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AND OCCUPATIONAL HEALTH
ZAGREB

1948-2008

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Institute for Medical Research and Occupational Health, 1948-2008: Report for 2004-2008

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Introduction

The Institute for Medical Research and Occupational Health is the second largest Croatian research centre. In a multi-disciplinary environment, about 100 scientific staff and postgraduate students work together to distinguish the Institute in biomedical and environmental research, toxicology, occupational health, chemistry, physics, and biology in Croatia and abroad.

60th Anniversary

In 2008, the Institute celebrated its 60th anniversary. The Institute was founded by a decree of the Yugoslav Academy of Arts and Sciences on 27 December 1947, upon the initiative of Andrija Štampar, who then presided over the Academy. The Institute celebrated the 60th anniversary with a scientific symposium entitled "Current Research in Occupational and Environmental Health" held in Zagreb on 28 November 2008. The symposium was organized together with the Croatian Academy of Sciences and Arts. The celebration was opened with a speech by Institute's director Ana Lucić Vrdoljak and by the president of Croatian Academy of Sciences and Arts, Milan Moguš. Congratulation speeches were then held by Dragan Primorac, Croatian Minister of Science, Education and Sports and Dražen Borčić on behalf of the Croatian Medical Council. The symposium programme was opened by Marko Šarić, Institute's long-time director and academician, who gave a comprehensive overview of the Institute's history and the role of Andrija Štampar in its foundation. Followed fourteen presentations by Institute's researchers who gave an overview of the ongoing research at the Institute. All presentations were published *in extenso* in *Archives of the Industrial Hygiene and Toxicology* (Arh Hig Rada Toksikol 2009;60:1-128).

Activities

Between 2004 and 2008, research was performed within scientific projects and programmes financed by the Croatian Ministry of Science, Education and Sports (MoSES) and other Croatian and international institutions. Between 2004 and 2006, research was conducted within the framework of 21 projects financed by the MoSES, and the projects were grouped in three programmes: Experimental Toxicology (director: Maja Blanuša), Environmental Pollution and Radioactivity (director: Vlasta Drevenkar), and Environmental Effect on Health (director: Božica Kanceljak-Macan). Since 2007, research continued within 23 projects financed by the MoSES. Nineteen projects were included in three scientific programmes co-ordinated by and performed at the Institute, as follows: Experimental Toxicology of Natural and Synthetic Substances (co-ordinator:

Ivan Sabolić), Environmental Pollution and Radioactivity (co-ordinator: Vlasta Drevenkar), and Environmental and Life-style Effects on Health (co-ordinator: Jelena Macan). Two projects were included in the scientific programmes of other institutions and two were independent.

Research was also conducted within collaborative projects with institutions from Croatia and the EU, and with international organizations such as World Health Organization (WHO), International Atomic Energy Agency (IAEA), and North Atlantic Treaty Organization (NATO) (Table 1).

Over the last five years the Institute's scientists published 280 research papers in journals indexed in *Current Contents* (CC) and *Science Citation Index* (SCI). This is a 42 % increase in average productivity in respect to the period 1999-2003. A particular boost in publication was seen in 2008 (Fig. 1). It is important to note that publications of the Institute's scientists have been cited about 830 times over the last five years.

The Institute has been recognised for professional services, especially in radiation protection and continuous monitoring of physical and chemical substances affecting environment. Occupational health is still as important for the Institute as it was 60 years ago, when the Institute was established. Analytical services include specific analysis such as quantification of heavy metals and pesticides in biological and environmental samples, measurement of air quality and pollution, testing for drugs, cytogenetic analysis, allergy testing, assessment of osteodegenerative changes, and psychological testing. Over the last five years professional services were contracted by the Ministry of Environmental

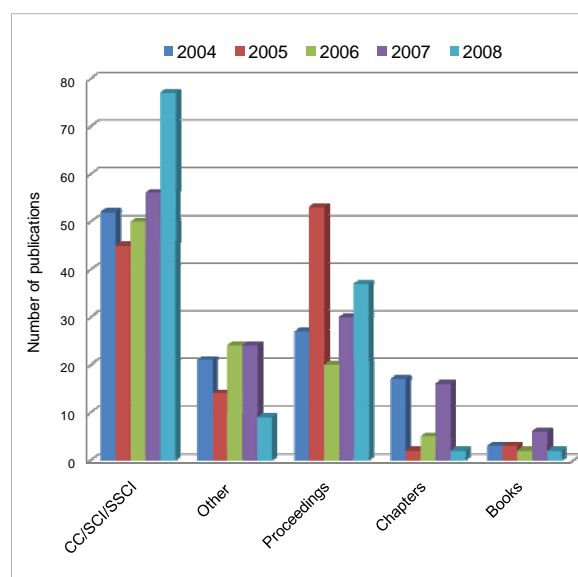


Fig. 1. Publication output of the Institute between 2004 and 2008

Table 1: The list of international projects conducted at the Institute between 2004 and 2008

Agency / Organisation	Project (participant from the Institute)
WHO	Global Environment Monitoring System Programme (GEMS/AIR): City Air Quality Trends (Vadić V) Global Environmental Radiation Monitoring Network (GERMON) (Kovač J and Senčar J)
EU	Assessment of environmental risk for use of radioactively contaminated industrial tailings (INTAILRISK), FP6 (2002-INCO-WBC-1), principal investigator Klerkx J, International Bureau for Environmental Studies, Brussels, Belgium: Critical pathways assessment (Kovač J) Cytogenetic Biomarkers and Human Cancer Risk, FP4 (QLK3-2000-02831) (project co-ordinator Norppa H, Finnish Institute of Occupational Health, Helsinki, Finland): Human Micronucleus Project, principal investigator Fenech M, CSIRO, Adelaide, Australia (Fučić A) European network on children's susceptibility and exposure to environmental genotoxicants, FP5 (QLK4-2002-02395), principal investigator Knudsen LE, Panum Institute, Copenhagen, Denmark (Fučić A) Policy interpretation network on children's health and environment, FP5 (QLK4-CT-2002-02198), principal investigator van den Hazel P, INCHEM, Hulpverlening Gelderland Midden, The Netherlands (Fučić A) Management and remediation of hazardous industrial wastes in the Western Balkan countries (INDUWASTE), FP&-INCO-Contact Number 515919, project co-ordinator: Klerkx J, International Bureau for Environmental Studies, Bruxelles, Belgium (Kovač J) Environmental risk from ionising contaminants: assessment and management (ERICA) (FI6 RCT-2003-506647), principal investigator: Larsson C-M, Swedish Radiation Protection Authority, Stockholm, Sweden (Prlić I) Health and Environment Network (HENVINET), FP6, project co-ordinator: Floisand I, Norwegian Institute for Air Research, Kjeller, Norway (Fučić A) Newborns and genotoxic exposure risks: Development and application of biomarkers of dietary exposure to genotoxic and immunotoxic chemicals and of biomarkers of early effects, using mother-child birth cohorts and biobanks (NewGeneris), FP6, project co-ordinator Kleinjans J, Maastricht University, Maastricht, The Netherlands (Fučić A) Expert team to support biomonitoring in Europe (ESBIO), FP6, project co-ordinator Johas R, BiPRO, Munich, Germany (Fučić A) A future for radioecology in Europe (FUTURAE), FP6, project co-ordinator Gariel J-C, Institute for Radiological Protection and Nuclear Safety, Clamart, France (Prlić I)
IAEA	Marine environmental assessment of the Mediterranean region (RER/7/003) (Franić Z) Air pollution monitoring in the Mediterranean region (RER/8/009) (Šega K)
NATO	Antidotes for the treatment of nerve gas agents poisoning (Reintegration Grant) (Kovarik Z) Butyrylcholinesterase and aldoximes: bioscavengers for detoxification of organophosphates (Collaborative Linkage Grant) (Kovarik Z)

Protection, Physical Planning and Construction, Croatian Environmental Agency, State Office for Radiation Protection, Ministry of the Interior, Hrvatske vode (national water management agency), and many others.

Despite the fact that the Institute is primarily a research institution, our scientists also participate in educational activities at the undergraduate as well as postgraduate level teaching on predoctoral and doctoral university courses. Under the supervision of the Institute's academic staff, 30 graduation, 5 M.Sc., and 21 Ph.D. theses were completed in 2004-2008.

The Institute is licensed to provide training to M.D. students who specialize in occupational health.

For many years, the Institute has been organising national and international scientific meetings, symposia, and workshops. Between 2004 and 2008, the Institute organised or co-organised the Symposium "General and Work Environment: Treatment and Prevention Aspects" (Zagreb, 2004), Third Professional Seminar "Advancement and Standardisation of Analytical Methods and Techniques for Air, Water, and Soil Pollution Measurements" (Milan, Italy; Paris, France; and

Idstein, Germany; 2004), Sixth Symposium of Croatian Radiation Protection Society (Stubičke Toplice, 2005), Regional Training Course on Heavy Metal Monitoring (Zagreb, 2005), Fourth Meeting on Air Protection (Zadar, 2005), Second Congress of Croatian Genetics (Supetar, 2005), Symposium: "Waste: Environmental and Health Issues" (Zagreb, 2005), Symposium "Immunological Reactions to Drugs" (Zagreb, 2006), Fourth Professional Seminar "Detection of Air, Water, and Soil Pollution by Specific Analytical Techniques: Introducing European Standards" (Amsterdam, The Netherlands and Germering, Germany; 2006), International Short Course "Environmental Management of Industrial Wastes in Western Balkan Countries" (Zagreb, 2006), Semi-annual working meeting of MEDICHEM members and satellite mini-symposium IMI/MEDICHEM (Zagreb, 2007), NATO Advanced Research Workshop "Decision Support for Natural Disasters and Intentional Threats to Water Security" (Dubrovnik, 2007), Fifth Meeting on Air Protection (Zadar, 2007), Fifth Professional Seminar "Detection of Air, Water, and Soil Pollution by Specific Analytical Techniques - Introducing European Standards" (Olten, Switzerland and Germering, Germany; 2006), the Symposium "Allergotoxic Research of the Environment in Croatia" (Zagreb, 2008), and the Symposium in Honour to Elsa Reiner (Osijek, 2008).

Organisation

Between 2004 and 2008, the Institute's management has changed. Since June 2008, the Institute has had a new director Ana Lucić Vrdoljak, and a new deputy director Davor Želježić. Until that time the Institute's director was Sanja Milković Kraus (since 1994). Since April 2005, Zdenko Franić has been the director's Quality Manager for the implementation of the ISO 9001:2000 quality management system. From 2004 to 2008, Institute's Management Board was changed three times. Since June 2008 it has included Krešimir Pavelić (chair), Alan Bosnar, Zdenko Franić, Vito Turšić, and Želimira Vasilić. Between October 2005 and June 2008 its members were Krešimir Pavelić (chair), Alan Bosnar, Krešimir Šega, and Želimira Vasilić. Before October 2005, the members were Zdenko Kovač (chair in 2004), Juraj Geber, Krešimir Šega, and Želimira Vasilić. Until September 2004, the chair of the Council of Scientist was Blanka Krauthacker, and Vlasta Drevenkar has chaired this body ever since.

On 31 December 2008, the Institute had nine laboratory units and two professional services. Their activities have been described in detail in this report. Several organisational changes took place between 2004 and 2008: Biomathematics Unit was closed at the beginning of 2004 and Analytical and Physical Chemistry Unit was closed in March 2008. A new Analytical Toxicology and Mineral Metabolism Unit

was set up in April 2007 upon Management Board decision to merge two research units Mineral Metabolism Unit and Clinical Toxicological Chemistry Unit. Institute has a small library which is open to researchers from other institutions. There is also a documentation section which keeps all Institute's publications, reports, and theses, and also accommodates the editorial office of *Archives of Industrial Hygiene and Toxicology*, an international scientific quarterly published journal by the Institute and indexed by major services such as Science Citation Index Expanded, PubMed, and Scopus.

In 2005, the Institute established a limited liability company Medicina rada Instituta za medicinska istraživanja i medicinu rada whose aim is to provide specific health care services. The company started to work in early 2008, and since then its director has been Sanja Milković-Kraus.

In mid 2008, the Institute took over from MoSES the management of a scientific research polygon at a hunting preserve "Šumbar" near Karlovac to study and evaluate environmental pollution of the locality. MoSES has financed the maintenance of the polygon.

On 31 December 2008, the Institute had 151 staff (117 women and 34 men). Ninety-two held university degree, 52 a Ph.D, while the rest were graduated students (a list of staff is given at the end of the report).

Several Institute's buildings were thoroughly renovated between 2004 and 2008. Occupational and Environmental Medicine Unit obtained new laboratories and offices and moved from the premises of the University Hospital Jordanovac. Laboratories of the Toxicology Unit, Molecular Toxicology, Analytical Toxicology and Mineral Metabolism Unit were also renovated into modern and pleasant working spaces.

Awards and recognitions

The Institute received recognitions for its research, collaboration, and other activities from the Croatian Society for Biochemistry and Molecular Biology (in 2006), the State Office for Nuclear Safety (in 2007), the Environmental Protection and Energy Efficiency Fund of the Republic of Croatia (in 2007). In 2004 Silver Award was given to Mladen Pavlović by the European Respiratory Society. The Croatian Medical Association awarded a diploma of honour to Jelena Macan, acknowledging her substantial contribution to the promotion of its professional, scientific and ethical values and principles. In 2007, the Hong Kong University of Science and Technology and the Chinese Academy of Sciences honoured Elsa Reiner with a plaque for her contribution in cholinesterase research.

Young scientists also received recognition for their research. Adrijana Košćec-Đuknić received Young Scientists Award for 2004 from the European Sleep Research Society. The Society of University Teachers,

Scholars and Other Scientists - Zagreb awarded Marija Ljubojević in 2004 and Ana-Marija Domijan in 2006. The Croatian Society for Biochemistry and Molecular Biology gave Annual Award for Young Scientists to Zrinka Kovarik in 2004 and to Goran Šinko in 2006.

In 2005 two diploma theses were awarded. The thesis by Butković S, prepared in the Clinical Toxicological Chemistry Unit and mentored by Alica Pizent and Vlasta Drevenkar, was awarded by Hrvatske vode as the best thesis in the field of water management. The Croatian Medical and Biological Engineering Society gave the second prize to the diploma thesis of Lončar J mentored by Vera

Garaj-Vrhovac, Mutagenesis Unit.

The Institute's Council of Scientists established in-house awards. In 2005 for the first time the award was given to young scientist and in 2006 for paper published in the *Archives of Industrial Hygiene and Toxicology* with at least 7 citations in SCI/SSCI/Scopus. Table 2 gives a list of all awardees between 2004 and 2008.

Zrinka Kovarik

Table 2 Annual awards for Institute's staff in five categories: (I) greatest number of papers published in journals indexed by Current Contents (CC), (II) paper published in a journal with the highest impact factor / rank, (III) award for young scientist for the greatest number of papers published in journals indexed by CC/SCI/SSCI, (IV) paper with at least 50 citations in SCI/SSCI/Scopus, (V) paper published in the Archives of Industrial Hygiene and Toxicology with at least 7 citations in SCI/SSCI/Scopus, and (VI) jubilee award for the most successful scientist in the period 2004-2008.

Year	Category					
	I	II	III	IV	V	VI
2004	Fučić A (4 CC)	Fučić A ^(a)		-		
2005	Cvijetić Avdagić S (6 CC)	Ljubojević M and Sabolić I ^(b)	Košćec- Đuknić A (2 CC)	Peraica M, Radić B, Lucić Vrdoljak A and Pavlović M (54 citations)	-	
2006	Peraica M (4 CC)	Varnai VM and Piasek M ^(c)	Domijan A-M (3 CC)	Fučić A (57 citations)	Telišman S (15 citations)	
2007	Kovarik Z (7 CC)	Liščić R ^(d)	Miličević A (5 CC)	Sabolić I (52 citations)	Peraica M and Domijan A-M (7 citations)	
2008	Kopjar N (7 CC)	Peraica M ^(e)	Domjan A-M (4 CC) Miličević A (4 CC)	Telišman S, Jurasović J and Pizent A (71 citations)	Ivičić N (9 citations)	Garaj-Vrhovac V

^(a) Neri M, Fučić A, Knudsen LE, Lando C, Merlo F, Bonassi S. Micronuclei frequency in children exposed to environmental mutagens: a review. *Mutat Res* 2003;544:243-54.

^(b) Ljubojević M, Herak- Kramberger CM, Hagos Y, Bahn A, Endou H, Burckhardt G, Sabolić I. Rat renal cortical OAT1 and OAT3 exhibit gender differences determined by both androgen stimulation and estrogen inhibition. *Am J Physiol - Renal Physiol* 2004;287:F124-38.

^(c) Blanuša M, Varnai VM, Piasek M, Kostial K. Chelators as antidotes of metal toxicity: Therapeutic and experimental aspects. *Curr Med Chem* 2005;12:2771-94.

^(d) Mukherjee O, Pastor P, Cairns NJ, Chakraverty S, Kauwe JSK, Shears S, Behrens MI, Budde J, Hinrichs AL, Norton J, Levitch D, Taylor- Reinwald L, Gitcho M, Tu PH, Grinberg LT, Liščić RM, Armendariz J, Morris JC, Goate AM. HDDD2 is a familial frontotemporal lobar degeneration with ubiquitin-positive, tau-negative inclusions caused by a missense mutation in the signal peptide of progranulin. *Ann Neurol* 2006;60:14-22.

^(e) Pfohl-Leszkowicz A, Tozlovanu M, Manderville R, Peraica M, Castegnaro M, Stefanovic V. New molecular and field evidences for the implication of mycotoxins but not aristolochic acid in human nephropathy and urinary tract tumor. *Mol Nutr Food Res* 2007;51:1131-46.

Analytical Toxicology and Mineral Metabolism Unit

Scientific staff:

Jasna Jurasović, head
Martina Piasek
Alica Pizent
Nataša Brajenović
Zorana Kljaković-Gašpić
Ivana Vinković Vrček
Irena Brčić Karačonji
Anja Katić
Maja Lazarus
Tatjana Orct

Technical staff:

Mladen Komesar
Snježana Mataušić
Krešimir Nekić
Vesna Triva

Associate scientists:

Maja Blanuša
Krista Kostial
Ljiljana Skender
Spomenka Telišman

In April 2007, *Mineral Metabolism Unit* and *Clinical Toxicological Chemistry Unit* merged into *Analytical Toxicology and Mineral Metabolism Unit*.

People who retired between 2004 and 2008: Maja Blanuša, Višnja Karačić, Ljiljana Skender, Spomenka Telišman, Marija Ciganović, Đurđa Breški

People who left between 2004 and 2008: Marija Šarić, Dijana Jureša, Darko Ilić, Igor Špoljarić, Andrea Tomljenović

Research activities

Exposure, intake and effects of toxic and essential elements

Principal investigator: Maja Blanuša 2002-2005, Martina Piasek 2006; collaborators: M. Piasek, S. Cvijetić Avdagić, V. M. Varnai, K. Kostial, D. Jureša, M. Šarić, M. Lazarus, T. Orct, Đ. Breški, M. Ciganović; project supported by the Ministry for Science, Education and Sports of the Republic of Croatia (MoSES)

Exposure to metals and their effects in pregnancy and postnatal period

Principal investigator: Martina Piasek 2007-, collaborators: J. Jurasović, V. M. Varnai, D. Jureša, I. Vinković Vrček, M. Lazarus, A. Katić, T. Orct, M. Blanuša, K. Kostial, M. Matek Sarić (Department of Medical Studies, University of Zadar), S. Stasenko (Clinical Hospital "Mercur", Zagreb); project supported by MoSES

Our research includes assessment of exposure, intake and organ distribution of toxic and essential metals and metalloids, and their biological effects in target organs/tissues. The assessment integrates element analysis in various environmental and biological samples. The research is focused on females of reproductive age and young mammals, including humans. Both show higher retention and vulnerability to toxic metals. We also investigate methods to decrease metal body burden using chelating agents and/or dietary supplements (calcium, selenium) in experimental rat models *in vivo*. Research from 2004 to 2008 includes the following findings.

Monitoring of metals

We quantified the relationships between metal mass fractions in soil, leaf litter, and ground beetles in the forest ecosystem of Medvednica Nature Park (A243). Metals have also been monitored in the ecosystem of a wider area of Nature Park Kopački rit using red deer (*Cervus elaphus*) as bioindicator organism (A145, B49).

Metal distribution and effects

We have completed a study of the effects of latent iron deficiency combined with cadmium exposure during pregnancy on trace elements in mother rats and foetuses. Decreases in maternal body weight, maternal and foetal liver weights, placental weights, and tissue iron were related to low iron in feed. Cadmium in organs increased; zinc in maternal liver and kidney also increased; and foetal zinc decreased. Lower intake of iron additionally increased maternal liver cadmium. Acute cadmium exposure in the last gestational trimester posed a risk to foetal viability, especially in combination with maternal low iron intake (A207).

We evaluated the distribution of manganese in organs of perinatally exposed pups and found that manganese increased in the pups' organs irrespective of the timing and length of exposure. When exposure ceased, organ manganese dropped, and other essential elements did not change. These results pointed out existence of an accurate regulation of manganese accumulation in the perinatal period (A130).

We obtained preliminary results on the distribution of organic mercury (thiomersal, a vaccine preservative, which contains ethyl mercury) compared to inorganic mercury (as chloride) in suckling rats. Mercury mass fraction was higher in the liver and kidneys after exposure to inorganic mercury, and in the brain and blood after exposure to thiomersal (A191).

We also assessed the effects of salt on bone health (B105), the effects of dietary mineral elements and acid load on bone mass in young rats (A239, E46), and calcium intake through food and water in women of different age (E21).

Cadmium as endocrine disrupting chemicals

We co-authored a chapter in a book on cutting-edge research on smoking, passive smoking in particular, and health. We presented literature and our own data on cadmium ion as a constituent of tobacco smoke that accumulates in human placental (trophoblast) cells where it suppresses progesterone release without apparent inhibition of tissue (syncytial) development (D23). We connected findings on health effects of passive smoking in children with an assessment of perinatal exposure and health risks of cadmium using human placental tissue (B83). We contributed to an extensive review article upon invitation. Our results and the data contributed by two other review co-authors using a different experimental paradigm add to novel concept of cadmium as an endocrine disruptor in women's reproduction (B14).

Methods to decrease metal retention

We evaluated the effects of a combined treatment with *meso*-2,3-dimercaptosuccinic acid (DMSA) and

calcium supplementation in reducing lead absorption and enhancing lead elimination in suckling rats. Calcium supplementation in milk, generally favourable at this age, reduced tissue lead when given during lead exposure, but had no effect when given after exposure. Administration of DMSA decreased lead in tissues. A major finding was that calcium supplementation could be efficiently applied during DMSA therapy (A266).

We assessed the efficiency of DMSA and/or calcium trisodium diethylenetriaminepentaacetate (CaDTPA) on cadmium mobilisation. Cadmium removal from rat body was better when the time between metal and chelating agent administration was shorter. Tissue zinc changed. Therapy with DMSA was more efficient, and the combination with both chelators gave best results (A163).

We evaluated the efficiency of DMSA and sodium 2,3-dimercapto-propane-1-sulphonate (DMPS) as mercury chelator in the presence of selenium in feed. Both chelating agents were efficient, and simultaneous administration of mercury and selenium caused redistribution of mercury in the organs. The results have shown that selenium decreases the efficiency of chelating agents for mercury removal from rat body (A109, E17).

We co-authored an invited overview of the clinical use and experimental aspects of chelating drugs as antidotes against metal toxicity. The article's major contribution is the evidence of age-dependency of chelating treatment, which includes our own experimental results (A20).

Martina Piasek

Reproductive health effects of metals in men

Principal investigator: Spomenka Telišman, 2002-2006; collaborators: J. Jurasović, A. Pizent, D. Ilić, I. Špoljarić, K. Nekić; project supported by MoSES

Reproductive health effects of toxic and essential metals in men

Principal investigator: Spomenka Telišman, 2007, Alica Pizent, 2008-; collaborators: J. Jurasović, A. Pizent, Z. Kljaković-Gašpić, B. Čolak (University Clinic "Vuk Vrhovac", Zagreb); project supported by MoSES

Between 2004 and 2008 we have continued to investigate possible combined effects of toxic and essential metals, including the contribution of age, smoking, and alcohol consumption on human health.

The effect of low-to-moderate lead (Pb) exposure on blood pressure was examined in industrial workers (A251). There was no significant difference in blood pressure between 100 Pb workers and 51 control subjects, possibly because the control subjects had relatively high blood Pb (BPb) levels and significantly higher body mass index (BMI) than Pb workers. After adjusting for potentially confounding variables by multiple regression (blood cadmium (BCd), serum Zn

(SZn) and Cu (SCu), age, intensity of smoking, alcohol consumption, and BMI), the results showed significant increase in systolic and diastolic blood pressure with increasing erythrocyte protoporphyrin (EP) in industrial workers. Long-term cumulative Pb exposure, which is better reflected by EP than by current BPb level, can significantly increase blood pressure in male workers moderately exposed to Pb.

In a chapter of the *Handbook on the Toxicology of Metals* we reviewed published studies on reproductive and developmental toxicity of metals (D7). The chapter provides up-to-date information on the adverse effects of Pb, Cd, mercury, manganese,

chromium, nickel, arsenic, aluminium, platinum, vanadium, lithium, and uranium on fertility, intrauterine growth, retardation, abortions, malformations, birth defects, and developmental effects. We also presented our own data, particularly on Pb- and Cd-related effects on human semen quality and male reproductive endocrine function.

The influence of Cd on reproductive parameters was examined in Croatian men with no occupational exposure to metals (A108). Increased exposure to Cd was mostly through smoking, and the difference in BCd was highly significant between 61 smokers and 62 nonsmokers. After adjusting for age, smoking, alcohol, and biomarkers of Pb, Cu, Zn, and Se, BCd was significantly associated with a decrease in testis size and an increase in serum estradiol, FSH and testosterone. The study results showed no significant correlation between BCd and the parameters of semen quality.

Reproductive toxicity of low-level Pb exposure was investigated in 240 Croatian men (A252). The values of BPb, BCd, SZn, SCu and serum selenium (SSe) were within the range common for general adult male population of Croatia and many other countries.

After adjusting for potentially confounding variables by multiple regression, we found a significant synergistic effect of BPb and BCd levels on increasing serum testosterone, and additive effect of a decrease in SSe on increasing serum testosterone. These results may have implications for the initiation and development of prostate cancer, because testosterone speeds up the progression of prostate cancer in its early stages.

A significant association was found between a particular atopy marker and/or ventilatory function parameter and SCu and Se-glutathione-peroxidase (GPx) in women, and between SZn, SSe and Cu, Zn-superoxide dismutase (SOD) in men (A208). These results were observed in apparently healthy subjects at metal and enzyme levels comparable to those of general populations of other European countries. The observed difference in response between the groups of men and women may partly be explained by different levels of relevant toxic and essential metals, and particularly by their combinations.

Alica Pizent

Nutrient cycle in the water column and water exchange at the water-sediment interface

Principal investigator: Ante Barić (Institute of Oceanography and Fisheries), 2002-2006; collaborator from the Institute: Z. Kljaković-Gašpić; project supported by MoSES

Role of plankton communities in the energy and matter flow in the Adriatic Sea

Principal investigator: Nada Krstulović (Institute of Oceanography and Fisheries), 2007-; collaborator from the Institute: Z. Kljaković-Gašpić; project supported by the MoSES

We investigated the role of plankton communities in the energy flow and organic matter cycling in the coastal and open Adriatic Sea, with particular attention to the spawning areas of small pelagic fish, mariculture areas, and other areas under significant anthropogenic influence.

Concentrations of essential (Cu, Zn) and toxic trace metals (Cd, Cr, Hg, Pb) were determined in the edible tissue of mussels collected from four shellfish farms in the Mali Ston Bay (A117). All values were

well below the permissible limits for fresh seafood. Evaluation of risks to human health associated with the consumption of mussels containing trace metals suggests that there is no health risk for moderate shellfish consumers. We have also published data on the levels of phosphorus and iron species in sediments from the Middle Adriatic area (A164, A165, C89)

Zorana Kljaković-Gašpić

Professional services

Analytical Toxicology and Mineral Metabolism Unit was set up in April 2007 upon Institute's Management Board decision to merge Mineral Metabolism Unit and Clinical Toxicological Chemistry Unit.

Between 2004 and 2008 we provided the following services for different clients, institutions (including academic ones), and industry:

- Analysis of body fluids and tissues for trace toxic (lead, cadmium, mercury, aluminium, silver) and essential elements (zinc, copper, selenium) by flame, graphite furnace, and cold vapour atomic absorption spectrometry (AAS);
- Measurement of δ -aminolevulinic acid dehydratase (ALAD) activity, erythrocyte protoporphyrin (EP) concentrations, and lead in

blood as biomarkers of lead exposure;

- Regular aluminium and mercury concentration controls of immunobiological preparations using graphite furnace and cold vapour AAS;

- Trace element (arsenic, copper, zinc, lead, cadmium, mercury, chromium, nickel and molybdenum) analysis in various environmental samples (particulate matter, water, sea sediments, soil) using flame, graphite furnace and cold vapour AAS;

- Analysis of benzene, toluene, ethylbenzene, and isomeric (*o*-, *m*-, *p*-) xylenes (BTEX) in human urine and blood using gas chromatography mass spectrometry (GC-MS);

- Analysis of phenylglyoxylic and mandelic acids (styrene and ethylbenzene metabolites) in human urine using a GC method with flame and photoionization detection;

- Analysis of lidocaine (used as anaesthetic or antiarrhythmic drug) in serum by GC-MS;

- Quantitative analysis of illicit drugs of abuse in hair and urine by GC-MS. Substances that we analysed include amphetamines, cannabinoids, opiates (morphine, codeine, heroin, 6-acetylmorphine), methadone, and cocaine. Moreover, in 2006, the Institute received a licence by the Ministry of Health and Social Welfare to perform these analyses.

All analytical methods are regularly checked for accuracy, precision and reproducibility by analysing certified reference standards and by participating in interlaboratory comparison programmes: National External Quality Assessment Scheme, Birmingham, UK (blood lead and cadmium and drugs of abuse in urine); Instituto Nacional de Toxicología y Ciencia Forenses,

Sevilla, Spain (drugs of abuse in hair); U.S. Department of Health and Human Services, Wisconsin State Laboratory Hygiene, Madison, WI, USA (erythrocyte protoporphyrin in blood); Trace Elements External Quality Assessment Scheme, Guilford, UK (serum copper, zinc, selenium and aluminium); IFA-Tulln Test System at the University of Natural Resources and Applied Life Sciences, Vienna, Austria, Department for Agrobiotechnology, IFA-Tulln, Center for Analytical Chemistry (trace metals and BTEX in water); Swedish National Food Administration, Uppsala, Sweden (trace metals in food).

In 2007, in collaboration with Environmental Hygiene Unit of the Institute, we also participated in Intercomparison exercise for heavy metals in PM₁₀ organized by EC Joint Research Centre, Institute for Environment and Sustainability, Ispra, Italy.

In September 2008, a thorough renovation started of the Unit's laboratories and offices to accommodate a recently purchased inductively coupled plasma mass spectrometer (ICP-MS) and a microwave autoclave high-pressure reactor system for complete digestion of samples for elemental analysis (first of that kind bought in Croatia). The ICP-MS has been bought from the Institute's funds and the microwave autoclave has been financed by MoSES. These instruments and the renovated laboratory with clean-room conditions will add value to our research and broaden the scope of services in trace and ultra trace multielement analysis.

Jasna Jurasović

Biochemistry and Organic Analytical Chemistry Unit

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People who retired between 2004 and 2008: Vera Simeon, Blanka Krauthacker

People who left between 2004 and 2008: Goran Tokić

Research activities

Pesticides, persistent and volatile organic pollutants in the environment

Principal investigator: Vlasta Drevenkar, 2002-2006; collaborators: V. Karačić, B. Krauthacker, Lj. Skender, Ž. Vasilić, S. Fingler Nuskern, S. Herceg Romanić, I. Brčić Karačonji, G. Mendaš Starčević, S. Stipičević, D. Kožul, R. Turk, M. Kralj, V. Triva, B. Tkalčević; project supported by MoSES

Organic pollutants in environment: distribution, interactions, human exposure

Principal investigator: Vlasta Drevenkar, 2007-; collaborators: B. Krauthacker, V. Karačić, Ž. Vasilić, S. Fingler Nuskern, S. Herceg Romanić, N. Brajenović, G. Mendaš Starčević, S. Stipičević, I. Brčić Karačonji, D. Kožul, M. Sambolec, Lj. Skender; project supported by MoSES

Conceptual model for biotechnical treatment of atrazine production waste water

Principal investigator: Dubravka Hršak (Ruder Bošković Institute), 2002-2005; collaborators from the Institute: V. Drevenkar, Ž. Vasilić, S. Fingler Nuskern, G. Mendaš Starčević, S. Stipičević; technological scientific/development project

Study of pesticide interactions with inorganic and organic substances in water and soil

Principal investigators: Vlasta Drevenkar and Lucija Zupančič-Kralj (Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia), 2004-2005; collaborators from the Institute: Ž. Vasilić, S. Fingler Nuskern, G. Mendaš, Starčević, S. Stipičević; Croatian-Slovenian bilateral cooperation programme in the field of science and technology

The aim of these studies was to extend the knowledge on the processes affecting the distribution, transport, and transformation of pesticides and volatile and persistent organic pollutants in the environment, as well as on specific biochemical indicators of human exposure to these chemicals. The studies were focused on (a) persistent, lipophilic, and bioaccumulative organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), (b) more polar chlorotriazine, methylthiotriazine and methoxytriazine herbicides and their dealkylated and hydroxylated degradation products, (c) chlorophenols, and (d) volatile micropollutants such as benzene, toluene, ethylbenzene, isomeric xylenes (BTEX) and nicotine.

Analytical procedures used in the studies were subject to improvement and critical evaluation.

Analytical methods

Conditions of microwave-assisted solvent extraction were optimised for accumulation of OC pesticides and PCB congeners from soil (E51) and pine needles and for further analysis by gas chromatography (GC) with electron capture or mass spectrometric (MS) detection.

A solid-phase microextraction procedure followed by GC analysis was developed for free concentration measurements of triazine compounds in aqueous phase before and after the sorption on a solid sorbent (E1).

Extraction with organic solvents and solid-phase extraction on different sorbents were compared for

accumulation of atrazine and its metabolites in urine. Sample preparation was optimised for final analysis with high performance liquid chromatography with UV diode array detector or coupled to tandem mass spectrometry (E28).

Dynamic headspace-purge and trap and headspace solid-phase microextraction (HS-SPME) procedures were developed and compared for GC determination of BTEX in urine with photoionisation and MS detection, respectively. HS-SPME-GC-MS method was recommended as faster and simpler for routine analysis of urinary BTEX (B9, B11).

Simple and rapid HS-SPME-GC-MS methods were developed to determine low concentrations of nicotine and cotinine in urine (A34) and of nicotine in hair. A comparison between HS-SPME and solvent extraction procedures revealed that ethyl acetate should be used for nicotine and cotinine extraction from hair and HS-SPME for their determination in urine. Cotinine concentrations determined in nonsmokers' urine by GC-MS analysis correlated significantly with concentrations measured by an enzyme-linked immunosorbent assay (ELISA).

Distribution and interactions of pollutants in the environment

Aromatic solvents (BTEX) were measured in municipal, well, river, lake, thermal, and marine water samples collected in different areas of Croatia (A33). In most samples BTEX concentrations were low and similar, and did not present any risk for the environment.

Distribution of OC compounds was investigated in aqueous and terrestrial ecosystems, including air (A143, B38, C45, E16), atmospheric particles (C159), coniferous tree needles (A98, A100, A101, A142, B38, B47, C44, C45, E16, E22), soil (A267, D9), and marine organisms from the Adriatic Sea (C70, E26).

Congener profiles of PCDDs/PCDFs were evaluated in ambient air samples collected at different sites in Zagreb in 1997-2000 (A143). The samples collected far from PCDD/PCDF sources exhibited a typical congener pattern dominated by highly chlorinated compounds. PCDF congener profiles changed markedly in air samples collected during a landfill fire.

Seasonal variations in the levels of PCB congeners (C159) and OC pesticides were studied in samples of inhalable atmospheric particles PM₁₀ collected in the northern part of Zagreb in 2000-2003. These levels were characteristic of global pollution.

A comparison between OC compound levels in pine needles and in ambient air confirmed pine needles as suitable passive bioindicators of air pollution (B38, C45). Differences in the distribution of OC pesticides and PCB congeners were investigated in one and two-year-old needles (A100, C44) collected at urban sites in Croatia (A98, A142) and in Croatian mountains (A101). The pattern of OC pesticides differed between mountain and urban sites

while the pattern of PCBs was similar.

PCB levels and patterns were evaluated in surface soil samples collected within and surrounding different airports and in the vicinity of partially devastated electrical transformer stations (A267). Contamination of airport soil with PCBs was not followed by an increase in the levels of PCDDs/PCDFs above the background values.

Mass fractions of OC pesticides and PCB congeners in the Mediterranean blue mussels (*Mytilus galloprovincialis*) (C70), sea turtles (*Caretta caretta*) (E26), and dolphins were characteristic of global environmental pollution. Sea mussels were sampled in collaboration with the Institute of Oceanography and Fisheries (Split), and samples of sea turtle and dolphin tissue were obtained from Croatian Natural History Museum.

Pollutant mobility in a water/soil system greatly depends on sorption interactions with organic and mineral soil constituents. Differences in sorption behaviour of chlorophenols between soil and terrestrial and marine sediments pointed to a different sorption mechanism and confirmed that the sorption capacity was related to the amount as well as type and origin of sorbent organic matter (A65). In batch experiments, triazines and chlorophenols were sorbed in native and organic free soils, humic acids isolated from soils, and in mineral sorbents in order to estimate the effect of organic and mineral soil fractions on sorption intensity and to assume sorption mechanisms. Partitioning/sorption of selected PCB congeners, OC insecticides, triazine and amide herbicides into dissolved humic acids, native soil and mineral sorbents was evaluated and compared by measuring their free concentrations in aqueous phase (A212).

Biodegradation pathway and mineralisation of atrazine by a bacterial community enriched from an agrochemical factory soil (261) was studied within the framework of a technological project aimed at optimising biotechnological treatment of wastewater in the production of atrazine. This study as well as the study of biodegradation of PCBs in soil by mixed bacterial cultures enriched from the PCB-contaminated soil (A205) and marine sediments (A9) was performed in collaboration with scientists from the Ruđer Bošković Institute.

Human exposure

Evaluation of long-term variations in exposure of general population in Croatia to persistent OC compounds was continued by analysis of OC pesticides and PCB congeners in human milk (A99, A271, E16) and serum (E16). OC compounds (pesticides, PCBs and PCDDs/PCDFs) in human milk have been monitored since 1977.

Atrazine metabolites were analysed in urine samples of exposed agricultural workers. Atrazine mercapturate was the only detected atrazine metabolite, distinguishing itself as a reliable and very sensitive

bioindicator of human exposure to atrazine (E28).

BTEX measurements in the urine of Zagreb general population provided reference values for environmental exposure. Smoking contributed significantly to the urinary BTEX concentration (A234, E5).

Exposure to environmental tobacco smoke (ETS) was assessed by determining nicotine (B10) and its specific metabolite cotinine in the urine (recent

exposure) and hair (long-term exposure) of nonsmokers. Nicotine and cotinine were analysed in urine samples of non smokers with or without ETS exposure (A34). Nicotine was also analysed in hair samples of children without any ETS exposure and children whose parents reported that someone in their environment smoked.

Vlasta Drevenkar

Mechanism of esterase interactions with organophosphorus compounds

Principal investigator: Vera Simeon, 2002-2004, Zrinka Kovarik, 2005-2006; collaborators: Z. Kovarik, G. Šinko, A. Bosak; project supported by MoSES

Interactions of organophosphates, carbamates and selected ligands with esterases

Principal investigator: Zrinka Kovarik, 2007-; collaborators: A. Bosak, M. Katalinić, G. Šinko, E. Reiner, Z. Radić (University of California at San Diego, USA); project supported by MoSES

Stereoselectivity of cholinesterases: kinetic studies of mechanisms of cholinesterase interactions with pharmacologically relevant compounds

Principal investigators: Zrinka Kovarik and M. Goličnik (School of Medicine, University of Ljubljana, Slovenia), 2005-2006; collaborators from the Institute: A. Bosak, M. Čalić, G. Šinko; Croatian-Slovenian bilateral cooperation programme in the field of science and technology

Expression and activity of cholinesterases in neuromuscular junction during organophosphate poisoning

Principal investigators: Zrinka Kovarik and T. Marš (School of Medicine, University of Ljubljana, Slovenia) 2007-2008; collaborators from the Institute: A. Bosak, M. Čalić, G. Šinko; Croatian-Slovenian bilateral cooperation programme in the field of science and technology

Antidotes for the treatment of nerve gas agents poisoning

Principal investigator: Zrinka Kovarik, 2005-2008; collaborators: A. Bosak, M. Čalić, G. Šinko; NATO Programme Security Through Science: Reintegration grant

Butyrylcholinesterase and aldoximes - bioscavengers for detoxification of organophosphates

Principal investigators: Zrinka Kovarik and K. Kuča (Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic), 2007-2009; collaborators from the Institute: S. Berend, A. Bosak, M. Čalić, A. Lucić Vrdoljak, B. Radić, G. Šinko; NATO Programme Security Through Science: Collaborative linkage grant

Our research between 2004 and 2008 was focused on interactions of nerve warfare agents, some pesticides and drugs, which structurally belong to organophosphates (OPs), carbamates, phenothiazins and flavonoids, with acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and paraoxonase (PON). The main goal was to expand the basic biochemical knowledge on esterases with an impact on health and environment. AChE has a vital function, as it terminates cholinergic neurotransmission by rapid hydrolysis of acetylcholine. The role of BChE and PON is not certain, but they exhibit a wide range of physiologically important hydrolytic activities, including drug metabolism, and interact with a broad range of substrates. It has been suggested that they scavenge anti-cholinesterase agents and thereby protect synaptic AChE from inhibition by these agents.

We studied the mechanism of enzyme-drug

interactions by measuring kinetics of catalytic enzymatic reaction. OPs inhibit AChE and BChE by esterifying their active site serine. Since oximes are known as antidotes able to reactivate both cholinesterases (ChE), we investigated interactions of the well known conventional pyridinium oximes 2-PAM and HI-6 with recombinant mouse AChE, its mutants, and BChE (A134, A137). Orientation of the oxime and conjugated phosphorus moiety on the serine is an important determinant of the reactivation. Because of optimised phosphorylation and reactivation rates, we proposed experimental conditions in which a mutant-oxime pair could work as a catalytic scavenger in continuous decomposition of OP (A139, A250).

We also studied reactivation of human native ChE inhibited by tabun or soman with conventional and a series of newly synthesised pyridinium oximes (A50, A51, A138, A140, A141, A153). Compounds with the

oxime group in *para*-position on the pyridinium ring were better reactivators of tabun- than of soman-inhibited acetylcholinesterase, but oximes with the oxime group in *ortho*-position were effective protectors of AChE against both tabun and soman inhibition. Semi-empirical calculations show that differences in reactivation rates can be explained by the steric hindrance within the oxime molecule (A140). Oximes react with acetylthiocholine, the substrate of ChE. We showed that the rate constants of the non-enzymatic reaction depend on molecular properties of the oximes (A244). We also emphasised some limitations of the Ellman spectrophotometric method for measuring ChE activity in the presence of oximes (A245).

The stereoselectivity of ChE was studied in carbamylation by bambuterol enantiomers, a selective BChE inhibitor (A29, A30, A136). The preference for (*R*)- over (*S*)-bambuterol observed primarily for ChE is dictated by residues of the acyl pocket and choline binding site (A29). The inhibition of ChE did not reveal enantiomeric preference for bicyclic amine quinuclidine-3-ol, its *N*-methyl or *N*-benzyl derivatives. However, AChE did not react with (*S*)-quinuclidine-3-ol acetate, but hydrolysed (*R*)-quinuclidine-3-ol acetate (A27, E4). Kinetic models for BChE inhibition by ethopropazine, a selective inhibitor of BChE, have been extended with ethopropazine enantiomers. BChE has a higher affinity for (*R*)-ethopropazine, while mutants of mouse AChE showed varying stereoselectivity (E48). We continued developing kinetic models to describe stereoselective interaction of BChE with ethopropazine to find a model that will better describe the mechanism of interactions between ChE and chiral

compounds than the models currently available in literature. We have proposed a method for cholinesterase activity measurement in whole blood based on BChE inhibition by ethopropazine (B98).

We studied the relationship between PON activity and lipid status in sera in the development of coronary diseases and oxidative stress (A225, A258-A260). PON activities were determined in healthy population and patients with type II diabetes. PON phenotypes AB and BB were related to the lipid status, which could indicate a risk of premature atherosclerosis in patients with type II diabetes (B40). A study of phenylacetate hydrolysis by human sera resulted in the confirmation of EDTA-sensitive and EDTA-insensitive esterases. We determined their catalytic constants for the hydrolysis of phenylacetate and their stability in aqueous media (A28).

We have had a fruitful collaboration with several researchers from Croatia and abroad. Recombinant enzymes were prepared at the Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego, La Jolla, USA. Oximes were synthesised at the Faculty of Science, University of Zagreb, Faculty of Military Sciences, Hradec Kralove, Czech Republic, and The Scripps Research Institute, La Jolla, USA. Cytotoxicity of oximes was measured in collaboration with GlaxoSmithKline Research Center Zagreb, Zagreb. Carbamate enantiomers were prepared at the Ruđer Bošković Institute, Zagreb. PON was studied in collaboration with colleagues from the Faculty for Veterinary Medicine and Faculty of Pharmacy and Biochemistry, University of Zagreb.

Zrinka Kovarik

Professional services

The Biochemistry and Organic Analytical Chemistry Unit provides analytical services to different institutions and laboratories engaged in environmental monitoring and protection. Organochlorine, organophosphorus, and triazine pesticides are measured in the surface, ground, and industrial waste waters. River sediments are analysed for organochlorine pesticides and atrazine. Most of surface water and river sediment analyses have been contracted by the national public utility Hrvatske vode. Triazine herbicides are analysed in agricultural soils. Polychlorinated biphenyls are determined in soils, waste oils, and similar waste materials. Aromatic solvents are determined in air samples,

pesticide residues in waste materials, and the most abundant organic compounds in air and water samples using gas chromatographic-mass spectrometric analysis.

Phenotyping of human plasma BChE is performed to detect the sensitivity of individuals to neuromuscular drugs. Paraoxonase activity in the presence of macrolides is measured in human serum. Cholinesterase activities are measured in animals suspected to have been exposed to anticholinesterase pesticides (organophosphates and carbamates).

Vlasta Drevenkar

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Research activities

Air pollution - exposure and health endpoints assessment

Principal investigator: Krešimir Šega, 2002-2006; collaborators: V. Vadić, J. Hršak, N. Kalinić, A. Šišović, I. Bešlić, G. Pehnc, M. Čačković, S. Žužul, A. Filipec, D. Lipovac, I. Balagović, V. Frković, Z. Frković, K. Pondeljak, M. Adžić; project supported by MoSES

Spatiotemporal distribution and origin of aerosols in urban surroundings

Principal investigator: Krešimir Šega, 2007-; collaborators: A. Šišović, N. Kalinić, M. Čačković, I. Bešlić, R. Godec, N. Periš (Institute of Public Health of Split and Split-Dalmatia County); project supported by MoSES

Air pollution monitoring in the Mediterranean region

National co-ordinator: Krešimir Šega, 2005-2008; supported by the International Atomic Energy Agency (IAEA)

Based on our measurements of daily concentrations of total suspended particulate matter (TSP) and black smoke (BS), and fractions of PM₁₀ and PM_{2.5} particle at several location in Zagreb, we estimated the following health endpoints: total mortality, cardiovascular mortality, respiratory mortality, hospital admissions due to respiratory disease, hospital admissions due to cardiovascular disease, hospital admissions due to asthma, hospital admissions due to chronic obstructive pulmonary disease (COPD), and hospital admission due to acute myocardial infarction. The obtained results suggested that fine particle fractions PM₁₀ and PM_{2.5} posed the main health threat to general population, while TSP and BS gave a false picture about health endpoints related to the particulate air pollution because of their negative concentration trends over the last decades (B108, C120, C121).

The influence of weather types in the continental part of Croatia on daily PM₁₀ concentrations and concentrations of metallic compounds in PM₁₀ were investigated. Most frequently PM₁₀ elevated concentrations were related to radiation weather type and south-eastern advection, while the lowest concentrations were recorded during the wind weather type (A17, A18, B7, E3).

Measurements of air pollution levels were

repeated at a large crossroads with high traffic density after its reconstruction. Concentrations of PM₁₀ particle fraction, nitrogen oxides, and polycyclic aromatic hydrocarbons (PAH) were monitored at six locations in the morning and afternoon over the week. Sample analysis and data assessment are ongoing (C2, C6).

PM_{2.5} particle fraction was continuously monitored to assess concentration distributions over time and space. The results show much higher annual concentration averages than recommended at all measuring sites, and significant seasonal and spatial differences in PM_{2.5} concentration levels (B7, C3, C4, C119, C123).

Mass concentrations of major acidic anions (chlorides, nitrates, and sulphates) in TSP and PM₁₀ particle fraction in Zagreb air were measured continuously and followed the order chloride < nitrate < sulphate. Significant correlations were found between TSP and investigated anions and between PM₁₀ and investigated anions, the latter showing a higher correlation coefficient. Annual average mass ratio of NO₃⁻ : SO₄²⁻ obtained in TSP and PM₁₀ was > 0.8, which suggests that mobile source emission was an important contributor to particle mass (A48, A49, B18, B19, C13, C14, C15, C17, C18).

Possible differences of particle-bound mass concentrations of polycyclic aromatic hydrocarbons

(PAHs) in PM₁₀ particle fraction were investigated in samples collected at two locations in Zagreb. Parallel measurements were carried out for one month in the winter and again in the summer. Mass concentrations of all measured PAHs were similar at both sampling sites, except for benzo-b-fluoranthene (BbF) and benzo-ghi-perylene (BghiP). Samples of PAHs were analysed using high performance liquid chromatography with a fluorescent detector (A246, A247, B111, B112, C130, C131, C133, C135).

Road traffic NO₂/NO_x emission ratio was estimated based on hourly averages of NO, NO₂, and O₃ concentration levels measured at one sampling site in Zagreb. Results show high seasonal dependence probably caused by weather conditions, sunlight, gas consumption in households and industry, and duration of summer holidays (C124).

Sampling and analytical methods were implemented for determining organic, elemental and total carbon content in airborne particles (A94, A95, C43).

Collaboration with co-workers in the project "Environmental Radioactivity and Radiation Protection" headed by G. Marović was continued by measuring total β-activity in daily PM_{2.5} samples collected at three measuring sites in Zagreb (B59, B60, C84).

In a collaborative project with the Faculty of Forestry, University of Zagreb entitled "Optimization of Energy and Safety Factors of Mechanical Woodworking", beech wood dust samples were collected at two woodworking facilities in order to determine the correction factor of the device for continuous determinations of aerosol mass concentration by photo-detection (A126, A127, C20).

Croatia, Albania, Bosnia & Herzegovina, Serbia, Montenegro, The Former Yugoslav Republic of Macedonia, Turkey, Cyprus, Greece, Malta, and Slovenia participated in the international project "Air pollution monitoring in the Mediterranean region" and created a regional database on concentrations of

particulate matter (PM) and heavy metal content in particles. The Institute hosted a training course on heavy metal determination in suspended particulate matter in Zagreb between 16 and 20 May 2005. IAEA in cooperation with the Institute of Nuclear Physics, Tirana, Albania organised a regional training course on "Validation, Evaluation, and Interpretation of Data Generated from Airborne Particulate Matter Measurements" in Tirana, Albania 23-27 June 2008.

In Zagreb, particle mass concentrations of suspended particulates were determined using the Gent air sampler. Twenty-four-hour samples were collected and mass concentrations for coarse and fine particle fractions determined and compared for the two seasons. This was followed by elemental composition analysis using energy dispersive polarised x-ray fluorescence (ED(P)XRF)-spectrometry with four secondary polarising excitation targets (analyses performed at IAEA Laboratories, Seibersdorf, Austria). We also presented and compared concentrations of the following elements Al, Si, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Cu, Zn, Pb, and Br. In order to analyse elements with low atom numbers, PIXE technique was used (analyses performed at the Ruđer Bošković Institute Zagreb).

Two training courses were organised related to the monitoring of radionuclides in the air: "Regional Training Course on Gamma Spectrometry for Monitoring of Radionuclides in Air" 10-14 April 2006, Aghia Paraskevi, Greece and "Regional Training Course on Advanced Gamma Spectrometry and Total Alpha/Beta Activity Measurement for Monitoring of Radionuclides in Air" 9-13 June 2008, Belgrade, Serbia.

An important outcome of the project was the creation of two regional databases, one related to particulate matter and heavy metal content in airborne particles and the other related to the radionuclides in the air.

Krešimir Šega

Carcinogenic and potentially carcinogenic pollutants in the air

Principal investigator: Vladimira Vadić, 2007-; collaborators: A. Šišović, M. Čačković, G. Pehnc, S. Žužul; project supported by MoSES

Literature sources were studied on arsenic, its distribution in the environment, population exposure, and the means of sampling from different environmental media, methods for determination and metabolism in humans.

Method for determination of arsenic levels in PM₁₀ particles in the air was studied in the laboratory. Arsenic concentrations were determined from samples of PM₁₀ particles using atomic absorption spectroscopy. Filters were destroyed by acid and

samples analysed using graphite and hybrid techniques. The obtained results were compared, and hybrid technique chosen for arsenic determination.

Destruction of samples by microwaves was tested in order to determine optimal conditions for the samples preparation. The investigation will continue with analysis of arsenic using inductively coupled plasma mass spectrometry (ICP-MS).

Arsenic in PM₁₀ particles was collected around Molve gass field at four measuring locations. Samples

of soil were also collected at the same measuring sites, and arsenic concentrations in glaysol, glaysol distric and glaysol vetric soil were high. The results showed that arsenic below limit values (C162).

Arsenic levels were investigated in PM₁₀ particles at five measuring sites in Zagreb. The study included horizontal and vertical distribution and seasonal variations.

Polycyclic aromatic hydrocarbons (PAH) (Flu, Pyr, BbF, BkF, BaP, BghiP and Ind) and their behaviour in the air were studied. Determination of BaAnt, Chrys, Phen and Ant started at one measuring site in the northern part of Zagreb (A247, C133, C135).

PAHs were measured at the "Podravina" gas field in the vicinity of Molve central gas plant during spring and summer seasons at four measuring sites (A95, C43).

Heavy metal composition in PM₁₀ particles was determined and concentrations were compared with the concentrations of TSP. In comparison with lead in TSP, 91-97 % of lead was found in the small PM₁₀ particles (A17, A18, C19, C53, C134, C156, C157).

Chemical composition of PM₁₀ and PM_{2.5} particles was studied with respect to the content of acidic components resulting from oxidation of primary and secondary pollutants in the air. Concentrations of acidic components were the highest in small particles.

Chemical composition of TSP and PM₁₀ particles was also studied with respect to acid components as indicators of pollutant sources. Annual average mass ratios of nitrates and sulphates found in TSP and PM₁₀ particles suggested that mobile source emission was an important contribution to air pollution by the particles mass. The results showed evidently higher contributions of nitrates and sulphates to overall PM₁₀ particles mass compared to TSP, while chlorides contributed slightly to the overall TSP mass (A49, C17, C18).

There is an ongoing study of photochemical smog

and oxidant levels in the air. The main constituent of photochemical smog is ozone. Ozone has direct harmful health effects. Due to its role in atmospheric chemistry, hydrogen peroxide is suggested as a better indicator of atmospheric oxidising capacity than ozone. Methods that successfully removed industrial SO₂ have led to the increase in H₂O₂ levels in the world and in Croatia. In the 1960s and 1970s, high levels of SO₂ and black smoke were measured in the air of Zagreb. However, over the last twenty years concentrations of SO₂, black smoke and total suspended matter have decreased, mainly as the result of lower coal consumption and introduction of natural gas for heating. The first H₂O₂ and ozone measurements in Croatia showed that both pollutants had characteristic daily variations with the highest values between 15:00 and 16:00 hours and between 14:00 and 17:00 hours, respectively. Over the day H₂O₂ volume fractions showed positive correlation with ozone ($r = 0.4$) and solar radiation ($r = 0.52$) and weak negative correlation with NO₂, CO, and BTEX. The influence of ozone as a strong oxidant on the environment and its role in atmospheric chemistry has well been described in literature. Measurements of H₂O₂ have become limited worldwide because equipment is sophisticated and expensive. For this reason computation models are being developed that calculate oxidant levels under determined environmental conditions and have an important role in assessing photochemical reactions and atmospheric processes. In 2008, we continued an analysis of photochemical models comparing measured and modelled H₂O₂ and ozone values in order to find optimal modelling parameters. Further investigation will focus on oxidant level modelling at different locations, under different meteorological conditions, and for different seasons (A3, B74, B75, B128, C102, C103, E38).

Vladimira Vadić

Professional services

Monitoring of air quality in urban and industrial areas in Croatia was started in Zagreb during the 1960s by our Unit and regional authority. Since the early 1970s, air pollution monitoring has gradually been introduced to other Croatian towns by regional Institutes of Public Health together with the regional authorities. All regional monitoring networks use the same methodology for air quality monitoring and are connected in one common network.

Coordination between regional monitoring networks continued from 2004 to 2008. Air quality was monitored in 39 Croatian towns, but only sulphur dioxide, black smoke, and deposited matter were measured in almost all towns.

Over the last five years, our Unit has been measuring SO₂, black smoke, deposited matter, PM₁₀ and metals Pb, Cd, As, Ni, and Mn in PM₁₀, PM_{2.5}, NO_x, ozone, BaP, sulphates, and NH₃ in Zagreb. Total suspended particulate matter (TSP) and metals Pb, Cd, and Mn in TSP had been measured until 2005.

Since 1970, the Unit has been submitting to the Croatian Ministry of Health annual reports on air quality in Croatian towns, categoring it according to the Law on Air Quality Protection and the Ordinance on Recommended and Limit Air Quality Values. Since 2005, the Unit has also been submitting annual reports to the Croatian Environmental Agency.

The Environmental Hygiene Unit is a WHO-

collaborating laboratory for air pollution and participates in the Global Environmental Monitoring System within the United Nations Environmental Programme. Every year, the Unit participates in QA/QC audit within the GEMS/AIR Programme.

Since 1991, the Unit has been included in the ecological monitoring at gas field Molve. H₂S, mercaptans, and mercury in the air, monitoring of pollutants in water and soil, and vegetation (used for animal food and forest vegetation) have been monitored by a large multidisciplinary team of researchers from the Institute for Medical Research and Occupational Health, Faculty of Agriculture, Faculty of Veterinary Medicine, Faculty of Forestry, and Institute of Public Health in Koprivnica of Koprivnica-Križevci county.

For one month of every season, the Unit monitors ammonia, hydrogen sulphide, mercaptans, and

meteorological parameters at the Zagreb central water purification system (CUPOVZ).

Air quality monitoring is going on in Potpićan which includes measurements of deposited matter, heavy metals, sulphates, ammonia, formaldehyde, and phenols.

PM₁₀, heavy metals, and BaP in PM₁₀ are measured at the measuring sites Zagreb-1 and Sisak-1 of the national monitoring network.

In 2008, the Unit started to measure PM₁₀ and heavy metals at a measuring site Galdovo in Sisak.

Measurements of deposited matter and heavy metals in it were performed in cement production plant Našicecement in Našice, in Slunj and in Vinkovci.

Vladimira Vađić

Molecular Toxicology Unit

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Research activities

Mechanisms of heavy metal toxicity in the kidney and reproductive tract

Principal investigator: Ivan Sabolić, 2002-2006; collaborators: C. M. Herak-Kramberger, M. Ljubojević, M. Škarica, E. Heršak, K. Šmaguc; project supported by MoSES

Mammalian renal transporters: gender differences and effects of toxic metals

Principal investigator: Ivan Sabolić, 2007-; collaborators: D. Breljak, M. Ljubojević, D. Balen, H. Brzica, N. Radović (Clinical Hospital "Dubrava" Zagreb); project supported by MoSES

In accordance with the proposed projects, several thematic groups were elaborated between 2004 and 2008. The data obtained were published in 14 peer-reviewed papers, 2 PhD theses, 4 BSc theses, 6 book chapters, and were reported at 23 national and 20 international congresses.

Localisation and sex differences (SD) in the expression and function of various transporters of organic anions (OA) and glucose were studied in kidneys and in a few other organs of rats and mice. OA transporters Oat1 (Slc22a6) and Oat3 (Slc22a8) were localised to the basolateral membrane (BLM) in specific nephron segments of the rat kidney (A151). In the cortex, the expression of both transporters exhibited SD [males (M) > females (F)] due to androgen stimulation and oestrogen inhibition. Furthermore, the expression of Oat2 (Slc22a7) protein and mRNA was studied in kidneys of variously treated rats and mice (A152). In adult rats, Oat2 mRNA was the highest in the outer stripe, whereas the protein was localised to the brush-border membrane (BBM) of the proximal tubule (PT) straight segment (S3), where it exhibited the F-dominant SD due to testosterone inhibition and oestradiol and progesterone stimulation. In mice, the expression pattern resembled that in rats. In other studies, the expression of Oats in rat, mouse, and human organs correlated with their function. Oat1 and Oat3 were localised and their roles in transporting serotonin metabolites characterised in the mouse brain and kidneys. Various serotonin metabolites proved to be good substrates for both Oats (A6). Furthermore, studies in cultured human adrenocortical cell line

showed that some cAMP-effectors can stimulate cortisol release by enhancing the expression of key enzymes of steroid biosynthesis and OAT3 (A4). In another two studies we re-evaluated the previously poorly known renal transporters from the SLC22 family in humans. A previously "unknown solute transporter 1" (UST1; *SLC22A9*) was characterised, found to operate as a multispecific transporter of OA in various renal tubules, and renamed to OAT8 (A270). The second transporter, hORCTL3 (*SLC22A13*), previously assumed to transport organic cations in humans, was proven to transport OA in PT and small intestine, and was renamed to OAT10 (A7). Finally, localisation and SD in the expression of Na⁺-glucose cotransporter SGLT1 were re-evaluated in the rat kidney, intestine, and a few other organs (A8, A228). The expression of SGLT1 protein along the nephron was correlated with its function and mRNA abundance; the transporter was largely localised to the BBM of S3, and exhibited SD (F>M) due to androgen inhibition. Beside in PT and small intestine, SGLT1 was detected in a few novel sites in the intestine, kidney, liver, and submandibular gland.

The effects of hypoxia and metal toxicity on the structure and function of the epithelium along the rat nephron were tested in studies *in vitro* and *in vivo*. Functional and structural stability of the kidney cortex slices was tested in optimal experimental conditions *in vitro* (A40). Impaired accumulation of *p*-aminohippurate in the tissue, loss of various membrane transporters, and loss of cytoskeleton and polarity in PT cells indicated a fast structural and functional deterioration of the slices incubated *in vitro*

for more than one hour, probably due to prolonged hypoxia. The effect of cadmium (Cd) and several other metals on the kidney structure, function, and expression of membrane transporters was tested in experimental nephrotoxicity in rats. Lipid organisation and dynamics was studied in various organelles isolated from the kidney cortex of control and Cd-treated rats (A282); Cd increased the lipid mobility and permeability for potassium without affecting the permeability for protons. We then compared the expression and cellular distribution of several BLM transporters, and the integrity of cytoskeleton in PT cells in acute and subchronic models of Cd nephrotoxicity (A227). PT of Cd-intoxicated rats exhibited damaged morphology and cytoskeleton, and loss of BLM invaginations and Na/K-ATPase. In a recent study in rats treated with various metals, the expression of P-glycoprotein Mdr1 (*Abcb1*) in the PT BBM was compared with accumulation of these metals in the renal cortex

(C114). Some accumulated metals (Cu, Mn, Zn, Ca, Mg, Al) did not affect, while cis-Pt, Pb, Cd, and Hg strongly enhanced the Mdr1 expression.

Various data related to SD in the mammalian kidney and metal nephrotoxicity were collected and discussed in two invited reviews. One (A230) described numerous information on sex-related differences in sex hormone receptors, organ morphology, enzyme activities, functions, transport of specific organic compounds, expression of membrane transporters, and toxicity in the kidneys of humans and experimental animals, and the other (A229) gave a summary of known mechanisms of nephrotoxicity, common to various metals. Current data indicate that toxic metals accumulate largely in the PT cells and cause oxidative stress-mediated functional and structural damage that results in reabsorptive and secretory defects.

Ivan Sabolić

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Research activities

Biomarkers of the cell genome damage in ecogenetic research

Principal investigator: Vera Garaj Vrhovac, 2002-2006; collaborators: D. Želježić, M. Đurinec, M. Milas, G. Tokić, M. Nikolić; project supported by MoSES

Mutagens and antimutagens in ecogenetic research

Principal investigator: Vera Garaj Vrhovac, 2007-, Collaborators: G. Gajski, M. Gavella (University Clinic Vuk Vrhovac Zagreb), B. Šarčević (University Hospital for Tumors Zagreb), V. Brumen (Andrija Štampar School of Public Health Zagreb), B. Šimpraga (Croatian Veterinary Institute Zagreb), T. Viculin (University Hospital for Tumors Zagreb); project supported by MoSES

The aim of our ecogenetic researches has been to determine mutagenicity and evaluate the risk associated with exposure to physical and chemical agents from occupational and living environment. Using *in vivo* and *in vitro* models, we evaluated the effects of chemical mutagens and ionising and non-ionising radiation on human genome, and evaluated antimutagenic properties of some natural compounds. We used different cytogenetic techniques such as analysis of chromosome aberration, sister-chromatid exchange analysis, micronucleus test, chromatid breakage assay, DNA diffusion assay, comet assay and its modifications. We evaluated the genotoxic and cytotoxic effects of low doses of pesticides and their metabolites on human peripheral blood lymphocytes and on different organ cells of experimental animals *in vitro* (A83, A272, A273, A274, A275, A276).

Chromosome aberrations as biomarkers of genome damage induced by radioactive isotopes, pesticides and their metabolites, drugs or cytostatics showed a strong deviation from control values and also showed interindividual differences which depended on mutagen concentrations and exposure time (A88, A119, A121, B31).

Recent micronucleus test measurements of cytogenetic damage were evaluated using new scoring criteria in addition to the micronucleus frequency; these include the number of nuclear bridges and buds

and other new biomarkers of cell damage. This measuring enables differentiation of clastogenic and aneugenic mode of action (A82, A91, A92.).

The comet assay based on the microgel electrophoresis of a single cell is a reliable, reproducible, and quick technique, and has proven useful for cytogenetic monitoring of effects *in vitro* in different cultures, experimental animals, and populations exposed to different chemical or physical mutagens at really low exposure doses. Using the comet assay in alkali conditions, we measured the level of primary DNA damage in leukocytes of occupationally exposed populations to physical agents (ultrasound, microwaves, X-rays) and to chemical agents such as tobacco dust. These tests showed a significant difference from control values. In addition to alkali conditions, the comet assay was also performed in a neutral medium giving an insight into different types of DNA damage in human cells such as sperm cells (A87, A89, A90, A120, A122, A235, A236, C33, C42).

We studied oxidative stress as possible mechanism of genomic alterations with a modification of the comet assay using formamidopyrimidine glycosylase (A85).

We tested the cytotoxicity of honeybee venom from *Apis mellifera* and of its two major constituents melittin and phospholipase A in different types of

tumor cells, including human laryngeal and cervical carcinoma cells and their drug resistant sublines, breast adenocarcinoma cells, colon adenocarcinoma cells, and glioblastoma, as well as in human embryonic kidney cells, normal fibroblasts, and

leukocytes to determine the exact mechanism of action of this natural compound (A84).

Verica Garaj Vrhovac

Genotoxicity of natural and anthropogenic agents

Principal investigator: Vilena Kašuba, 2002-2006; collaborators: R. Rozgaj, N. Kopjar, M. Milić; project supported by MoSES

Genotoxicity of chemical and physical agents of natural and anthropogenic origin

Principal investigator: Vilena Kašuba, 2007-; collaborators: A. Fučić, R. Rozgaj, N. Kopjar, D. Želježić, M. Milić, M. Mladinić, S. Pepeljnjak (Faculty of Pharmacy and Biochemistry, University of Zagreb), I. Milas (University Hospital for Tumors Zagreb), V. Pavlica (University Hospital for Tumors Zagreb), F. Stipoljev (General Hospital "Sveti Duh" Zagreb); project supported by MoSES

The main focus of our research done over the last five years was genotoxic effects of man-made chemical and physical agents.

Occupational exposure to ionising radiation (A78, A91, A81, A121, B31, C39, C57, C58) and ultrasound (A78, A90) was confirmed to significantly increase chromosomal aberrations. In unexposed population cytogenetic damage was assessed using the micronucleus assay (MN) (A91), structural chromosomal aberration (CA) test, and alkaline comet assay (A119). A follow-up study has shown that increased MN frequency and CA in peripheral blood lymphocytes can help to predict the risk of cancer in humans (A24-A26, A77). We published a review of genomic damage in children accidentally exposed to ionising radiation (A79), and estimated the developmental and transplacental genotoxic effects of fluconazole (A80).

Using centromeric probes and the fluorescence *in situ* hybridisation (FISH) technique, we have found out that a portion of micronuclei containing centromeric region significantly increased after long-term exposure to carbofuran (A277). To assess the genome damage in tobacco industry workers, we used the MN, SCE, and CA tests. It was significantly increased, and smoking showed an additive effect to SCE values. Higher risk was seen in women (A167).

We also estimated the genome damage in cancer patients receiving radiotherapy (A87, A88, C35) and adjuvant chemotherapy (A120, A122). The effects of therapeutic irinotecan concentrations on human leukocytes were assessed using the alkali and neutral comet assay, fluorescence cell-survival assay, MN assay, CA assay, SCE, and FISH. We additionally measured the activity of acetylcholinesterase (AChE) in erythrocytes. Our results confirm irinotecan cyto- and genotoxicity, and its strong inhibitory effect on AChE. Oxime HI-6 has been found to reduce the inhibitory effect of irinotecan, with an acceptable level of cyto/genotoxicity to human cells (A123, A216). Antidotal effects of tenocyclidine (TCP) were

also investigated and primary DNA damage of leukocytes and brain cells assessed in rats treated with sublethal doses of soman. Good protective ability of TCP to AChE was confirmed, with an acceptable level of cyto/genotoxicity (A154, A203, A215).

We used the alkaline comet assay to investigate DNA damage in different rat cells and organs (brain, liver, kidney). Adamantyl derivative TAMORF proved to be much more successful in alleviating poisoning symptoms in rats than physostigmine. These antidotes were not toxic for rats in tested concentrations (A154). Using cytogenetic and molecular markers in peripheral human blood lymphocytes *in vitro*, we have established that very low concentrations of tryptophol can cause apoptosis, DNA and chromosome damage, and can slow down cell division (A129).

We also studied the influence of different polymerisation modes by LED curing units on the toxicity of dental composite materials in human lymphocytes *in vitro* (A118). Mutagenic, cytogenetic, and genotoxic effects of waste waters from a phosphogypsum facility were investigated on bacterial and cell lines (A192). Using the alkaline comet assay, we assessed the integrity of DNA in erythrocytes of fish *Cobitis elongata* affected by water pollution (A124). The comet assay was used to investigate the ability of fumonizine B₁ (FB₁) to induce DNA damage in rat kidney cells. A simultaneous increase in the sphinganine:sphingosine ratio points out the metabolic cohesion of sphingolipids with FB₁ genotoxicity (A59, A61, A242). We have proven the genotoxic effects of FB₁, beauvericin, and ochratoxin A, alone or in combination, in porcine kidney PK15 cells (A242).

To see how aluminium affects genome integrity we tested its effects in green and brown hydra. We observed a high level of predictivity with the comet assay, which depended on aluminium concentration in water. These results point out possible application of hydra as a bioindicator of water pollution (A133).

Lead acetate genotoxicity in suckling rats were estimated (A115). The effects of amiphostine and melatonin on human peripheral blood lymphocytes were investigated using the MN assay *in vitro*. In these experimental conditions no genotoxicity of these radioprotectors was detected. This calls for further investigation of these compounds *in vivo* and of their potential use in the clinic (A12, B45). The radioprotective effects of propolis and quercetin in gamma-irradiated mice and *in vitro* were evaluated using the alkaline comet assay (A11, A12, A13, A190, A193). The cytotoxicity of low-molecular

weight metabolites of yeast *Candida albicans* was also estimated (A128).

Herbicide norflurazone was tested for toxic effects in freshwater invertebrate *Polyces felina* Daly using the alkaline comet assay. It induced higher mortality, changes in mobility, as well as morphological and histological changes in treated versus control animals, and increased DNA damage in planarian cells (A102). The same invertebrate was also investigated in toxicological and cytogenetic studies (A111-A113).

Vilena Kašuba

Professional services

The Mutagenesis Unit has been providing biomonitoring and occupational exposure assessment services and advice to individuals, institutions, and public authorities for more than 25 years. Cytogenetic analyses are regularly performed for workers exposed to physical mutagens (ionising and nonionising radiation) and mutagenic/carcinogenic chemicals in healthcare institutions and industry. Over the last five years the number of cytogenetic analyses has varied as follows: 2004: 970 analyses; 2005: 591 analyses; 2006: 359 analyses; 2007: 345 analyses and 2008: 135 analyses. The drop reflects changes in national regulatory requirements over this period.

The methodology and protocols used in cytogenetic analyses are in accordance with current International Atomic Energy Agency (IAEA)

Technical reports and International Programme on Chemical Safety (IPCS) guidelines for the monitoring of carcinogens in humans. Biomarkers used in human biomonitoring and *in vitro* studies are structural chromosome aberrations, sister chromatid exchange (SCE) analysis and cytokinesis-block micronucleus (CBMN) assay in peripheral blood lymphocytes; fluorescence *in situ* hybridisation (FISH), and comet assay or single cell gel electrophoresis. *In vivo* rodent micronucleus assay is also used for experimental purposes and in animal studies. In the Mutagenesis Unit expert services have also been provided in cytogenetic testing of different drugs.

Nevenka Kopjar

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Research activities

Allergic disorders of the respiratory system and skin

Principal investigator: Božica Kanceljak-Macan, 2002-2005, Jelena Macan, 2006; collaborators: S. Milković-Kraus, D. Plavec, J. Macan, A. Gudelj-Gračanin, K. Janković; project supported by MoSES

Allergotoxic effects of general and occupational environment

Principal investigator: Jelena Macan, 2007-; collaborators: S. Milković-Kraus, Lj. Prester, V. M. Varnai, I. Maloča, A. Ljubičić Čalušić, I. Sabolić Pipinić, B. Kanceljak-Macan, M. Vučemilo (Faculty of Veterinary Medicine, University of Zagreb), S. Cvitanović (Clinical Hospital Center Split), A. Čavlović (Faculty of Forestry, University of Zagreb); project supported by MoSES

Between 2004 and 2008, our research included a retrospective cohort study evaluating the prevalence of atopy markers in the period 1985-1999, assessment of exposure to common and occupational allergens in inland and coastal Croatia, and exposure to histamine in living and working environments.

Retrospective cohort study of atopy marker prevalence in Croatian adult population between 1985 and 1999

We analysed a 15-year trend in the prevalence of atopy markers (elevated total IgE, positive skin prick test to common aeroallergens and positive atopic symptoms) for 721 Croatian adults (445 men and 276 women) examined for allergies within a pre-employment screening scheme. The study showed an increasing trend in the prevalence of elevated total IgE combined with positive atopic symptoms in men (OR 2.44, 95%CI 1.39-4.29, $p=0.002$) but not in women (A158).

Exposure to common and occupational allergens in inland and coastal Croatia

In samples of settled dust, gathered in households and working environments from inland and coastal parts of Croatia, we determined the type of dust mites and allergen levels of pyroglyphid mites. Enzyme immunoassay (ELISA) was introduced to determine *Dermatophagoides pteronyssinus* (Der p 1) and *Dermatophagoides farinae* (Der f 1) mite allergens. ELISA showed satisfactory precision (intra-assay CV<6.9%, inter-assay CV<13.3%), accuracy (91-93%), and sensitivity (2 ng/mL) (B86). Analysis of dust samples from fishing boat cabins showed significant exposure to pyroglyphid mites. This is the first time that pyroglyphid mites have been identified as potential occupational allergens for fishermen. Non-pyroglyphid mites have been confirmed as occupational allergens in various rural environments like barley, hay, animal food warehouses, and pigsties (B54).

We measured August-October levels of *Ambrosia elatior* pollen in air samples taken in the south of Croatia (Sinj and Split areas), and its proportion in total pollen count peaked in the first week of September with 12 %. During this peak, patients sensitised to *A. elatior* reported a significantly higher intensity of allergic rhinitis and asthma symptoms than in the beginning of the pollen season, confirming the significance of this pollen in the incidence of pollen allergies for the inhabitants of the south of Croatia (A47).

Azithromycin was identified and described as a new occupational allergen. We described a case series including clinical characteristics, diagnostic pathways, and risk factors in occupational allergic contact dermatitis caused by azithromycin in pharmaceutical workers. Results suggest that daily manipulation with powdered azithromycin and intermediates is a main route of sensitisation. In addition, workers reported work-related symptoms of urticaria, rhinoconjunctivitis, laryngitis, and/or dyspnoea, which might be related to azithromycin hypersensitivity (A177).

Allergy testing in a group of workers from tobacco industry (cigarette manufacture) established tobacco allergens and non-pyroglyphid mites as occupational allergens in this industry (A279).

Protective working clothes were evaluated as possible cause of skin disorders among industrial workers, including allergic and irritative textile

dermatitis as an inflammatory skin disease related to wearing clothes or other aspects of manipulation with textile (production, processing, maintenance, and sale) (B55). An investigation of the cause of skin eruptions connected with wearing protective clothing in a group of female industrial workers confirmed that irritative contact dermatitis was caused by chemical irritation by residual alkali in the clothing after washing and by mechanical irritation (A214).

Exposure to histamine in living and occupational environment

Histamine was analysed as an environmental toxin and not as an endogenous mediator of allergic reactions. Initially, histamine from fish flour was identified as the cause of occupational poisoning in a group of harbour workers. For the next 40 months histamine levels were monitored in samples of stored fish flour. Histamine maintained highly toxic level (>2 g/kg) if stored at 4-8 °C for more than 3 years. If stored at ambient temperature (24 °C) and unprotected from light these levels were unchanged or maintained for at least 3 months. The results confirmed our earlier hypothesis that fishmeals with high content of histamine are a potential long-term health hazard for poultry and other animals if used as feed, and also for men manipulating it (A157).

Jelena Macan

Occupational stressors and workers' health

Principal investigator: Milica Gomzi, 2002-2006; collaborators: Jasminka Bobić, Rajka Luzar, project supported by MoSES

In 2004-2006, our research project attempted to identify numerous health symptoms and work-related disorders and injuries in relation to age, kind of activity (woodworking industry, cement production, office work), exposure to biomechanical loads, and stress at work. Our findings indicate a subtle but powerful association between stress, health, and physical exercise. Stress associated with depression encourages fatigue and lowers resistance to illness. In further research, we focused on the nature and significance of workers' mental models of coping with stress. In workers professionally exposed to xylene, frequent complaints about loss of memory correspond to the length of exposure but not to the standardised psychological tests results (A21).

Workplace violence is a serious occupational hazard and public health issue. For injured and threatened workers, workplace violence can lead to medical treatment, absence from work, and decreased productivity (B32).

The prevalence of Post Traumatic Stress Disorder (PTSD) symptom clusters was examined in armed robbery victims (AR) at their work place and in male ex-prisoners of war (ex-POW). The results indicate

greater occurrence of full PTSD in the AR group, as well as re-experience and arousal symptoms combination in those with partial PTSD, while the ex-POW group dominantly showed arousal symptoms as isolated cluster, followed by a combination of re-experience and arousal symptoms (A22). The colour preference on Luescher Colour Test was analysed in male ex-prisoners of war with PTSD. Increased preferences for violet and green and decreased preference for red were discussed in terms of "coping strategies" and "projective hypotheses" (A23).

Passive smoking increases sensitivity to environmental irritants and respiratory infection. Our follow-up study has demonstrated a significant relationship between household smoking and rates of doctor-diagnosed upper respiratory illness in schoolchildren exposed to tobacco smoke in families in which both parents are smokers (B83).

General physical environment of a workplace is associated with a risk of health disorders. We found that those who rated their physical environment (temperature, air quality, lighting, and acoustics) as poor had increased prevalence of health symptoms. Tight, mechanically ventilated working and living

space as well as daily noise may cause discomfort and health complaints (B34).

Plant noise exposure brings a serious risk of hearing disorders and injuries in woodworkers. The relation between the level of noise exposure and mean injury rate differed between the kinds of activities which led to injury, that is, whether the involved material handling or use of power tools. Although use of hearing protection should be encouraged, replacing and modifying equipment to decrease noise at the source should be the first priority. However, when resources are limited, we recommend that education about injuries and interventions should target younger working population, those who work longer hours, power tool operators, and particularly workers who have physical impairments (B33).

Musculoskeletal work-related disorders are extremely common as a result of repetitive movements, awkward postures, force, and other occupational conditions and ergonomic hazards. The effect of prolonged work in a restricted and/or standing position is multiplied by the presence of high levels of muscular tension and job strain during work (B35).

This research will continue by examining the effects of physical, psychosocial, and ergonomic factors on workers' safety and health of the musculoskeletal system.

Milica Gomzi

Effects of general and work environment on the musculo-skeletal system

Principal investigator: Selma Cvijetić Avdagić, 2007-; collaborators: J. Bobić, M. Gomzi, M. Blanuša, S. Grazio (Clinical Hospital "Sestre milosrdnice" Zagreb), L. Krapac (Polyclinic for Rheumatic Diseases, Physical Medicine and Rehabilitation "Dr. Drago Čop" Zagreb); project supported by MoSES

Epidemiological data show that the prevalence of osteoporosis in Croatia is similar to other European countries, while the prevalence and incidence of osteoporotic fractures is slightly higher (D8, B16, B17). Our study on geographical and racial differences in bone mineral density (BMD) showed distinct global differences in BMD in the hip and spine BMD both in men and women, approximating one standard deviation between populations with the highest and lowest BMD. These differences persisted after adjustment for age, sex, and body size (A166).

The influence of age, anthropometry, and postmenopause on calculated bone mineral apparent density (BMAD) was investigated in a large number of healthy subjects (A44). The results suggested that BMD depended on the bone size and body size, and that different BMD could be the consequence of difference in these parameters. The assessment of BMD in a healthy female student population showed that the peak bone mass was achieved before the age of twenty, but later in the long-bone cortical skeleton (A46). Body weight and physical activity were the most significant positive predictors of bone density in all measured sites.

As the recurrent calcium urolithiasis is often associated with calcium metabolism disorders, BMD was assessed in patients with urolithiasis in order to determine factors that could have influenced the changes in bone density over a one year period (B15). Bone mass reduction in patients with urolithiasis did not differ significantly from controls, and was mostly related to age, hyperuricosuria, and calcium dietary restriction, but not to increased calcium excretion. Assessment of metabolic parameters in patients with urolithiasis showed 25 % higher mean ionic activity

product of calcium and oxalate ions IAP(CaOx) than in controls (A5, B2). IAP(CaOx) and calculated Ogawa indices were recommended for estimating the potential risk of calcium oxalate stone formation.

We analysed the association of nutrients and lifestyle modifiers with BMD in healthy postmenopausal women. Moderate alcohol consumption was positively associated with bone mass, while mineral water and high fibre intake were beneficial for weight and body mass index. Significant differences in dietary habits were found in schoolchildren and students in relation to high or low physical activity (A42).

Progression of hand osteoarthritis in rural population aged 54 to 56 years was the most prominent in the distal interphalangeal joints in women and in the proximal interphalangeal joints in men. Mechanical factors, probably occupational workload, were associated with the more rapid progression of the disease (A45).

Our analysis of proprioception in people with whiplash injury showed that they incorrectly perceived the position of their own head (A263). Therefore, rehabilitation should include the correction of proprioception and head coordination. Monitoring recovery of femoral muscles after knee injury using ultrasound measurement of muscular volume, showed that ultrasound is an appropriate method for determining muscular atrophy during immobilisation and also for evaluating muscular restitution during physical therapy (A262).

Selma Cvijetić Avdagić

Problem sleepiness: psychophysiological and behavioural aspects

Principal investigator: Biserka Radošević Vidaček, 2002-2006; collaborators: A. Koščec-Đuknić, M. Bakotić; project supported by MoSES

Problem sleepiness: sociocultural, behavioural and psychophysiological aspects

Principal investigator: Biserka Radošević Vidaček, 2007-; collaborators: A. Koščec-Đuknić, M. Bakotić; project supported by MoSES

Research on problem sleepiness mainly shifted our interest from the context of working time to the context of school time. However, both working time and school time, which were the focus of our studies, had a common aspect: the arrangement of time in shifts. The majority of our studies dealt with sleep and daytime functioning in a school system involving two rotating shifts. Such a school system is distinct from school systems in many other countries in which school always starts in the morning. Conclusions on relations between reduced and irregular sleep, delayed sleep phase and poor sleep quality on the one hand, and poor academic performance and daytime functioning in adolescents on the other, have so far been based on the results of studies conducted in countries in which school starts in the morning. As the two-shift school system can have a specific impact on sleep and wakefulness, we have started to study Croatian adolescents attending school one week in the morning and the other in the afternoon. The aim of these studies is to see if healthy sleep is possible in different school shifts and to identify developmental, psychological, sociocultural, and situational factors related to sleep characteristics and daytime functioning in adolescents.

Our field and laboratory studies of adolescents analysed developmental changes of morningness-eveningness preferences over adolescence; relative value of different sleep preferences and characteristics for prediction of indices of daytime functioning in adolescents; interindividual differences in paying off the sleep debt from morning shift days by means of napping or extension of main sleep in situations with less external constraints; intraindividual variations in sleep patterns and daytime sleepiness over a week of morning and a week of afternoon shifts; impact of living with parents on students' sleep patterns on weekdays and weekends; effect of different activities performed in bed before sleep start on characteristics of subsequent sleep; sleep parameters obtained for school days and weekends by means of two methods - sleep diaries and actigraphs; subjective and physiological indices of daytime sleepiness; and efficiency in performing tasks at the end of week with morning shift schedule and week with afternoon shift schedule (E20).

We synthesised the results of our studies that examined the relationship between sleep characteristics and daytime functioning in students attending school in two rotating shifts and compared them with a few other studies that examined adolescents attending school in two permanent or rotating shifts (B24).

In order to explore whether parents' engagement in shift work affected the sleep habits of their adolescent children who attended school in two shifts we analysed data of primary and secondary school students of both gender, age range 11-18 years, whose parents were both employed. Parents' working schedule significantly affected the sleep patterns of secondary school adolescents, i.e. wake-up time and sleep duration on school days when they attended school in the morning shift, as well as bed time and bed time delay on weekends. A significant interaction between parents' working schedule, adolescents' gender and type of school (primary vs. secondary) was found for sleep extension on weekends after afternoon shift school. This study showed that parental involvement in shift work had negative effects on sleep of secondary school adolescents. It contributed to earlier wake-up time and shorter sleep in a week when adolescents attended school in the morning, as well as to greater bedtime irregularity (A217).

The role of various biological, behavioural and social factors in regulation of sleep and wakefulness in adolescence was discussed. Different approaches attempting to implement results of studies examining daytime sleepiness of adolescents were examined (A132).

Various forms of sleep deprivation and the effects of sleep deprivation on recovery sleep, physiological parameters, daytime sleepiness and performance in cognitive and psychomotor tasks were discussed. Special consideration was given to importance of individual differences in some characteristics related to sleep and wakefulness, like morningness-eveningness, sleep need, individual level of sleepiness, the ability to fall asleep quickly, and so called "vulnerability to sleep loss", which may be of practical importance for predicting the effects of sleep deprivation in everyday life (B3).

In addition to studies of sleep and wakefulness in the context of two-shift school system, we studied 24-hour variations of subjective activation. The study examined the validity of R. Thayer's activation model regarding 24-hour variations of two dimensions of activation (energetic and tense arousal) and their relations with variations of indices of physiological activation and performance efficiency. The activation was measured by means of Thayer's Activation-Deactivation Adjective Check List, which uses descriptors such as sleepy, drowsy, wide-awake, tired, wakeful, lively, and energetic to measure energetic arousal, and descriptors such as tense, intense, fearful, jittery, clutched up, still, and calm to measure tense

arousal. The study showed that the 24-hour variation of energy had a strong circadian component, which was related to variation of body temperature. A circadian component was not observed in 24-hour variations of tension. The results also indicated that in conditions when energy and tension varied within a normal circadian range of values higher energy was

associated with lower tension. These findings were not consistent with Thayer's hypothesis that energy and tension are positively associated at low and intermediate levels of activation (A131).

Biserka Radošević Vidaček

Dementia: electrophysiological and genetic study

Principal investigator: Rajka Liščić, 2002-2006; collaborator: M. Poduje; project supported by MoSES

Frontotemporal dementia with motor neuron disease

Principal investigator: Rajka Liščić, 2007-2008; Croatian-Slovenian bilateral cooperation programme in the field of science and technology

Frontotemporal dementia

Principal investigator: Rajka Liščić, 2007-; collaborators: S. Kovačić (General Hospital Zabok), I. Martinić Popović (Clinical Hospital "Sestre milosrdnice", Zagreb); project supported by MoSES

Within the project *Dementia: Electrophysiological and Genetic Study*, we described a new neurophysiological method for the registration of the Stroop Color-Word Test in healthy volunteers in collaboration with the Institute for Clinical Neurophysiology, University Medical Centre Ljubljana. The Stroop Color-Word Test is used for investigating frontal lobes, and has been modified for electrophysiological diagnosis of dementia.

Frontotemporal dementia (FTD) is the second most common form of dementia in people under the age of 65 years after Alzheimer's disease. Therefore, within the last two projects we focused our efforts on FTD, a focal, non-Alzheimer form of dementia, clinically characterised as behavioural or aphasic variant, and later in the course of the disease with dementia and parkinsonism. Clinical FTD may be associated with a variety of different neuropathological entities. During my 12-month Fulbright scholarship we described a new mutation on granulin (GRN) gene in hereditary dysphasic disinhibition dementia family 2 (HDDD2), a variety of FTD, in collaboration with Washington University, School of Medicine, St. Louis, MO, USA (A185). It describes the molecular genetic cause of familial frontotemporal lobar degeneration (FTLD) with ubiquitin positive inclusions (FTLD-U), and is a major scientific discovery that helps to understand the mechanisms and the way progranulin mutation might cause familial FTLD. We also performed a retrospective analysis of neuropathology of FTLD (1988-2005) according to the consensus criteria by McKhann and colleagues (2001), and recommended an extension of the McKhann's criteria. Very important for the clinical work is our finding of clinical and psychometric differences between Alzheimer's disease and FTD in an early stage of the

disease, again in collaboration with Washington University School of Medicine, St. Louis, MO, USA (A149). We described another new mutation on GRN gene (A10) in Hereditary Dysphasia Disinhibition Dementia 1 (HDDD1), which is another form of FTLD. This important discovery describes the molecular genetic cause of familial dementia and helps to understand the mechanisms and the way GRN mutation might cause familial FTLD.

Major discoveries have been made in the recent past in genetics, biochemistry, and neuropathology of FTLD. TAR DNA-binding protein (TDP-43), encoded by the TARDBP gene, has been identified as the major pathological protein of FTLD, with ubiquitin-immunoreactive inclusions with or without familial amyotrophic lateral sclerosis (ALS) and sporadic ALS (A150). On the level of neurotransmitters, serotonergic and catecholaminergic systems regulate behavioural functions and are involved in the aetiology of polygenic neuropsychiatric disorders such as FTLD. Cognitive impairment in ALS has been demonstrated in up to 50 % of ALS patients. Therefore, our aim was to identify gene polymorphism coding for proteins involved in serotonin synthesis (tryptophan hydroxylase), catecholamine synthesis (DBH), active neurotransmitter uptake (catecholamine and serotonin transporter), and neurotransmitter degradation (MAO-A, MAO-B, COMT), with the emphasis on executive function in ALS patients. A sizable proportion of ALS patients' showed behavioural and cognitive changes within the spectrum of FTD impairment. However, further studies on a larger sample are needed to confirm it.

Rajka Liščić

Morbidity and mortality risks in adult Croatian population

Principal investigator: Mladen Pavlović, 2002-2006; collaborators: N. Čorović, M. Malinar; project supported by MoSES

Morbidity and mortality risk assessment in Croatian population: a follow-up study

Principal investigator: Mladen Pavlović, 2007-; collaborators: N. Čorović, D. Šimić (Central State Administrative Office for E-Croatia Zagreb); project supported by MoSES

Between 2004 and 2008 we continued with the fourth phase of a project investigating chronic diseases in Croatian population. Survival analysis in relation to cardiovascular mortality and smoking and coffee drinking habits (A197, A281, B72, B73). Apart from that, we continued to collect data on residential status and vital statistics about the subjects participating in our study on chronic illnesses. We relied on available data from local registry offices and parish offices, and on telephone contacts or contacts with participants' relatives. In 2005, the participants were queried about their health status, and their subjective feeling of health. Just as in earlier research, for ethical reasons we excluded persons who were not able to respond due to illness or who had been hospitalised and persons who changed residence or moved to old people's home. With our database updated, we asked the participants to take one more examination taking care that the sample remained representative.

The 2006-2007 medical examination was conducted in the same fashion as in 1972 and 1982. We extended the basic questionnaire on chronic illnesses that was designed according to the IX International Classification of Illnesses and Causes of Death with additional questions related to older age, the mini-mental state examination (MMSE) or SF-36 (CASP 19; Croatian version) for quality of life of older persons. We also performed a smell test for isopropyl alcohol. The rest of the questionnaire dealt with marital status, possession of driving license, residence, hours spent walking, and medicines used, classified into 11 categories according to the WHO Anatomical Therapeutic Chemical (ATC) classification. The part of the questionnaire on diet was extended to include Mediterranean food (olive oil, pelagic fish, river and lake fish, beer, wine,

spirits). Preparation activities for supplement to coding and electrocardiograph results were conducted as well as ventilatory function test according to predicted values for aged Croatian population. Consultations on additional anthropometric variables as height and weight, waist circumference and thorax circumference with difference according to inhale was introduced.

We also conducted preparation activities for ultrasound densitometry of the heel bone for participants from Zagreb, Omiš, and Split. In the autumn of 2006 and 2007 we clinically examined 396 of 754 invited participants who were earlier examined in 1969, 1972, and 1982. Analysis of lipids and basic biochemical tests including creatinine, alkaline phosphatase, activity of alpha 1-antitrypsin, proteins in serum, acidum uricum, CRP, urea, gamma GT, sugar in blood, calcium, and phosphor in serum, and urine ketones, proteins and glucose were performed at the Institute for Clinical and Experimental Medicine, Prague. Selenium, zinc and copper concentration as well as the activity of glutathione peroxidase were measured in blood samples collected from participants from Vis, Virovitica, Zagreb, Split, and Omiš. Prostate-specific antigen was also determined in the serum of men. All data were entered into databases under a code number that had existed since 1969 to protect participant's privacy. We are about to publishing preliminary data and subjective health assessment along with data related to the Mediterranean diet and metabolic syndrome. Preliminary results indicate statistically significant regional differences in serum selenium and zinc.

Mladen Pavlović

Professional services

Between 2004 and 2008, the Unit of Occupational and Environmental Medicine performed medical examination of healthy subjects and patients within research activities, and operated as an outpatient clinic providing specific diagnostic services, and medical and psychological examinations related to occupational health. Medical procedures were focused on determining occupational diseases and assessing working ability. Particular attention was given to the evaluation of occupational allergic diseases.

Diagnostic procedures included bone densitometry, allergological testing, lung function testing, ECG, exercise testing, psychological examinations, and biochemical measurements. Additionally, Professional services of this Unit included court expertise in the field of occupational medicine, toxicology, and clinical psychology.

Jelena Macan

Radiation Dosimetry and Radiobiology Unit

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Research activities

Biomedical effects of radiofrequency microwave radiation

Principal investigator: Ivančica Trošić, 2002-2006; collaborators: M. Mataušić-Pišl, I. Pavičić, N. Horš; project supported by MoSES

Biological markers of activity of electromagnetic nonionising radiation

Principal investigator: Ivančica Trošić, 2007-; collaborators: S. Milković-Kraus, I. Pavičić, M. Suša, M. Mataušić-Pišl, I. Bušljeta Prusac (Belupo Pharmaceuticals and Cosmetics, Koprivnica), S. Pažanin (Institute for Marine Medicine, Split); project supported by MoSES

Exposure to radiofrequency microwave radiation (RF/MW) from electroenergetic and radar systems, RTV networks, telecommunications, and medical and household devices significantly exceeds naturally occurring exposure. Animal model studies might give a comprehensive insight into the biological effects of radiation, while cell culture research might provide a better look at action mechanisms. We used the erythrocyte micronucleus assay to investigate the extent of genetic damage in bone marrow red cells of rats exposed to RF/MW radiation and found that it increased the frequency of polychromatic erythrocytes (PCEs). RF/MW also had a transient effect on proliferation and maturation of erythropoietic cells in the rat bone marrow, and we observed sporadic appearance of micronucleated immature bone marrow red cells (A253). Further, to observe the erythropoietic changes in rats subchronically exposed to the nonthermal RF/MW irradiation, rats were submitted to the same experimental conditions as above in order to be irradiated for 2, 8, 15 and 30 days. Bone marrow smears were examined to determine absolute counts of anuclear cells and erythropoietic precursor cells. In the exposed animals, erythrocyte count, haemoglobin, and haematocrit increased in peripheral blood on irradiation days 8 and 15. Concurrently, anuclear cells and erythropoietic precursor cells significantly decreased in the bone marrow on day 15, but micronucleated cell frequency increased. In the applied experimental condition, RF/MW radiation might cause disturbance in red cell maturation and proliferation, and induce micronucleus

formation in erythropoietic cells (A38). An *in vivo* mammalian cytogenetic test (MN) was used to investigate the extent of genetic damage in bone marrow red cells of rats exposed to RF/MW radiation. Rats were exposed to a 2.45 GHz continuous RF/MW field for 2 h daily, 7 days a week, at a power density of 5-10 mW/cm². RF/MW irradiation has a transitory cytogenetic effect manifested by micronucleus formation in erythropoietic cells (A254). To evaluate the effects of radiation on erythropoiesis in rats exposed at whole body SAR of 1.25 ± 0.36 W/kg, we observed the kinetics of PCEs and micronucleated (MN) PCEs in bone marrow and peripheral blood. Rats were exposed to the frequency field of 2.45 GHz, SAR of 1.25 W/kg for 2 hours a day, 7 days a week. Obtained findings were considered to be indicators of radiation effects on bone marrow erythropoiesis that was consequently reflected in the peripheral blood. Dynamic haemopoietic was restored by the end of the experiment, which indicates possibility of activation process to the selected experimental conditions of subchronic RF/MW exposure (A257). We investigated the blood-forming system in rats after whole-body microwave exposure, with particular reference to lymphocytes. Rat exposure was similar to conditions described above. SAR was 1-2 W/kg. Cell response was assessed from the number and the type of bone marrow nuclear and peripheral blood white cells. A significant decrease in lymphoblast count was observed on experimental day 15 and 30, whereas other examined parameters did not significantly differ from control animals. Our findings suggest that the

stress response by the blood forming system after microwave exposure could be a sign of adaptation rather than of dysfunction (A254). We tested the assumption that RF/MW affects the synthesis of melatonin, a regulator of hematopoiesis and immunological response, by determining the excretion of melatonin metabolite (aMT6s) in nocturnal urine of rats. A decline in aMT6s was observed from experimental day 8 to 11. The aMT6s level remained consistently low until the end of the experiment, but not significantly lower than the control values. Under the experimental conditions described above, repeated 2.45 GHz irradiation could act as a stressor and therefore influence rat melatonin balance (C140).

Electromagnetic radiation (EM) of microwave frequencies in the range of 300 MHz-3000 MHz (MW) has become one of the major physical environmental pollutants. This raises concern about health hazards related to the interaction between microwave EM fields and life processes. Depending on the field strength, frequency, wave form, modulation, and duration of exposure, microwave radiation is absorbed in the molecules and manifests itself as a change in vibration energy or heat. Biomarkers of susceptibility and of the effect of low-intensity microwave radiation have been investigated using complex approach that includes cell cultures, and experimental animals. The mechanisms of MW radiation activity at the cellular and subcellular level have been examined *in vitro* using continuous cell cultures. Investigation has been continued by research of cellular and intracellular response to applied doses and exposure time of particular physical agents. The influence of 864 MHz electromagnetic field on proliferation, colony forming ability, and viability of continuous cell line V79 was evaluated. Cells were exposed to 864 MHz continuous wave for 1, 2, and 3 hours at an average SAR of 0.08 W/kg. Cells irradiated for two or three hours showed a significant decrease in growth on experimental day 3. The colony-forming ability and viability of the irradiated cells did not significantly differ from control. Applied RF/MW irradiation significantly affected cell proliferation kinetics but not viability or ability of V79 cells to form colonies (A194).

We also evaluated and compared the effects of 864 MHz and 935 MHz radiofrequency/microwave (RF/MW) fields on the growth, colony-forming ability, and viability of V79 cells. Cells were exposed to continuous wave frequencies of 864 MHz and 935 MHz for 1, 2 and 3 hours. SAR was 0.08 W/kg for the 864 MHz or 0.12 W/kg for the 935 MHz field. The growth of the 864 MHz-irradiated cells significantly decreased after two- and three-hour exposure 72 hours after irradiation. Similar was observed 72 hours after

exposure to 935 MHz microwaves for three hours. Colony-forming ability and cell viability of V79 cells exposed to 864 MHz or 935 MHz microwaves did not significantly differ from control cells. The two applied RF/MW fields showed similar effects on the growth, colony-forming ability, and viability of V79 cells. Cell growth impact was time-dependent for both fields (A195).

In addition, we evaluated whether low-level, ultra high frequency (UHF) irradiation of 935 MHz influenced the structure and growth of V79 cells. UHF field was generated inside a Gigahertz Transversal Electromagnetic Mode cell (GTEM-cell) with a Hewlett-Packard signal generator. The electric field strength was 8.2 ± 0.3 V/cm and the average SAR was 0.12 W/kg. Cell samples were exposed to a 935 MHz continuous wave frequency field for 1, 2, and 3 h. In comparison with control cells, the microtubule structure clearly altered after 3 h of irradiation. A significantly decreased growth was noted in cells exposed for 3 h three days after irradiation. It seems that the 935 MHz, low-level UHF radiation affects microtubule proteins, which consequently may obstruct cell growth (A196).

Basic investigations called attention of dentists to evaluate the time and dose dependence effect of halogen blue light from Elipar @TriLight, ESPE Dental AG Germany, since the success of filling duration depends on appropriate composite polymerization. A quartz-tungsten-halogen lamp emits radiation wavelengths 400-515 nm. We evaluated the effects of halogen light from a dental curing unit on cell viability, colony-forming ability, and proliferation of V79 cells. The light was set at the medium mode (M), exponential (E) and standard (S) illumination mode for 20, 40 and 80 seconds. Cell viability was not affected by the blue light regardless of exposure time and light mode. Colony-forming ability was slightly, but not significantly lower in treated cells than in control cells. Cell proliferation was lower in cells exposed to mode M for 80 s on post-exposure day 3 and 4. On the same post-exposure days, proliferation of cells exposed to modes E and S was significantly inhibited after 20, 40, and 80 s of exposure. It is still unclear whether disrupted cell function and no significant decrease in colony-forming ability of V79 cells, in addition to significant time- and dose-dependent inhibition of cell proliferation are owed to the dental curing blue light activity and/or changes in temperature during the experiment *in vitro* (A107).

Ivančica Trošić

Professional services

Next year, the Radiation Dosimetry and Radiobiology Unit will have 50 years of providing consulting and professional services in the domain of radiation protection, personal dosimetry, quality assurance, and control of ionising radiation sources to individuals occupationally exposed to ionising radiation, medical institutions, and public authorities.

The Unit has developed a new active electronic dosimeter in collaboration with ALARA Ltd. company. This device is versatile and can be used for many purposes, including personal dosimetry, scientific validation, and professional measurements. Over the last five years the Unit has invested in new equipment to meet requirements for ISO 17025:2007 accreditation for personal and environmental TLD dosimetry. This is a step forward in harmonization with the EU directives on procedures for regular monitoring of exposure to external ionising radiation and to regaining licence to perform radiation protection technical services. We continued to invest in professional education of all staff members, scientific and professional, in order for them to implement new radiation protection safety measures in everyday life, to keep up with state-of-the-art measurement techniques, to be aware of new ionizing and nonionising radiation sources and all other new technologies affecting individuals and environment.

Over the last five years we had continued measuring and identifying natural radioactivity and radioactive elements in fallout, air, water, soil, food and feed, and coal. In a few special projects we focused on naturally occurring radioactive materials enhanced by our industries. We had also extensively measured nonionising radiation exposure to mobile radio telecommunication technologies.

The Unit has been licensed for its services by the Ministry of Health and Social Welfare, Ministry of Environmental Protection, Physical Planning and Construction, and the Ministry of Agriculture, Forestry and Water Management.

Ivica Prlić

Between 2004 and 2008, the Unit had been providing 470 immunological analyses of human sera with a double agar gel immunodiffusion test using antigen-antibody complexes. The analysis included specific immunological response to occupational and general allergens. We had also been monitoring, detecting, and identifying fibrogenic dusts, and in particular all types of asbestos in bulk materials. 215 bulk materials were analysed for asbestos by type and presence. The analysis was performed according to a standardised method for stereo- and polarizing microscopy described in the MDHS 77-HSE document "Method for the Determination of Hazardous Substances; series 77 – Asbestos in bulk materials", ISO 9000, i BS ISO/IEC 17025. The Unit is included in the Asbestos in Materials Scheme (AIMS) under the guidance of the Institute of Occupational Medicine, Health and Safety Laboratory, Sheffield, Great Britain. At the moment, AIMS Round 37 is in procedure. Within this scheme our Institute has been appointed as the national institution for asbestos monitoring and identification in bulk materials.

Ivančica Trošić

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Research activities

Environmental radioactivity and radiation protection

Principal investigator: Gordana Marović, 2002-2006; collaborators: J. Kovač, N. Lokobauer, Z. Franić, M. Bronzović, M. Surić-Mihić, M. Maračić, T. Meštrović, B. Petrinec, I. Prlić, J. Senčar, E. Sokolović, B. Kmezić, Z. Kubelka, Lj. Petroci, S. Hajdarović, N. Iveković; project supported by MoSES

Environmental radioactivity and radiation protection

Principal investigator: Gordana Marović, 2007-; collaborators: Z. Franić, J. Kovač, M. Surić Mihić, N. Lokobauer, M. Vrtar (Clinical Hospital Center Zagreb), N. Kovačević (Clinical Hospital Center Zagreb), D. Šinka (Enconet International d.o.o. Zagreb); project supported by MoSES

Monitoring of environmental radioactivity started in 1959 as part of a continuing monitoring program designed to provide information about the presence of radionuclides in the Croatian environment. Research activities conducted under the monitoring programme have included the accumulation and translocation of natural and fission radionuclides in environmental samples. A major focus has been placed on the transfer of biologically significant radionuclides to the food chain and water, and on assessment of potential human doses resulting from direct and indirect ingestion of radionuclides.

Data on the presence and movement of radionuclides in the environment and within ecosystems were obtained by analysing samples from the terrestrial and marine environment - fallout, air, soils, drinking water, sea, and surface waters. In order to assess the body burden in Croatian population through the presence and accumulation of radionuclides in the food chain, levels of radioactivity were measured in samples of food stuffs and animal feed (A66, A68, A70, A71, C26, C80, C81, C83).

Radiation doses received by the Croatian population by ingestion of fission radionuclides through the food chain were of special importance in order to set up appropriate measures and effective actions to be implemented in case of a nuclear or radiological event and/or broad contamination by nuclear material. We have continued to improve,

standardise, and harmonise analytical methods on radioactivity measurements in various media.

Radiocaesium activity in selected marine bioindicator organisms and carp were investigated in cooperation with a project headed by Z. Franić. We estimated the effective ecological half-life of ¹³⁷Cs in mussels and the concentration factor for carp. Annual effective dose received by an adult Croatian through consumption of these organisms is rather small (A69, C29).

Mean residence time of the Adriatic seawater has been estimated using ⁹⁰Sr as a radioactive tracer. We presented an overview of the ongoing and future radioecological investigations in the Adriatic Sea and coastal areas along with a detailed work plan for further investigations of seawater circulation utilising radiotracers, which will contribute greatly to the management of wastewater and other communal waste in the Adriatic area (A67, B28, C22).

Within investigations of naturally occurring radionuclides in water systems, special attention has been paid to the activity concentrations of ²²⁶Ra in tap, mineral, and river waters. Some of these waters were not suitable for consumption due to increased activity concentrations of natural radionuclides. The effects of ²²⁶Ra concentration in drinking water were investigated and a model was designed to measure continuous exposure to ²²⁶Ra through water consumption over a long time. We also developed a mathematical model

to assess doses received through continuous exposure to low-dose radiation (A19, A35, C7, C8).

The growing popularity of bottled water consumption in recent years has raised the issue of treatment and removal of ^{226}Ra in water factories in Croatia. We estimated the doses received through water consumption with regard to age and sex. Estimated burdens make it possible to predict exposure control measures and protection for individuals or population at risk in cases of prolonged exposure (A36, B12, B13, C9, C10).

Mosses, lichens, and some mushroom species are sensitive bioindicators of radioactive contamination for various ecosystems, particularly in the event of a nuclear accident and uncontrolled emission of fission products. We summarised the results of systematic, long-term measurements of fission radionuclide activities in mosses, some edible mushroom species, and grass. Radiocaesium activity in mosses and mushrooms were in good correlation with fallout activities. Regarding the risk assessment for the Croatian population arising from consumption of mushrooms (*Boletus edulis* and *Cantharellus cibarius*), the collective effective dose was found to be quite low. Mushroom consumption was not a critical pathway for the transfer of radiocaesium from fallout (A162, C30, C86, C88).

Disposal of waste has been studied with particular attention on possible long-term exposure to radiation as well as technologically enhanced natural radioactivity associated with electrical power production and production of mineral fertilisers. Safety criteria for materials used in building construction were set, based on the external hazard index, H_{ex} , and radium equivalent activity, Ra_{eq} , of sampled ash and building material (A161, B27, B61, C61-C67, C82, C87).

In cooperation with a project headed by K. Šega, we have reviewed investigations of radioactive matter in the air as a part of systematic investigation of environmental radioactivity in the context of existing Croatian regulations. Despite the constant presence of radioactive matter in the air, background exposure doses as well as activity concentrations of monitored radionuclides have never exceeded national limits. The annual effective dose determined for ^{137}Cs and ^7Be inhaled from air was very small (A72, B59, B60, C24, C25, C27, C84, C85).

We have also continued developing measures for rapid and effective minimisation of radioactive contamination in normal and accidental situations (C118).

Gordana Marović

Radioecology of the Adriatic Sea and coastal areas

Principal investigator: Zdenko Franić, 2007-; collaborators: G. Marović, G. Branica, B. Petrinec, D. Kubelka (State Office for Radiation Protection Zagreb), N. Leder (Hydrographic Institute of Republic of Croatia Split); project supported by MoSES

The Adriatic Sea is a small and relatively shallow body of water, with specific water circulation and almost land-locked position between the high mountains of the Balkan and Italian peninsulas. It is therefore expected to be more sensitive to anthropogenic and natural radionuclides than the rest of the Mediterranean. In order to assess the impact on human health and the environment, investigation of main pollution sources and exposure pathways is essential. It is also important for plan, establish and implement efficient radioprotection measures for the marine environment and coastal areas, and to optimise the management of the Adriatic Sea as integral ecosystem and important economic resource.

Time trends of absorbed dose rates in air and sea, inventories and activity concentrations of radionuclides in seawater and coastal areas were studied and compared with regional trends (A67, A72, C24, C25, C85).

Samples of bioindicator organisms including pilchards (*Sardina pilchardus*), musky octopusses (*Ozaena moschata*), and mussels (*Mytilus galloprovincialis*) were collected and analysed. Radiation doses received through the intake of radionuclides in these marine foodstuffs were small

and comparable with other foodstuffs in Croatia, such as carp (*Cyprinus carpio*), potatoe (*Solanum tuberosum*), and wheat (A66, A68-A71, A162, C26, C29, C30, C80, C81, C83, C86, C88).

Collaborators on the project embarked on a scientific radioecological-oceanographic cruise (*International Scientific Cruise to Adriatic and Ionian Seas*) from 24 September to 5 October 2007, organised by the International Atomic Energy Agency (IAEA) within the project IAEA RER/7/003 (*Marine Environmental Assessment of the Mediterranean Sea*). The cruise ship was a research vessel Palagruža, owned by the Hydrographic Institute of the Republic of Croatia, which accommodated 30 IAEA scientists from Albania, Bosnia and Herzegovina, Croatia, Greece, Italy, Malta, Montenegro, Slovenia, Romania and Ukraine. The research route included Croatian territorial waters with sampling locations in the Channel of Brač, Jabuka Pit, Island of Brusnik, Palagruža Sill, the South Adriatic Pit, but also a few sampling locations in Greece, Albania, and Montenegro and two sampling stations in international waters at the inflowing and outflowing vein of the Strait of Otranto.

Samples of sediment core and surface seawater

were collected at 10 locations, and seawater samples from the various depths of the water column on six locations. Seawater temperature, salinity, and density were measured at all locations. At four locations sediment samples were collected from the seabed from the depths of up to 40 cm. Sampling locations Jabuka Pit – Palagruža Sill – South Adriatic Pit – Albania form a line along the Adriatic axis. Sediment samples have been divided into 2 cm thick slices for a closer look into the sedimentation processes. Subsequent investigation will include gamma-spectrometry and other methods investigating the penetration rate of fission radionuclides into deeper sediment layers. Sediment analysis is still in progress.

Preliminary estimation of the Adriatic sea-water turnover time using fallout ^{137}Cs as a radioactive tracer has confirmed the findings of a similar analysis performed using ^{90}Sr as a radioactive tracer. In both studies the turnover time was less than five years (A67, C22).

For non-radiological characterization, we collected samples of surface water at locations Jabuka Pit, near the island of Brusnik, and South Adriatic Pit. At several other locations we took samples from greater

depths (B28).

Field gamma-spectrometry was performed on the islands of Brusnik, Palagruža, and Vis, and the samples were also analysed in a laboratory. The results showed that background radiation on the islands of Brusnik and Jabuka is slightly greater than on the islands of Palagruža and Vis. Brusnik is partly and Jabuka entirely of magmatic origin, with higher concentrations of natural radionuclides than other, mostly limestone islands. The external hazard index and radium equivalent activity for middle-Dalmatian islands were considerably higher than on the other islands. However, external gamma dose rates measured on these islands correspond to annual doses that are less than 1 mSv (C105, C106). Radiochemical treatment of samples, gamma-spectrometry, and other analyses are in progress.

Over the first two years of the project, special attention has been paid to quality assurance and quality control of laboratory practice when dealing with radioecological samples (B30, C27, C34).

Zdenko Franić

Professional services

Measurements of environmental radiological contamination by the coal-fired power plant Plomin were continued within a research contract with Hrvatska Elektroprivreda. Field measurements and sampling were carried out at the site of ash and slag deposit in the area around the plant as well as in the coastal area and in the Bay of Plomin. A decrease in the levels of radionuclide contamination was observed due to restoration taken at the waste deposit site.

Intense agricultural production with an increased use of fertilisers containing radioactive elements contributes to the level of technologically enhanced natural radioactivity. We continued our research of radionuclide concentrations in waste waters from a phosphate fertiliser plant in collaboration with fertiliser company Petrokemija Kutina.

Radioactive contamination originated by Nuclear Power Plant Krško (Republic of Slovenia) has been monitored at several locations in the north-west Croatia, including calculation of doses. As part of

the programme of nuclear emergency planning, our emergency response team performed periodic field exercises to practice field measurements in case of nuclear accident. These measurements are within the framework of the project Monitoring and Assessment of Radioactive Contamination Originating from Nuclear Power Plant Krško.

The Institute has established continuous collaboration with several research institutions abroad. Over the past years, intercalibration programmes were carried out within the international collaboration with the World Health Organization (WHO), International Atomic Energy Association (IAEA), Nuclear Power Plant Krško, Jožef Štefan Institute, Ljubljana and the Institute of Occupational Safety (Zavod za varstvo pri delu) of the Republic of Slovenia.

Gordana Marović

Toxicology Unit

Scientific staff:

Božica Radić, head
Maja Peraica
Ana Lucić Vrdoljak
Suzana Berend
Dubravka Flaajs

Technical staff:

Marija Kramarić
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Associate scientist:

Radovan Fuchs

People who left between 2004 and 2008: Ana-Marija Domjan, Radovan Fuchs, Vilim Žlender

Research activities

Anticholinesterase poisons – biological effects of new antidotes

Principal investigator: Božica Radić, 2002-2006; collaborators: A. Lucić Vrdoljak, S. Berend, M. Kramarić, J. Mileković; project supported by MoSES

Therapeutic effect of newly synthesised compounds in organophosphorous poisoning

Principal investigator: Božica Radić, 2007-; Collaborators: A. Lucić Vrdoljak, S. Berend, R. Fuchs (MoSES), J. Lovrić (Medical School, University of Zagreb); project supported by MoSES

Due to a wide use of organophosphate (OP) compounds and carbamates in modern agriculture, millions of people are exposed to these compounds through air, water, or food. In particular circumstances, this exposure can cause manifest health problems such as acute or chronic poisonings. One of the toxic effects of anticholinesterase compounds such as the highly toxic nervous warfare agents (soman, sarin, tabun, VX) and carbamates is irreversible inhibition of hydrolytic enzymes, that is, the vitally important acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). AChE inhibition results in the accumulation of acetylcholine (ACh) at the synaptic cleft of the cholinergic neurones, leading to overstimulation of cholinergic receptors. Symptoms of poisoning include salivation, tremors, diarrhoea, and respiratory failure. As a result, the common cause of death is suffocation (D24). The current standard treatment of OP poisoning includes combined administration of a cholinesterase reactivator (oxime), a muscarinic cholinergic receptor antagonist (atropine sulphate), and pre-treatment with reversible carbamate AChE-inhibitor such as pyridostigmine. Our research focused on antidotes with nucleophilic characteristics, the oxime group, and other compounds that could have act against anticholinesterase poisoning. We investigated the antidotal effect of tenocyclidine (TCP) and its adamantyl derivative (TAMORF) - compounds with potential activity in N-methyl-D-aspartate (NMDA) receptors. These compounds weakly inhibited AChE, without reactivating or protecting AChE inhibited by soman *in vitro* (A215). However, *in vivo* results have shown that TAMORF could be a good alternative to current pretreatment, as it had no side effects. TAMORF metabolism in rats suggests that only a part is metabolised while the other part remains unchanged. TAMORF is a promising source molecule for further modifications

and for the development of more potent antitumour or radioprotective substances or of OP-poisoning antidotes (A154, A203). In addition, we investigated the antidotal effect of mono-pyridinium compounds (A155) and bispyridinium oximes in soman and tabun poisoning (A50). A group of K-oximes was investigated *in vitro* and *in vivo* in mice and rats. Although they were relatively toxic to mice, all oximes showed an improvement in the therapy of tabun poisoning (A15, A141). With those that showed the best pharmacological properties as reactivators and protectors we expanded experiments by applying them in combination as pretreatment and therapy (A153). Low acute toxicity and high reactivating potency *in vitro* and *in vivo* single out K048 as a promising oxime for future investigation.

We have begun a research with irinotecan, an anticancer prodrug that has been approved for the treatment of colon cancer. Cancer patients receiving irinotecan may have acute cholinergic side effects. *In vitro* experiments suggest that this is due to direct inhibition of AChE. We therefore investigated the efficiency of the HI-6 oxime in protecting and/or reactivating human erythrocyte AChE inhibited by irinotecan. We also investigated its cyto/genotoxicity *in vitro*. HI-6 increases AChE activity after irinotecan inhibition. The results obtained encourage further investigation of HI-6 *in vivo*, as well as its development for possible application in chemotherapy (A123, A216).

Furthermore, we have been studying AChE activity and assessing genome damage in workers employed in carbofuran production (A277, A278).

One part of our research focused on the role of the enzyme paraoxonase and BChE in lipid metabolism in rats (A31, B51).

Božica Radić

Effect of mycotoxins on humans and animals

Principal investigator: Radovan Fuchs, 2002-2006; collaborators: M. Peraica, A.-M. Domijan, V. Žlender, M. Matašin; project supported by MoSES

Toxic effects of mycotoxins on humans and animals

Principal investigator: Maja Peraica, 2007-; collaborators: R. Fuchs (MoSES), A.-M. Domijan, V. Žlender, D. Flajs; project supported by MoSES

The most common contaminants of grains in temperate climates, including Croatia are moulds of the *Fusarium*, *Penicillium* and *Aspergillus* genus. These moulds produce a number of mycotoxins and among them the most frequent are fumonisins, zearalenone (ZEA), and ochratoxin A (OTA). We have introduced methods for OTA determination in beans (A58) and ZEA in wheat (E9). ZEA was found in all samples of wheat collected in Slavonija, but its concentration was very low. In bean samples collected in various parts of Croatia the concentration of OTA was low, and OTA-positive bean samples were found in the central and northern Croatia, while those from the southern Croatia (Dalmatia and Primorje) were OTA-free. In maize samples collected in all maize-producing counties FB₁ was found in 100 %, ZEA in 84 %, OTA in 39 %, and FB₂ in 6 % of samples (A57, B20). The co-occurrence of the three mycotoxins was high (37 %), and the mean concentration of single mycotoxins was low.

The concentration of OTA was determined in white and red wines collected all over Croatia (B21). All red wines contained OTA, and its mean concentration was higher than in white wines. OTA concentration in wines from Croatia is lower than in the neighbouring countries. Our method was evaluated in an interlaboratory study performed by the Association of Enological Laboratories from Porto (Portugal).

The mechanism of OTA and FB₁ toxicity was studied on a rat model. Methods for the measuring parameters of oxidative lesions of macromolecules (proteins, lipids, and DNA) were introduced and used for studying the effect of mycotoxins in rats (A56, A59-A62, B23, E11). We have found that OTA produces apoptotic cells in the rat kidney and that their appearance depends on the dose and the length of exposure (B22). OTA-induced genotoxic lesions were measured using the comet assay in the kidney of rats treated with multiple OTA doses (A59, A275). All parameters of DNA lesions (tail length and intensity, as well as the tail moment) increase with OTA concentration in the kidney.

Multiple FB₁ treatment increased the concentration of sphinganine (Sa), sphingosine (So), and their ratio (target molecules in FB₁ toxicity), which both caused DNA damage measured with comet test (tail length, tail intensity and tail moment) in kidney and liver homogenates (A61). In both organs this happened before MDA and protein carbonyl concentrations increased and before catalase activity of decreased, showing that oxidative stress is not crucial in FB₁ genotoxicity. The effect of a single FB₁ dose on DNA lesions measured with the comet assay in kidney homogenate showed time and dose dependence (A202). Apoptotic cells were found in the cortical and outer medullar part of the kidney. The effect of FB₁ on glutathione concentration and activity of mitogen-activated protein kinases and the expression of heat shock proteins was studied in the kidney and liver (A226). FB₁ affected the oxidative balance in the cell, causing tissue-specific activation and expression of molecules involved in oxidative stress.

OTA and FB₁ increase oxidative stress, and their damaging effect on DNA is synergistic even at as low doses as those in the European-type diet (A60). Using the standard and modified comet assay with formamido-pyrimidine-DNA-glycosylase enzyme that breaks DNA at the site of oxidative lesion, we have found that both OTA and FB₁ cause oxidative DNA damage, but this is not the only mechanism of their genotoxicity (A59).

A part of our research focused on the aetiology of endemic nephropathy was performed in collaboration with the Institute of Public Health of the Brodsko-Posavska County. In it we determined OTA concentration and Sa/So ratio in human urine samples collected from the same residents of endemic and control villages in 2000 and 2005. The frequency of OTA-positive samples was higher in the residents of the endemic area than in controls. However, OTA concentrations were not significantly different between the two populations. The Sa/So ratio was also higher in the urine of the endemic area residents.

Maja Peraica

Professional services

The Toxicology Unit measures blood and plasma cholinesterase activity in people exposed to anticholinesterase pesticides, as cholinesterase activity is a direct biomarker of toxic effects caused by anticholinesterase agents (organophosphates or carbamates). We also provide toxicological evaluation

of public health pesticides by determining basic acute toxicity *in vivo*. Testing includes acute oral, dermal, and primary skin and eye irritation and sensitisation.

Božica Radić

Other Research Projects

Effect of physical and chemical agents - cytogenetic biomarkers

Principal investigator: Aleksandra Fučić, 2002-2006; project supported by MoSES

My study of the impact of physical and chemical agents in humans and in animals during growth and development has shown that children and young animals are particularly vulnerable to genotoxicants. This confirms recent research findings and calls for development of a new environmental protection legislation to reduced health risks for the children (A25, A37, A186, A189, A264, A265). An analysis of chromosome aberrations showed that this method can predict increased cancer risk (A24, A74, A75, A78, B27). However, sister chromatid exchange showed no such predictivity (A24). Exposure to depleted uranium in war circumstances did not significantly change the incidence of leukaemia and lymphoma in children (A144). Research within the project resulted in

additional collaboration in European Framework Programmes, as follows: European network on children susceptibility and exposure to environmental genotoxicants (FP5); Policy interpretation network on children's health and environment (FP5); Health and Environment Network (FP6); Newborns and genotoxic risks: Development and application of biomarkers of dietary exposure to genotoxic and immunotoxic chemicals and of biomarkers of early effects, using mother-child birth cohorts and biobanks (FP6); and Expert team to support biomonitoring in Europe (FP6).

Aleksandra Fučić

1-Aminocyclopropanecarboxylic acids: coordination chemistry

Principal investigator: Nenad Raos, 2002-2006; collaborators: L. Mak, A. Miličević, former Analytical and Physical Chemistry Unit; project supported by MoSES

Development of models for the estimation of stability constants

Principal investigator: Nenad Raos, 2007-; collaborators: A. Miličević; project supported by MoSES

The aims of both projects (the second being a continuation of the first) were (1) to investigate the structure of chelates with 1-aminocyclopropane-1-carboxylic acid (acc) and interpret experimental results using the molecular-mechanics method; (2) to develop and apply the overlapping spheres (OS) method; and (3) to develop models for the estimation of stability constants of coordination compounds using topological indices.

In cooperation with the Faculty of Science, University of Zagreb we solved the crystal structure of copper(II) chelate with acc (A106). The structure turned to be quite unusual; it was a trimer consisting of one diaqua-trans- and two cis-biscomplexes.

The overlapping spheres method was first applied to find low-energy conformations, and then to estimate stability constants of coordination compounds.

The search for low-energy conformations is a classical problem in conformational analysis, especially in molecular-mechanics approach. The problem stems from the fact that molecular mechanics is capable of finding out conformations, but cannot tell in advance which of them will have low conformational energy. Thus we developed an original algorithm based on the OS method. We placed the central sphere with radius R_v at a defined

position in a molecule and then applied repulsive potential proportional to the overlapping volumes of atoms penetrating the central sphere. The algorithm was successfully checked on branched alkanes and on the *trans*-(*N*-Boc-acc-OH)₂Cu complex (A219). The OS method was also used to estimate stability constants of copper(II) and nickel(II) chelates with naturally occurring amino acids and diamines (A168, A170, A220). The central sphere was placed at various positions in molecule. The best results were obtained with central atom position.

In applying topological indices to estimate stability constants of coordination compounds (A171, A175), we developed a new approach; instead of varying topological indices, we varied graph, i.e. constitutional formula. For our calculations we used graphs of the ligand (L), mono- (ML) and bis-complex (ML₂), as well as mono-complex with two additionally bonded ligand water molecules (ML^{aq}) and complexes with additional bonds between central atom and side chain (ML^{ort}), mimicking this way strong non-bonding interactions within addition to copper(II) and nickel(II) complexes with amino acids, their derivatives, diamines and triamines (A172), we also estimated stability constants with dipeptides (A174), where each chelate ring of the complex was treated separately. It was also possible to estimate

stability constants of one class of compounds from the calibration line developed on another (A173) and to theoretically evaluate results obtained by two experimental methods (A221).

A general review of issues involved and methods

used was published in a review article (B64). The second project yielded one Ph.D. thesis (E31).

Nenad Raos

Transition metals with bioligands: interaction and modelling

Principal investigator: Jasmina Sabolović, 2002-2006; collaborators: G. Branica, M. Marković, former Analytical and Physical Chemistry Unit; project supported by MoSES

Transition metal complexes with bioligands: modelling and interaction

Principal investigator: Jasmina Sabolović, 2007-; collaborators: G. Branica, M. Marković, S. Tomić (Ruder Bošković Institute, Zagreb) V. Gomzi (Ruder Bošković Institute, Zagreb); project supported by MoSES

Metal complexes with various bioligands have been studied experimentally and theoretically. Complexes dissolved in aqueous and non-aqueous solvents were analysed using voltammetry and EPR spectroscopy, whereas their solid-state structures were determined using X-ray diffraction. We have developed new molecular mechanics vacuum-like force fields applicable to both gas-phase (isolated molecules) and condensed-phase simulations (crystal, aqueous solution) to predict and simulate structural and thermodynamical properties.

We used voltammetric techniques to study the interaction between lead and ascorbic acid at physiological ionic strength, different pH values, and different ratio of metal and ligand concentrations, and have published our finding on stability constants (A32).

Using the cyclic voltammetry, we determined redox potentials for the copper(II) complexes with aliphatic L-amino acids and their *N,N*-dimethyl derivatives in non-complexing electrolyte (NaCl₄) under physiological conditions (0.15 mol L⁻¹). We investigated the effects of *N,N*-dimethyl groups on electrochemical properties of the studied copper(II) complexes. As we expected, dimethylated copper complexes showed lower stability, deduced from redox potential shifts, than unsubstituted copper complexes. We tested our hypothesis that different electrochemical behaviour can be explained by steric reasons and aliphatic-aliphatic interactions.

We found a significant correlation between topological indices based on graph theory and stability constants experimentally determined using glass electrode potentiometry and square wave voltammetry for a series of copper(II) complexes with glycine, L-alanine, and their *N*-alkyl derivatives (A221).

The X-ray crystal and molecular structures of bis(*N,N*-diethylglycinato)copper(II) (A232), aquabis(*N,N*-dimethylglycinato)copper(II), aquabis(*N,N*-dimethylglycinato)copper(II) dihydrate (A233), and of the copper(II) complexes with L-valine and L-isoleucine were determined. Earlier we developed a molecular mechanics model (MM) and force field (named FFW) that reliably reproduced experimental crystal and quantum-chemically

calculated vacuum structures by geometry optimisation of anhydrous and aqua copper(II) complexes with aliphatic amino acids, with either *trans*- or *cis*-CuN₂O₂ coordination polyhedron.

We used FFW for conformational analysis of anhydrous bis(*L-N,N*-dimethylvalinato)copper(II) and bis(*L-N,N*-dimethylleucinato)copper(II) and surrounded with four water molecules to examine the possibility of the lowest energy conformers to bind a water molecule to the copper(II) coordination sphere (A179). MM results suggested that different EPR spectra of the two complexes, dissolved in different solvents and studied at different temperatures, could be explained by steric reasons.

We did conformational analysis to study the effects of steric aliphatic-aliphatic interactions in the coordination polymer of bis(*N,N*-diethylglycinato)copper(II) *in vacuo* and in the crystal lattice (A232). Combined experimental and theoretical results showed that intermolecular aliphatic-aliphatic interactions affected changes in molecular conformation and in the copper(II) coordination sphere during crystallisation.

We also applied FFW to study the effects of hydrogen bonding in the crystal structures of aquabis(*N,N*-dimethylglycinato)copper(II) dihydrate and aquabis(*N,N*-dimethylglycinato)copper(II) (A233). Different hydrogen bonding patterns in these crystal modifications did not visibly affect the molecular structure of the copper(II) complex. Experimental and theoretical results suggested that the main effect of two water molecules of crystallisation in the dihydrate modification was to stabilise the crystal packing via hydrogen bonding, while similar pyramidal copper(II) coordination geometry in both modifications was due to axially coordinated water molecule and its intermolecular interactions.

In collaboration with colleagues from the University of Innsbruck, we studied the mechanism for *cis-trans* isomerisation of bis(glycinato)copper(II) in the gas-phase based on high-level quantum chemical calculations (A249). The results showed that *cis* to *trans* interconversion occurred without breaking the bonds.

We developed a new force field whose

parametrisation was based on experimental crystal data and quantum-chemically obtained vacuum structures of a series of copper(II) complexes with aliphatic α -amino acids and their N-alkyl derivatives and an SPC/E water model. Whereas FFW has proved reliable for MM simulations *in vacuo* and in crystal, the new force field is also reliable for MD simulations of solvated complexes in aqueous solution.

We developed force fields for modelling nine *tris*(acetylacetonato)metal(III) complexes in crystalline surroundings to examine the hypothesis if experimental enthalpy of fusion values could be connected with the in-crystal potential energies, and we showed that the connection existed (A231).

Jasmina Sabolović

Oligoelements in biological matrices and multielement profile quality control

Principal investigator: Nikola Ivičić, 2007-; Collaborators: B. Momčilović, A. Benutić (Croatian National Institute of Public Health, Zagreb), J. Pongračić (Croatian National Institute of Public Health, Zagreb); project supported by MoSES

We continued to study the relationship between human depression and multielement profile of distinct biological matrices such as hair and whole blood. Our prospective, randomised, double-blind, clinical-epidemiological study of the multielement profile involved 48 depressed subjects (15 men and 33 women) and 48 apparently healthy control subjects (23 men and 25 women). All research was conducted at the Centre for Biotic Medicine in Moscow, Russia, strictly observing the Declaration of Helsinki. We analysed 40 elements in hair and whole blood using the inductively coupled plasma mass spectrometry (ICP-MS) at the. The multielement profile of depressed subject was distinctly different from control subjects, and showed higher accumulation of trace elements Al, Ag, B, Ba, Cd, Ga, La, Mg, Mn, S, Sr, Ti, V i W in the hair and of so-called macro-elements sodium and potassium in the whole blood (A184, C94). To our knowledge, this is the first demonstration of the biomolecular basis of human depression (A184, C94). At the same time, we observed selenium, copper, and iodine deficiency in depressed subjects, i.e., the lack of elements that were hypothetically associated with human the depression. Thus, copper is essential for the synthesis of the epinephrine, selenium is essential in enzymes controlling the uptake of iodine to the thyroid gland, and iodine is essential for the hormonal function of the thyroid gland. We demonstrated iodine deficiency

to be the principal nutritive determinant associated with human depression (A183, B66). We also showed that lymphocyte DNA damage that accompanied the physical sport activity depended on the athlete's nutritional status, which reflected in hair multielement profile (C160). In our study of calcium metabolism in perimenopausal women, we observed a phenomenon that we named the "calcium paradox", i.e. that simultaneous supplementation with the calcium, magnesium, and vitamin D was followed by counter-intuitive fall of hair calcium, supposedly due to increased calcium deposition in the skeletal mineralised tissue under the given conditions of supplementation (A209, B67).

We also studied theoretical implications of the same isotope mass number within and between elements on mass spectrometric analysis, and developed a new conceptual "fish skin" model (Russ. *cheschuya*) to describe these relationships (B66).

A study of the human whole blood multielement profile revealed that the homeostasis of bioelements in the human body varies with every element within that homeostasis system. This observation allows for a new conceptual approach that nears holistic interpretation of complex interactions between bioelements in the human body (A182).

Nikola Ivičić

Metal metabolism

Principal investigator: Berislav Momčilović, 2002-2006; collaborator: N. Ivičić, former Analytical and Physical Chemistry Unit; project supported by MoSES

We studied hair and blood multielement profile in subjects suffering from depression in a prospective, randomized, double blind, clinical-epidemiological study. The results indicate a strong aetiological link to the euthyroid nutritional iodine deficiency and/or metabolic impairment. The multielement profile analysis of Hg, Se, and I showed the increased hair

Hg in association with low iodine, but variable Se concentration. In contrast to the now prevailing view of Hg toxicity as a result of high binding affinity to Se, our research points to the iodine nutritional status and/or metabolism as the primary target of human Hg toxicity. Furthermore, the multielement profile analysis of hair and blood showed higher Ca, Mg, Sr,

and Si content as an early warning sign of osteoporosis at the time when DEXA (dual x-ray absorptiometry) findings were inconclusive. Dental alloys are widely used in oral and orthopaedic prosthetics. Our research in collaboration with the Zagreb University Dental School demonstrated that manufacturers of dental alloys do not report their composition in full (A52). Metal ion leaching from Au/Pt dental alloy showed a large release of Cr and only minuscule amounts of Au; indicating that the allergy associated with Au is in fact to be associated with the non-recognized Cr.

In collaboration with the University of North Dakota Department of Physics, we showed that the distribution of radon daughters ^{214}Po (alpha emitter) and ^{214}Bi (beta emitter) varied considerably between different human brain structures in people suffering Alzheimer's disease (AD) (A181). Therefore, the radiation risk to the amygdala and hippocampus, two brain structures controlling emotions and memory respectively, is more than two orders of magnitude higher than that to the paleo cerebral structures of locus ceruleus and nucleus basalis. The internal dose of ^{214}Po alpha particles to amygdala is sufficient enough to destroy this brain structure within but a few years. In AD, both ^{214}Po and ^{214}Bi radon daughters

accrue selectively in the brain cell proteins whereas lipids are not infested, except for amygdala.

A new method for Mo analysis in whole human blood using differential pulsed anodic stripping voltammetry (DPASV) was developed at the Institute's Analytical and Physical Chemistry Unit. The results were compared with those obtained by another Institute's Unit using electrothermal atomic absorption spectrometry (ET-AAS) and by the Center for Biotic Medicine (CBM), Moscow, Russia, using inductively coupled plasma mass spectrometry (ICP-MS).

There was a high congruence between the results of all three different analytical methods (ANOVA $P > 0.05$ for comparing all three methods and between any two methods). Beside these departments and laboratories at home and abroad, we collaborated with the Energy and Environment Research Center, Grand Forks, ND, USA on the application of an experimental dose rate idiorrhhythmic model, to study metabolic and toxicological implications of temporary high intake of Hg from whale meat in the diet of isolated island communities.

This report was taken from the Activity Report for 2006 (Arh Hig Rada Toksikol 2007;58:99-100)

Metals – biocompatibility and stress in alopecia, depression, and diabetes

Principal investigator: Berislav Momčilović, 2007-; collaborators: A. Čelebić (School of Dental Medicine, University of Zagreb, Zagreb), J. Stipetić-Ovčariček (School of Dental Medicine, University of Zagreb, Zagreb), V. Stančić (Clinical Hospital "Sestre milosrdnice", Zagreb), R. Poljak-Guberina (Privat Dental Practice, Zagreb), S. Morović (Clinical Hospital "Sestre milosrdnice", Zagreb), Z. Mršić-Krmpotić (University Hospital for Tumors, Zagreb), T. Radionov (Psychiatric Hospital "Vrače", Zagreb), R. Zorica (University Hospital for Tumors, Zagreb); project supported by MoSES

We reported the importance of the multielement profile of hair and whole blood in diagnostics and dietary supplementation in depression, osteopenia/osteoporosis, and occupational exposure to the free radical-generating xenobiotics, based on our own clinical material from a prospective, randomized, double blind, clinical-epidemiological study (D22). Depressed subjects (B66) have a distinctly different multielement profile than the healthy subjects. Heavy metals like silver have a strong tendency to accumulate in the hair of depressed subjects (B67). The number of sister chromatid exchanges (SCE) in the lymphocytes of registered medical nurses working with cytostatics (free-radical-generating xenobiotics) was inversely related to blood Se, Sr, Mn, and Cu, indicating the protective role of these elements in decreasing the risk of cytostatics and other free-radical-generating xenobiotics in human health (A97).

These results were presented as three invited lectures at international meetings. Moreover, multielement profile appears to be a very sensitive indicator for early detection of osteopenia/osteoporosis and for the assessment of the metabolic response to dietary supplementation with calcium, magnesium, and vitamin D. Our study revealed a seasonal variation of whole body radon daughter ^{214}Bi and environmental radon in the air we breath, with high values occurring in the winter, and lower values in the summer; the whole body concentration of radon daughter ^{214}Bi was higher in the human body than in the surrounding air (A182). The results of this research were implemented in a postgraduate programme for medical and dental practitioners

This report was taken from the Activity Report for 2007 (Arh Hig Rada Toksikol 2008;59:R45)

Immunotoxic effects of indoor bioaerosols and lifestyle

Principal investigator: Božica Kanceljak-Macan, 2002-2005; collaborators: M. Bakotić, J. Bobić, M. Gomzi, J. Jurasović, A. Košćec-Đuknić, J. Macan, M. Mataušić-Pišl, S. Milković-Kraus, I. Pavičić, A. Pizent, B. Radošević Vidaček, S. Telišman, I. Trošić, R. Turk, V. M. Varnai; project supported by the Institute for Medical Research and Occupational Health

The aim of this project was to establish occupational indoor exposure to biological hazards and to see which lifestyle factors contribute to immunological disorders associated with this exposure. We measured endotoxin levels in environmental samples (air or settled dust). Endotoxin is considered a good marker of indoor bacterial contamination and also an environmental immunomodulator that may play a role in the occurrence of allergic disorders (B123). The standard method for endotoxin measurement is the chromogenic *Limulus* amoebocyte lysate (LAL) bioassay, and we introduced and validated an endpoint variation, which yielded a good linearity of the standard curve ($r = 0.983$), recovery (92 and 110%), and repeatability (CV 0 to 8.5 %) (B122).

In order to study the influence of the indoor environment on health, well-being, and work effectiveness, we investigated non-specific symptoms known as the Sick Building Syndrome (SBS) in office buildings workers.

Endotoxin levels were determined in settled dust samples from the workplaces and households of librarians, and were evaluated with regard to the prevalence of SBS symptoms. The presence of one or more work-related SBS symptoms was reported in 65.2 % of analysed librarians (eye irritation in 61 %, throat irritation in 38 %, sneezing in 32 %, nose irritation in 30 %, cough in 16 %, and cold in 14 %). Endotoxin levels were similar in dust samples from the library and households of librarians, suggesting that occupational exposure to endotoxin cannot explain the occurrence of SBS symptoms in librarians (C79).

We then investigated the contribution of somatic, psychological, and environmental factors to SBS symptoms in women office workers from three

companies. Women working in air conditioned buildings reported SBS symptoms more often than those working in buildings with natural ventilation. Neuroticism and physical condition as certain aspects of psychological profile were strongly associated with reported SBS symptoms in both groups of office workers. Only psychological factors were established as predictors of SBS symptoms, while somatic variables, including markers of atopy, failed to predict SBS symptoms (A96). Symptoms which are the consequence of psychosocial stressors may render a person more sensitive to otherwise normally tolerated physical and chemical factors in the environment. Persons of greater emotional stability report better quality of life, less SBS symptoms in the working environment, and better work organisation. Furthermore, they have better simple reaction time scores to visual stimuli.

We studied the association of age, smoking, alcohol, erythrocyte superoxide dismutase (SOD), whole blood glutathione peroxidase (GPx), blood lead (BPb) and cadmium (BCd), and serum copper (SCu), zinc (SZn) and selenium (SSe) with atopic status and ventilatory function in 166 women and 50 men with no occupational exposure to metals or other xenobiotics. In women, the increase in IgE was associated with a decrease in SCu. In men, the increase in non-specific bronchial reactivity and in the risk of positive SPT was associated with a decrease in SSe. Additionally, the decrease in FVC% and FEV₁% in men were associated with a decrease in SZn. These results were observed at metal levels comparable to those in general populations worldwide (A208).

Božica Kanceljak-Macan

Professional Units

Poison Control Centre

The Poison Control Centre of the Institute for Medical Research and Occupational Health in Zagreb is the only toxicological information centre in Croatia, serving a population of about 4.5 million inhabitants. It was founded in 1971 by the Department for Occupational Diseases and Toxicology of the Institute, and ever since the average number of calls has been increasing from about the initial 100 a year to more than 1400 in 2008. The Centre provides a wide range of services accessible to the general public, medical professionals, and health authorities. The most important is our 24-hour emergency phone service (+385-1-2348-342), providing basic first aid information to general public and to medical care personell in the event of poisoning. It also provides information on the toxic effects of poisoning or exposure including treatment advice for health professionals. The Centre's database, comprising information on 3000 commercial products most frequently involved in poisoning episodes, is updated on the regular basis.

From 2004 to 2008, the Centre received a total of 6418 telephone enquiries about suspected poisoning incidents, averaging to 1300 calls a year. Most calls come from medical professionals and more than 90 % concern acute poisoning incidents. Consultations on health risks from chemical exposure at the workplace were given to occupational health physicians in 59 cases (B115). The Centre also performed

toxicological risk assessments for 532 plant protection and biocidal products placed on the market in Croatia.

The Centre has been publishing annual activity reports in a science journal *Archives of Industrial Hygiene and Toxicology* since 2006. Several lectures on toxicology and poison control were delivered to plant protection and pest control manufacturers and users and to emergency physicians, paediatricians, and pharmacists at the scientific and professional meetings. The Centre also provides expert toxicological assistance to the Croatian Ministry of Health and Social Welfare within the scope of activities of the Committee on Hazardous Chemicals and has participated in the preparation of legal acts and guidelines for registration and control of hazardous chemicals and biocides. We intensified our collaboration with the Ministry of Agriculture, Fishery, and Rural Development on harmonisation and implementation of the EU legislation regarding plant protection products. Centre's experts were involved in toxicological training provided within the two CARDS twinning projects on "Further development in capacity building in the area of plant protection products" by the Pesticide Safety Directorate in York, UK.

Jelena Macan and Rajka Turk

Laboratory Animal Unit

Institute's Laboratory Animal Unit breeds Wistar rats (HsdBrlHan:WIST) in accordance with the national Animal Welfare Act and other applicable regulations, guidelines and policies. Animals are bred in strictly controlled conditions, under surveillance of a veterinarian, and then used for scientific research. The living conditions foster good health and comfort of animals. Animals receive standard GLP-certified laboratory food and water *ad libitum* with altering 12h light and dark cycles. The facilities are cleaned every week to reduce the risk of external contamination. Croatian legislation forbids animal experiments that have not been approved by the Institutional Animal Care and Use Committee (IACUC) and National Animal Research Authority, appointed by the Ministry of Agriculture, Republic of Croatia. The approval is issued to a researcher at the Institute who requested it for the experiment. During experiments, the veterinarian checks on all the animals included in the experiment on the daily basis. At the end of an

experiment, animals are put down with carbon dioxide (CO₂) or other recommended method. In the past five years, 6315 animals of both sexes were bred and used in scientific experiments conducted at the Institute or other research institution in Croatia. Breeding and maintenance costs are covered by selling animals. A health monitoring program, which includes a check for zoonoses, is regularly performed in collaboration with the National Veterinary Institute.

Mirjana Mataušić-Pišl and Vedran Micek

Archives of Industrial Hygiene and Toxicology

Since 1950, the Institute has published a quarterly scientific journal *Arhiv za higijenu rada i toksikologiju - Archives of Industrial Hygiene and Toxicology*. In the period 2004-2008, *Archives* was the official journal of the Croatian Medical Association - Croatian Society on Occupational Health, Croatian Toxicological Society, and Slovenian Society of Toxicology.

Archives publishes contributions relevant to all aspects of environmental and occupational health and toxicology (scientific papers, professional papers, reviews, short communications, case reports, observations, technical papers and letters to the editor). Announcements, book reviews and meeting reports are also accepted. In the period 2004-2008 *Archives* published 195 manuscripts, and their distribution by category is shown in Table 1.

In 2008, *Archives* has been accepted for coverage in Science Citation Index Expanded® (Thomson Reuters). We expect to receive an impact factor in the 2010 Journal Citation Reports, to be published in late spring/early summer 2011. The journal is also indexed by Medline/PubMed, Scopus, Animal Science Database, Biological Sciences (CSA), BIOSIS Previews, GreenFile, INIS, Pollution Abstracts, Veterinary Science Database, and Water Resources Abstracts.

Over the past five years the editors in chief of *Archives* were:

- Radovan Pleština in 2004; Vol. 55
- Elsa Reiner and Marko Šarić in 2005; Vol. 56
- Blanka Krauthacker between 2006 and 2008; Vol. 57, 58 and 59 (No. 1, 2)
- Nevenka Kopjar since 2008; Vol. 59 (No. 3, 4)

Archives has had an international Advisory Editorial Board and an Executive Editorial Board.

Between 2004 and 2008 the members of the Advisory Editorial Board were Black A (Australia) 2004-2006; Aliev G (USA) 2008-; Bajgar J (Czech Republic) 2008-; Blasiak J (Poland) 2008-; Borzelleca J (USA) 2004-2006; Borron SW (USA) 2008-; Budihna MV (Slovenia) 2004-; Cikrt M (Czech Republic) 2004-; Doctor BP (USA) 2008-; Drobne D (Slovenia) 2008-; Evans HL (USA) 2004-; Godnić-Cvar J (Austria) 2007-; Hsieh YHP (USA) 2008-; Ilich-Ernst J (USA) 2004-; Kendrovski V (Macedonia) 2007-; Hayes AW (USA) 2008-; Kežić S (The Netherlands) 2007-; Kostial K (Croatia) 2004-; Lotti M (Italy) 2004-; Matković V (USA) 2008-; Mišurović A (Montenegro) 2007-; Pavelić K (Croatia) 2007-; Petz B (Croatia) 2004-2005; Plavšić F (Croatia) 2004-2006; Pleština R (Croatia) 2005-2006; Prpić-Majić D (Croatia) 2004-2005; Radenković M (Serbia) 2007-; Reiner E (Croatia) 2007-; Rico A (France) 2004-2006; Rumboldt Z (Croatia) 2004-; Šarić M (Croatia) 2007-; Šega K (Croatia) 2004-2006; Valić F (Croatia) 2004-; Winker R (Austria) 2008-.

The Editorial Executive Board consisted of Bogadi Šare A (Croatia) 2004-2006; Bradamante V (Croatia) 2008-; Cvijetić Avdagić S (Croatia) 2007-; Dodić-Fikfak M (Slovenia) 2008-; Franić Z (Croatia) 2005-; Gomzi M (Croatia) 2004-2006; Jurasović J (Croatia) 2007-2008; Kopjar N (Croatia) 2004-2008; Lucić Vrdoljak A (Croatia) 2008-; Milković-Kraus S (Croatia) 2004-2008; Peraica M (Croatia) 2004-2006; Piasek M (Croatia) 2004-; Radošević-Vidaček B (Croatia) 2004-2006, 2008-; Šega K (Croatia) 2007-2008; Trebše P (Slovenia) 2004-2008; Vasilčić Ž (Croatia) 2004-.

Table 2. Distribution of manuscripts by categories published in the Archives between 2004 and 2008

Category	2004	2005	2006	2007	2008	Total
Original scientific paper	3	14	14	15	13	59
Short communication	2	5		2	11	20
Observation	1	4	7	-	4	16
Case report	-	-	-	-	2	2
Professional paper	-	-	8	6	3	17
Technical paper	-	-	5	1	1	7
Conference paper	14	1	-	-	-	15
Review	8	11	13	18	5	55
Note	1	1	-	-	2	4
Total	29	36	47	42	41	195

Associate Editors of *Archives* were Elsa Reiner and Marko Šarić (Vol. 55, 2004 and Volume 57, 2006). Assistant Editor was Želimira Vasilić (Vol. 56-58, 2005-2008). Technical Editor was Želimira Vasilić. Copyeditor for articles in English was Dado Čakalo (English), and Branko Erdeljac was the language reviser for Croatian.

ISI Web of Science® cited altogether 52 manuscripts published in *Archives* in the period 2004-2008 (sum of citations: 94). The authors of manuscripts published in *Archives* came from 27 countries (Figure 2). Figure 3 shows the proportion of

authors from the Institute in the total number of Croatian manuscripts published in *Archives*.

The publishing of *Archives* is supported by the Ministry of Science, Education and Sports of the Republic of Croatia.

All research articles published in the journal are permanently available on-line free of charge at <http://hrcak.srce.hr/aiht> or <http://www.versita.com/science/medicine/aiht>.

Nevenka Kopjar

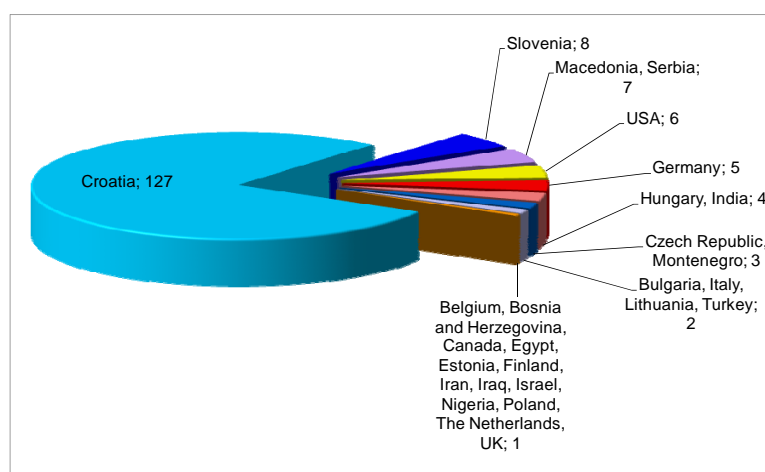


Fig. 2. Distribution of countries that the corresponding authors came from in period 2004-2008

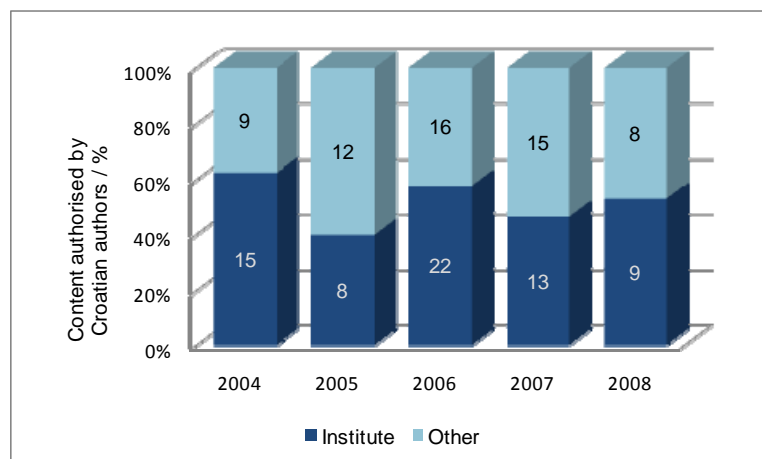


Fig. 3. Proportion of Institute's authors with respect to total number of Croatian manuscripts published in the Archives

List of Publications 2004-2008

A. Papers indexed in CC/SCIE/SSCI databases

- A1. ACKER K, KEZELE N, KLASINC L, MÖELLER D, PEHNEC G, ŠORGO G, WIEPRECHT W, ŽUŽUL S. Atmospheric H₂O₂ measurement and modeling campaign during summer 2004 in Zagreb, Croatia. *Atmos Environ* 2008;42:2530-42.
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- E35. NIKOLIĆ M. Učinak spolnog ciklusa na ekspresiju organskog anionskog prijenosnika OAT1 u bubrezima ženki štakora (Expression of renal organic anion transporter OAT1 in hormonal cycle of female rats) [Ph.D. thesis]. Zagreb: Faculty of Science, University of Zagreb; 2007. Mentor: Sabolić I.
- E36. PAVIČIĆ I. Biološki pokazatelji učinka radiofrekvencijskog mikrovalnog zračenja (935 MHz) na V79 stanice u kulturi (Biological markers of radiofrequency microwave radiation (935 MHz) effect on V79 cell culture) [M.Sc. thesis]. Zagreb: Faculty of Science, University of Zagreb; 2005. Mentor: Trošić I.
- E37. PAVIČIĆ I. Djelovanje radiofrekvencijskog zračenja na strukture citoskeleta stanica u kulturi (Influence of radiofrequency radiation on cytoskeleton structure of cells in culture) [Ph.D. thesis]. Zagreb: Prirodoslovno-matematički fakultet Sveučilišta u Zagrebu; 2008. Mentor: Trošić I.
- E38. PEHNEC G. Uloga ozona i peroksida u oksidacijskom kapacitetu atmosfere iznad Hrvatske i oksidativnom stresu (The role of ozone and peroxide in the oxidizing capacity of the atmosphere over Croatia and in oxidative stress) [Ph.D. thesis]. Zagreb: Faculty of Science, University of Zagreb; 2007. Mentors: Vađić V, Klasinc L.
- E39. PETEK MJ. Procjena biološke učinkovitosti tenocilkidina i adamantanskog derivata TAMORF-a u uvjetima *in vitro* i *in vivo* (Evaluation of biological efficiency of tenocyclidine and its adamantane derivative TAMORF *in vitro* and *in vivo*) [Ph.D. thesis]. Zagreb: Faculty of Science, University of Zagreb; 2008. Mentor: Lucić Vrdoljak A.
- E40. PLEŠA N. Procjena genotoksičnosti irinotekana metodom komet testa (Assessment of irinotecan genotoxicity using the comet assay) [Graduation thesis]. Zagreb: Faculty of Science, University of Zagreb; 2005. Mentor: Kopjar N.
- E41. RAVLIĆ S. Genotoksični učinak metabolita DDT-a u kulturi ljudskih limfocita primjenom mikronukleus testa (Assessment of DDT metabolites genotoxicity in the human lymphocyte culture using micronucleus assay) [Graduation thesis]. Zagreb: Faculty of Science, University of Zagreb; 2006. Mentor: Garaj-Vrhovac V.
- E42. RUDEŠ K. Djelovanje okratoksina A na proteinske karbonile u jetri štakora (The effect of ochratoxin A on protein carbonyls in rat liver) [Graduation thesis]. Zagreb: Faculty of Pharmacy and Biochemistry, University of Zagreb; 2006. Mentors: Domijan A-M, Žuntar I.
- E43. RUK D. Kemijska i genotoksična svojstva otpadnih produkata nastalih u proizvodnji čelika (Chemical and genotoxic properties of wasteby-products of steel production) [M.Sc. thesis]. Zagreb: Faculty of Science, University of Zagreb; 2007. Mentor: Garaj-Vrhovac V.
- E44. SOKOLOVIĆ M. Genotoksični učinci T-2 toksina na genom limfocita periferne krvi pilića u uvjetima *in vivo* (Genotoxic effects of T-2 toxin of chicken peripheral lymphocyte genome in the *in vivo* conditions) [Ph.D. thesis]. Zagreb: Faculty of Science, University of Zagreb; 2007. Mentor: Garaj-Vrhovac V.
- E45. ŠAFRANIĆ A. Procjena genotoksičnosti triptofola primjenom mikronukleus testa (Evaluation of tryptophol genotoxicity using micronucleus assay) [Graduation thesis]. Zagreb: Faculty of Food Technology and Biotechnology, University of Zagreb; 2006. Mentor: Kopjar N.
- E46. ŠARIĆ M. Utjecaj mineralnih sastojaka i kiselosti prehrane na koštanu masu mladih štakora (Effects of dietary mineral elements and acid load on bone mass in young rats) [Ph.D. thesis]. Zagreb: School of Medicine, University of Zagreb; 2007. Mentor: Piasek M.
- E47. ŠIMIĆ M. Određivanje omjera sfinganin/sfingozin u urinu kao pokazatelj izloženosti fumonizinima (The ratio sphinganine/sphingosine in urine as indicator of exposure to fumonisins) [Graduation thesis]. Zagreb: Faculty of Pharmacy and Biochemistry, University of Zagreb; 2006. Mentors: Domijan A-M, Žuntar I.
- E48. ŠINKO G. Inhibicija kolinesterazâ enantiomerima etopropazina (Inhibition of cholinesterases with ethopropazine enantiomers) [Ph.D. thesis]. Zagreb: Faculty of Science, University of Zagreb; 2007. Mentor: Simeon V.
- E49. ŠOŠTARIĆ N. Inhibicija acetilkolinesteraze i butirilkolinesteraze piridinijevim oksimima, 2-PAM i HI-6 (Inhibition of acetylcholinesterase and butyrylcholinesterase by pyridinium oximes 2-PAM and HI-6) [Graduation thesis]. Zagreb: Faculty of Science, University of Zagreb. Mentors: Simeon V, Kovarik Z.
- E50. ŠTIMAC A. Utjecaj promjene primarne strukture acetilkolinesteraze na inhibiciju s karbamatom metaproterenola (Amino acid residues controlling the inhibition of acetylcholinesterase by metaproterenol carbamate) [Graduation

- thesis]. Zagreb: Faculty of Science, University of Zagreb; 2008. Mentors: Kovarik Z, Weygand-Durašević I.
- E51. TURKALJ J. Mikrovalna ekstrakcija postojanih organoklorovih spojeva iz tla (Microwave-assisted extraction of persistent organochlorine compounds from soil) [Graduation thesis]. Zagreb: Faculty of Science, University of Zagreb; 2006. Mentors: Vasilić Ž, Drevenkar V.
- E52. VELIKIĆ G. Terapijska djelotvornost derivate piridin klorida pri trovanju somanom. (Therapeutic effect pyridinium chloride derivatives against soman poisoning) [Graduation thesis]. Zagreb: Faculty of Pharmacy and Biochemistry, University of Zagreb; 2006. Mentor: Lucić Vrdoljak A.
- E53. VISKOVIĆ T. Utjecaj odabranih parametara na biosintezu zearalenona s pomoću plijesni *Fusarium graminearum* ZMPBF 1244 (The effect of selected parameters on the biosynthesis of zearalenone using *Fusarium graminearum* ZMPBF 1244) [Graduation thesis]. Zagreb: Faculty of Food Technology and Biotechnology, University of Zagreb; 2004. Mentors: Domijan A-M, Duraković S.
- E54. VRANJKOVIĆ B. Određivanje koncentracije okratoksina u mediju i LLC-PK1 staničnom lizatu (Determination of the ochratoxin A in media and lysate of LLC-PK1 cells) [Graduation thesis]. Zagreb: Faculty of Pharmacy and Biochemistry, University of Zagreb; 2004. Mentors: Domijan A-M, Petrik J.
- E55. VUCINIC D. Ridistribuzione delle particelle e delle fibre instillate per via tracheale nei polmoni di ratti Wistar (Redistribution of particles and fibres instaled through trachea into lungs of Winstar rats) [Graduation thesis, in Italian]. Milano, Italia: Università Degli Studi di Milano, Facoltà di Scienze Matematiche Fisiche e Naturali. Corso di Laurea in Scienze Biologiche; 2004. Mentors: Trošić I, Comolli R.
- E56. ŽLENDER V. Učinci okratoksina A na ekspresiju prijenosnika organskih aniona Oat1, Oat2, Oat3 i Oat5 u bubregu štakora (Effects of ochratoxin A on the expression of organic anion transporters Oat1, Oat2, Oat3 and Oat5 in rat kidney) [Ph.D. thesis]. Zagreb: Faculty of Science, University of Zagreb; 2007. Mentor: Sabolić I.

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Čačković, Mirjana
Čorović, Naima
Drevenkar, Vlasta
Fingler Nuskern, Sanja
Flajs, Dubravka
Franić, Zdenko
Fučić, Aleksandra
Gajski, Goran
Garaj-Vrhovac, Verica
Godec, Rajka
Gomzi, Milica
Herceg Romanić, Snježana
Ivičić, Nikola
Jurasović, Jasna
Kašuba, Vilena
Katalinić, Maja
Katić, Anja
Katić, Jelena
Kljaković Gašpić, Zorana
Kopjar, Nevenka
Koščec-Đuknić, Adrijana
Kovač, Jadranka
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Kožul, Darija
Lazarus, Maja
Liščić, Rajka
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Ljubojević, Marija
Ljubičić Čalušić, Anita
Macan, Jelena
Marković, Marijana
Marović, Gordana
Mataušić-Pišl, Mirjana
Mendaš Starčević, Gordana
Milić, Mirta
Miličević, Ante
Milković-Kraus, Sanja
Mladinić, Marin
Pavičić, Ivan
Pavlović, Mladen
Pehnec, Gordana

Peraica, Maja
Petrinec, Branko
Piasek, Martina
Pizent, Alica
Prester, Ljerka
Radić, Božica
Radošević-Vidaček, Biserka
Raos, Nenad
Rozgaj, Ružica
Sabolić, Ivan
Sabolić Pipinić, Ivana
Sabolović, Jasmina
Samolec, Marija
Stipičević, Sanja
Surić Mihić, Marija
Šega, Krešimir
Šinko, Goran
Šišović, Anica
Tomljenović, Andrea
Tončić, Josip
Trošić, Ivančica
Vađić, Vladimira
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Vasilić, Želimira
Vinković Vrček, Ivana
Želježić, Davor
Žužul, Silva

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Prlić, Ivica
Roić, Branka
Stankić Drobnjak, Spomenka
Skoko, Božena
Turk, Rajka
Zorić, Mate

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Dasović, Vjieran
Filipec, Ana
Frković, Zvonimir
Idrizi, Selvije
Gečić, Irma
Heršak, Eva
Horš, Nada
Janković, Katarina
Komesar, Mladen
Kralj, Mirjana

Kramarić, Marija
Kubelka, Zorica
Kujundžić Brkulj, Marija
Lieberth, Marija
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Mataušić, Snježana
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Mileković, Jasna
Nekić, Krešimir
Nikolić, Maja
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Sokolović, Enis
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Šmaguc, Kata
Tkalčević, Biserka
Triva, Vesna

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Đurđević, Dragica
Ferenčak, Verica
Horvat, Milica
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Rustić, Sanja

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Knežević, Smilja
Mankić Perković, Ljiljana
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Novoselec, Snježana
Posavec, Ivan
Slivak, Anica
Smolčić, Štefica
Štrk, Jelena
Vidović, Barica
Vuković, Marica
Zajec, Julijus
Župetić, Božidar
Živanović, Kristina

Associate scientists

Blanuša, Maja
Kanceljak-Macan, Božica
Kostial, Krista
Krauthacker Blanka
Reiner, Elsa
Simeon, Vera

Šarić, Marko
Telišman, Spomenka

Retired between 2004 and 2008

Adžić, Mira
Barić, Janka
Blanuša, Maja
Breber, Nada
Breški, Đurđa
Ciganović, Marija
Frković, Vesna
Hajdarović, Stane
Hršak, Janko
Iveković, Nediljka
Kalinić, Nataša
Kanceljak-Macan, Božica
Karačić, Višnja
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Lokobauer, Nevenka
Malinar, Marta
Poduje, Marija
Pondeljak, Katarina
Sajko, Mirjana
Simeon, Vera
Skender, Ljiljana
Švast, Blaženka
Telišman, Spomenka
Varga, Božena

Left between 2004 and 2008

Bračanov, Vlatka
Bronzović, Maja
Čepelak, Slobodan
Domijan, Ana-Marija
Đurinec, Martina
Ferenčak, Zlatan
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Pažur, Daniela
Plavec, Davor
Ramić, Snježana
Suša, Martina
Šarić, Marija
Šimić, Diana
Šimunić, Suzana
Škarica, Mario
Tokić, Goran
Špoljarić, Igor
Žlender, Vilim