nations Environment Programme (UNEP)	V. Vađić (1973–2014), G. Pehneć (since 2015)
The Danube Air Nexus (DAN), EC-JRC Project (since 2013)	Joint Research Centre	K. Šega
<b>Radiation Dosimetry and Radiobiology Unit</b>		
EAN NORM; European ALARA Network for Naturally Occurring Radioactive Materials	Project Coordinator IAF	I. Prlić
Contract no. TREN/H4/51/2005 of the European Commission (EC) (since 2005)	Radioökologie GmbH, Dresden, Germany	I. Prlić
IAEA Technical Cooperation Project CRO/3/002 – Establishing a national radioactive waste storage and processing facility. WP 2: Establishment, implementation and supervision of an electronic system for monitoring the flow of low level radioactive medical materials within a health-care institution, from their delivery through usage to their safe disposal (since 2009)	IAEA	I. Prlić
IMI_FondNEK: Radiological monitoring programme for extended location of Radioactive Waste Storage Facility (study, 2016)	Fund for financing the decommissioning of the Krško Nuclear Power Plant and the disposal of NEK radioactive waste and spent nuclear fuel	I. Prlić

## 4. PROFESSIONAL UNITS



### 4.1. Laboratory Animal Breeding Unit

#### EMPLOYEES

##### HEAD

Vedran Micek, DVM, professional associate

##### ASSOCIATE

Mirjana Mataušić Pišl, PhD, DVM

##### TECHNICAL STAFF

Kata Šmaguc, technician

#### ACTIVITY

The Laboratory Animal Unit of the Institute breeds laboratory rats, strain HsdBrIHan: Wistar, in accordance with the Animal Welfare Act (OG 102/17) and other applicable laws, guidelines, and policies. Animals are bred under strictly controlled conditions, under surveillance of authorised personnel (DVM), and then used as a model in experimental research. The Unit has facilities consistent with legislation and guidelines concerning the breeding and housing of laboratory animals.

From 2016, the Laboratory Animal unit is authorized for performing *in vivo* experiments for a ten-year period. The living conditions of animals are appropriate and contribute to their health and welfare. The housing, feeding, animal care, and experimental procedures are managed by a veterinarian in accordance with contemporary veterinary practices. The animals are kept in steady-state micro environmental conditions and fed with standard GLP certified laboratory food and water *ad libitum* with altering 12 h light and dark cycles. Sanitation of facilities is performed on a weekly basis in order to reduce the possibility of any external contamination. A health monitoring program, which includes checks for zoonoses, is regularly performed in collaboration with the National Veterinary Institute.

In the context of projects funded by the Croatian Science Foundation, scheduled *in vivo* experiments were performed for four projects: AGEMETAR and OPENTOX (Chapter 16.1.A.1.), MycotoxA (Chapter 16.1.B.1.), and dislipiDHA - *Nutritive modulation of docosahexaenoic acid in diabetic dyslipidemia* (45, 62, 81, 264, 266, 280, 292). One original article (32) and two conference abstracts (238, 304) were published in collaboration with external partners (Department of Biotechnology University of Rijeka and National Veterinary Institute, Zagreb).



## 4.2. Poison Control Centre

### EMPLOYEES

#### HEAD

Rajka Turk, MSc, research advisor in science

#### ASSOCIATES

Researchers of the Occupational and Environmental Health Unit (Chapter 2.5)

### ACTIVITY

The information service of the Poison Control Centre received 2,150 calls from health institutions and professionals in Croatia regarding acute poisoning incidents. Following requests from the industry, 148 toxicological evaluations were prepared as well as 28 reports for the registration of pesticides according to the Plant Protection Products Act and Regulation (EU) No. 1107/2009 on placing of plant protection products on the market. Following enquiries from the industry, 62 evaluations for the purpose of biocidal products authorization according to the Biocidal products Act and Regulation (EU) No. 528/2012 concerning the placement on the market and use of biocidal products.

Collaboration with the Agency for Medicinal Products and Medical Devices of Croatia in monitoring of drug poisonings (pharmacovigilance) continued. In collaboration with the Croatian Institute for Emergency Medicine and Croatian Institute for Toxicology and Antidoping, we finalized the Guidelines for Emergency Medical Services in Case of Chemical Accidents. Further work on the Phytosanitary Information System was continued with the Ministry of Agriculture.

Annual reports of the Poison Control Centre continued to be published in the journal *Archives of Industrial Hygiene and Toxicology* in English and Croatian (186). A professional paper on occupational poisonings recorded at the Poison Control Centre in 2016 was also published (108) and presented at the occupational medicine and sport specialists meeting. A popular articles on the harmful effects of chemicals were published in the popular science publications (110, 165). Lectures on toxic effects of drugs were delivered to biology/chemistry and public health teachers via meetings of County Professional Teacher Councils and in collaboration with the Agency for Vocational Education and Training and Adult Education (Chapter 11.B.1.). Publication of short communications for the general public on recent poisoning incidents and their prevention was initiated at the Institute's website (195-198).

## 5. RESEARCH AREA "ŠUMBAR"

### ● HEAD

Josip Tončić, MSc, DVM, professional associate in science

### ● ACTIVITY

The Research Area "Šumbar" is located east of the city of Karlovac (GPS coordinates: 45.5297, 15.6322) with a zone of 2,153 ha mainly covered by an English oak (*Quercus robur*) and hornbeam (*Carpinus betulus*) forest. "Šumbar" is a unique ecosystem in which the activities of safeguarding, control, and improvement of the habitat's stability are undertaken. Within the scope of these activities, a very important activity is the environmental study of water, soil, air, and biological material, which is related to natural and anthropogenic environmental pollution and the main goal is to preserve a healthy habitat.

A background ionising radiation monitoring RS 131 HP Ionization Chamber: Reuter Stokes type was performed continuously. The entire measuring system is connected to the telecommunication system, which allows real-time online data monitoring. Measurements were performed with mobile ALARA devices at various microlocations. All collected data were later processed, evaluated, and correlated in the Radiation Dosimetry and Radiobiology Unit. Part of the proposed activities in the scope of the Horizon 2020 EUROATOM (Integrating Radiation Protection Research in the European Union) call ref: NFRP-07-2015 programme was also carried out at "Šumbar".

Measurements with HORIBA APNA-360 (Ambient NO<sub>x</sub> Monitor), HORIBA APOA-360 (Ambient O<sub>3</sub> Monitor) and Sven Leckel Sequential Sampler SEQ47/50, which measure NO, NO<sub>2</sub>, NO<sub>x</sub>, O<sub>3</sub> and PM<sub>2.5</sub> were continued within the scope of the programme of monitoring air pollution and quality.

## 6. COMPANY OWNED BY THE INSTITUTE

### **Occupational Health Polyclinic of the Institute for Medical Research and Occupational Health Ltd., Ksaverska cesta 2, Zagreb**

#### **DIRECTOR**

Prim Jelena Macan, PhD, MD, permanent scientific advisor (90 % of working hours at the Institute, 10 % at the Polyclinic)

#### **ASSOCIATE**

Franka Šakić, senior technician (90 % of working hours at the Institute, 10 % at the Polyclinic)

#### **BUSINESS RESULTS**

The professional activity of the Polyclinic continued operating in 2017 providing services in the domain of occupational and sports medicine, and internal medicine. The outpatient clinic provided a total of 144 medical services for 96 customers. Also, 14 professional opinions and 23 work ability assessments were delivered as part of first and control medical examinations. Two occupational diseases were reported. An occupational medicine specialist delivered 14 judicial-medical expert opinions for the Administrative Court in Zagreb. The Psychotherapy Office led by A. Bjelajac, PhD, psychologist and psychotherapist, started working within the company in Sep 2017. The company operated positively in 2017.

## 7. PUBLISHING

The Institute is the publisher of the scientific journal *Arhiv za higijenu rada i toksikologiju - Archives of Industrial Hygiene and Toxicology* [ISSN 0004-1254 (print), ISSN 1848-6312 (online)].

The *Archives* is the official journal of four Croatian and one Slovenian association, and is a regular member of the Committee on Publication Ethics (COPE). The Editorial staff are members of the Mediterranean Editors and Translators and European Association of Science Editors. Articles from the fields of occupational health, toxicology, ecology, chemistry, biochemistry, biology, pharmacology, and psychology are edited in line with modern standards. The journal publishing is financially supported by the Ministry of Science and Education and through subscriptions. The *Archives* is issued four times a year.

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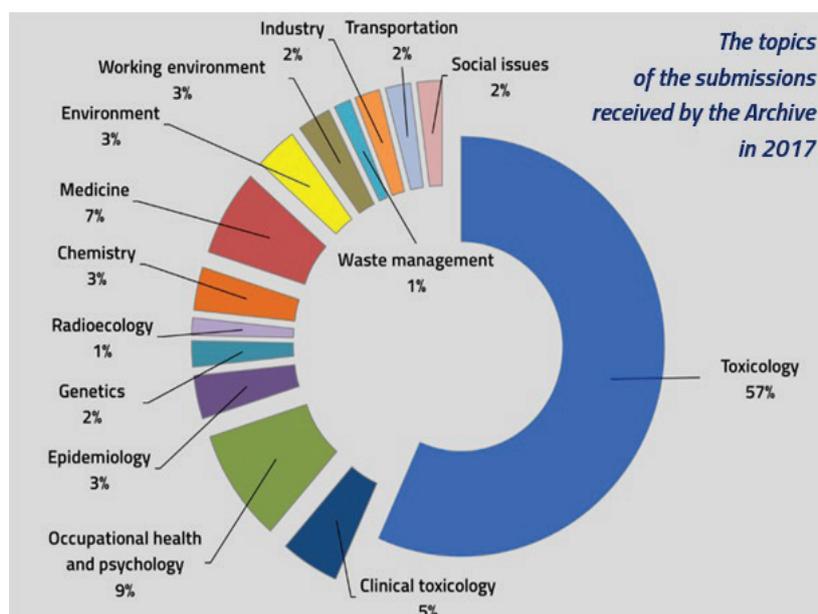
General information about the journal *Arhiv za higijenu rada i toksikologiju - Archives of Industrial Hygiene and Toxicology*

The *Archives* is indexed in SCI-Expanded, Medline/PubMed, Scopus, and 18 other databases, and is currently ranked within the third quartile (Q3) of the *Public, Environmental & Occupational Health* category and the fourth quartile (Q4) of the *Toxicology* category (source: InCites Journal Citation Reports®, Jun 2017). The Impact Factor for 2016 is 1.395, which ranks it fifth overall among Croatian journals, and its 5-year IF is 1.320. The h-index of the *Archives* at the end of 2017 was 22.

During 2017, the Editorial Office of the *Archives* received a total of 150 submissions, most of which were submitted through the journal's online system available at <https://arhiv.imi.hr> and the remainder by e-mail ([arhiv@imi.hr](mailto:arhiv@imi.hr)). The most submissions were original articles (79 %), while the other contributions were review papers (12 %), case reports (7 %), and letter to the Editor or notes (1 %). The submissions were made by authors from 29 different countries.

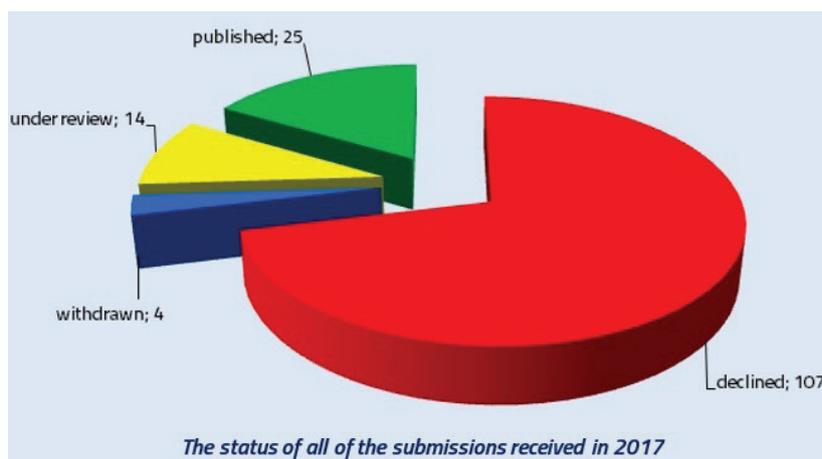


More than two thirds of submissions covered topics from toxicology and clinical toxicology, while occupational health and psychology followed with a somewhat smaller number of submissions. Each submission is screened for plagiarism by the iThenticate Plagiarism Detection Software. The contained Crossref Similarity Check is used to check the authenticity of a submission against a vast database of scientific literature published worldwide. Access to the aforementioned software system is enabled through the journal's cooperation with its online publisher De Gruyter Open. Only a low share of papers was detected to contain a significant amount of plagiarised text/data (less than 10 % of submissions).



The Editor in Chief has sent a total of 359 invitations to review to researchers and professionals working in relevant fields, both from domestic institutions and international. More than one third of invitations (39 %) resulted in an obtained reviewer's opinion (five invitations on average per reviewed submission).

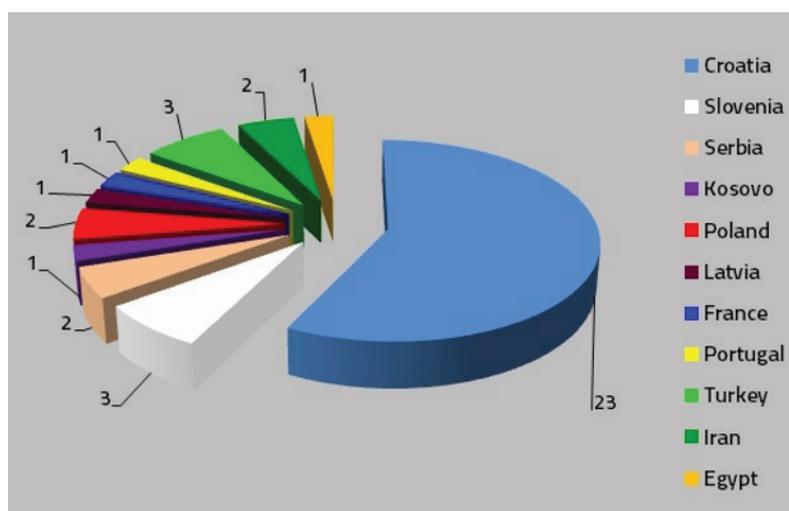
Of the total number of received submissions, rejections on grounds of insufficient quality were made on 107 occasions, while a smaller number of submissions was withdrawn by the authors. The decision to reject was made at the level of the Editorial Office for over two thirds of rejected submissions, while the remainder was rejected following negative reviews. Considering only the submissions processed and decided upon in 2017 ( $n = 136$ ), the rejection rate amounts to a high 82 %. A part of the received submissions was published in regular issues over the year.



Over the course of the year, four regular issues of Volume no. 68 were published, containing 40 articles (73 % of original articles, 23 % of reviews, and 4 % of letters to the Editor or technical papers) and 7 other document types: *New editions, In memoriam, Announcements, Reports, and News*. The last issue of the Volume also published abstracts from the Inter-County Professional Meeting of Healthcare Educators "Challenges in the Medical Nurse and Technician Professions from the Perspective of Occupational Health and Radiation Dosimetry" held at the Institute (24 Nov 2017). The largest number of authors among those submissions was from Croatia and other European countries.



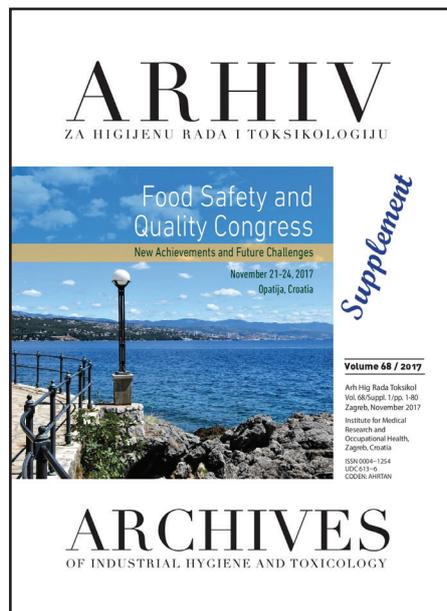
*The cover pages of the regular Archives' issues published in 2017*



*Distribution of articles published in 2017 by country of author*

In November 2017, a Supplement issue was published comprising 101 abstracts from the Food Safety and Quality Congress with international participation "New Achievements and Future Challenges", held in Opatija (21 - 24 Nov 2017). The publication of the Supplement was funded by the Andrija Štampar Teaching Institute of Public Health, Zagreb. The abstracts were copyedited by Ž. Pavlaković and M. Herman, while the layout and technical editing was done by M. Herman.

The journal's editors work continuously to promote the reputation of the journal domestically and internationally, which includes contacts with researchers and professional associations.



Activities regarding the practical education of authors who publish in the *Archives* were continued. The journal's editors also participate in the postgraduate specialisation study in occupational and sports medicine for medical doctors held at the Institute. Within the course "Professional Diseases, Occupationally Related Diseases and Professional Toxicology" the workshop "How to write and publish a good case report or series" (lecturers: J. Macan, D. Čakalo, I. Brčić Karačonji, and N. Kopjar). During 2017, the journal's website and online submission system were redesigned (<https://arhiv.imi.hr>), as was the *Archives'* website within the Institute's homepage (<https://www.imi.hr/en/publishing/>).

The entirety of the *Archives'* old volumes was scanned and made available at the Portal of Scientific Journals of the Republic of Croatia - HRČAK, which has increased the visibility of our journal and made it even more accessible to

the domestic and international scientific community. By clicking the link <https://hrcak.srce.hr/aiht>, visitors can view our journal's contents as far back as 1946, the first year of its publication. Full text articles are also available on website: <https://www.degruyter.com/view/j/aiht>.

## 8. Publications by the Institute's employees

CATEGORY	TOTAL
<b>8.1. Original articles, reviews, and professional papers (+ accepted for 2018)</b>	<b>127 (+ 35)</b>
Indexed in WoS	70
Indexed in WoS and accepted for publication in 2018	35
Indexed in other databases	4
Articles in non-indexed journals	29
Articles from meetings held in Croatia	22
Articles from meetings held abroad	2
<b>8.2. Books and journals (+ accepted for 2018)</b>	<b>21 (+ 2)</b>
Author/editor of a publication	2
Articles/chapters in a publication	15
Articles/chapters in a publication accepted for 2018	2
Journal or book of abstracts editor	4
<b>8.3. Other publications</b>	<b>13</b>
Printed editions	9
Online editions	4
<b>8.4. Theses</b>	<b>13</b>
Authored by the Institute's employees	6
Authored by candidates with mentors/co-mentors from the Institute	7
<b>8.5. Abstracts from meetings held in Croatia</b>	<b>43</b>
Indexed in WoS	6
Abstracts in other journals and books of abstracts	36
Abstracts in online editions	1
<b>8.6. Abstracts from meetings held abroad</b>	<b>59</b>
Indexed in WoS	19
Abstracts in other journals and books of abstracts	37
Abstracts in online editions	3
<b>8.7. Professional reports</b>	<b>26</b>
National projects, contracts, and partnerships	25
International projects, contracts, and partnerships	1
<b>TOTAL PUBLICATIONS IN 2017 (+ accepted for 2018)</b>	<b>302 (+37)</b>

### 8.1. ORIGINAL ARTICLES, REVIEWS, AND PROFESSIONAL PAPERS

#### Indexed in WoS

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