

ПРОГРАМА

9:00 – 9:30 РЕГИСТРАЦИЯ

9:30 – 9:40 ОТКРИВАНЕ

9:40 – 10:00 **ПЛЕНАРЕН ДОКЛАД**

EUROPEAN REGISTRATION OF TOXICOLOGISTS IN BULGARIA

Prof. Christophor Dishovsky, MD, PhD, DSc, ERT

President of Bulgarian ERT Commission

President of Bulgarian Toxicological Society

10:00 – 12:30

УСТНИ ПРЕЗЕНТАЦИИ

Председател: Проф. д-р М. Мичева, ERT

Секретар: Доц. д-р В. Цанкова, ERT

МОНИТОРИНГ, ДИАГНОСТИКА И ПРОГНОЗА НА ИНТОКСИКАЦИИТЕ

ETIOLOGY OF FATAL ACUTE EXOGENOUS POISONING - EPIDEMIOLOGICAL STUDY OF TWO YEARS PERIOD

Radenkova-Saeva J.

ТОКСИКОЛОГИЯ НА БИОЛОГИЧНО АКТИВНИ ВЕЩЕСТВА ОТ ПРИРОДЕН И СИНТЕТИЧЕН ПРОИЗХОД

COMPARING THE INDUCTIVE CAPABILITY OF DIOSGENIN AND PHENOBARBITAL IN RATS

Kondeva-Burdina M., Mitcheva M., Nikolov S., Krasteva

TOXICITY EVALUATION OF NANO-SIZED CARRIERS FOR DRUG DELIVERY

Virginia Tzankova, Magdalena Kondeva-Burdina, Denitsa Aluani, Yordan Yordanov,
Borislav Tzankov, Krassimira Yoncheva, Nikolay Danchev

IN VITRO AND IN VIVO EVALUATION OF PROTECTIVE EFFECTS OF FREE AND NANOENCAPSULATED QUERCETIN

Denitsa Aluani, Yordan Yordanov, Vladislav Batchev, Virginia Tzankova, Magdalena
Kondeva-Burdina, Krassimira Yoncheva

EFFECTS OF PURE AND MICELLAR PROPOLIS ON CYTOTOXICITY AND PROTECTION AGAINST H₂O₂-INDUCED OXIDATIVE STRESS IN VITRO

Y. Yordanov, D. Aluani, V. Tzankova, K. Yoncheva, P. Petrov, B. Trusheva, V.
Bankova

IN VITRO/IN VIVO EXPERIMENTAL STUDY ON SOME METABOLIC INTERACTIONS WITH AMPHETAMINE – POSSIBLE MECHANISMS

Vessela Vitcheva, Rumyana Simeonova, Magdalena Kondeva-Burdina, Mitka
Mitcheva

IN VITRO EFFECTS OF NEWLY SYNTHESIZED DERIVATIVES OF CAFFEINE-8-THIOGLYCOLIC ACID ON ISOLATED RAT SUBCELLULAR FRACTIONS

Alexandra Kasabova, Magdalena Kondeva-Burdina, Javor Mitkov, Maya Georgieva,
Virginia Tzankova, Alexander Zlatkov

PHARMACOGENOMICS OF ADVERSE DRUG REACTIONS: IMPLEMENTING PERSONALIZED MEDICINE

Stanislav Yanev and Maria Popova

METAL [ZN(II), CU(II), CO(II), NI(II)] COMPLEXES OF MELOXICAM AND ISOXICAM: TOXIC EFFECTS IN CULTURED HUMAN AND ANIMAL CELLS

Lora Dyakova, Tanya Zhivkova, Milena Georgieva, Robert Khairallah, Pencho Beykov, Georgi Miloshev, Gabriela Marinescu, Daniela-Cristina Culita, Luminita Patron, Radostina Alexandrova

CU(II) AND CO(II) COMPLEXES WITH THE SAME SCHIFF BASES EXPRESS DIFFERENT RATE OF CYTOTOXICITY

Desislav Dinev, Milena Georgieva, Tanya Zhivkova, Lora Dyakova, Katya Popova, Ivaylo Dankov, Nikola Simeonov, Georgi Miloshev, Gabriela Marinescu, Daniela-Cristina Culita, Luminita Patron, Radostina Alexandrova

ADVERSE EFFECTS OF E280 ON CNS AND PROTECTIVE EFFECT OF ELLAGIC ACID ON RAT'S BEHAVIOR

Popatanasov A., Tancheva, L., R. Kalfin

ПРОФЕСИОНАЛНА ТОКСИКОЛОГИЯ

RISK MANAGEMENT OF THE CHEMICAL EXPOSURE AT PORT VARNA WEST

Karolina Lyubomirova, Lidia Hristova, Nevena Tzacheva

РЕГУЛАТОРНА ТОКСИКОЛОГИЯ

FOURTH LIST OF INDICATIVE OCCUPATIONAL EXPOSURE LIMIT VALUES PURSUANT TO COUNCIL DIRECTIVE 98/24/EC, AND AMENDING COMMISSION DIRECTIVES 91/322/EEC, 2000/39/EC AND 2009/161/EU

Venko Georgiev, Svetla Tzolova, Zhivka Halkova

SIGNIFICANCE OF TOXICOLOGICAL STUDIES IN THE SAFETY ASSESSMENT OF GMO FOOD IN LINE OF REGULATION № 503/2013

Tzveta Georgieva

STUDY OF TOXICOLOGICAL CHARACTERISTICS OF CERTAIN FOOD COLORS

R.Klisurov and L.Tancheva

12:30 – 13:00

ОБЯД

13:00 – 14:00

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STUDY OF ACUTE POISONING WITH CHLOPHAZOLIN FOR A PERIOD OF ONE YEAR

Stoyanova R.D., J. Radenkova-Saeva

ТОКСИКОЛОГИЯ НА БИОЛОГИЧНО АКТИВНИ ВЕЩЕСТВА ОТ ПРИРОДЕН И СИНТЕТИЧЕН ПРОИЗХОД

ISOTHERMAL TITRATION CALORIMETRY (ITC) AND DIFFERENTIAL SCANNING CALORIMETRY (DSC) FOR INVESTIGATION OF INTERACTION OF DEXAMETHASONE WITH BLOOD PLASMA PROTEINS

Anelia Zasheva, Silviya Abarova, Boris Tenchov

PROTECTANT DRUG EFFICACY AGAINST SCOPOLAMINE-INDUCED DEMENTIA IN MICE, A DSC APPROACH

Silviya Abarova, Alexandra Marinkova1, Rumiana Koynova, Lubomir Traikov, Lyubka Tancheva, Boris Tenchov

BEHAVIORAL EFFECTS OF NEW NEUROTENSIN ANALOGUES ON EXPERIMENTAL MODEL OF PARKINSON'S DISEASE

Maria Lazarova, Svetlana Stoeva, Lyubka Tancheva, Tamara Pajpanova, Reni Kalfin

DIFFERENTIAL SCANNING CALORIMETRY - METHOD FOR DETECTION OF NEURODEGENERATIVE DISEASES

Stella Zaharinova, Silviya Abarova, Lyubka Tancheva, Svetlana Stoeva, Rumiana Koynova, Boris Tenchov

ПРОФЕСИОНАЛНА ТОКСИКОЛОГИЯ

ASSESSMENT OF ASBESTOS CONTAMINATION IN AREAS OF FORMER PRODUCTIONS AND ASBESTOS-CONTAINING EQUIPMENT IN THE COUNTRY

Savina Dimitrova and Emiliya Mavrodieva

HYGIENIC CHARACTERISTIC OF INSULATION WOOLS FROM MAN-MADE MINERAL FIBERS

E. Mavrodieva, S. Dimitrova, D. Stankova, G. Paunova L. Mechkueva

STUDY ON THE AIR POLLUTANTS IN THE WORKING ENVIRONMENT WITH LASER CUTTING OF PAPER AND PLASTIC PRODUCTS

M. Tsoneva, K. Bozhilova, H. Kopcheva, R. Georgieva

SYNOPSIS OF LEGISLATION IN THE REPUBLIC OF BULGARIA IN THE FIELD OF MONITORING AND PROTECTING THE WORKERS FROM THE EFFECTS OF CHEMICAL FACTORS IN THE WORKPLACE

Tsonka Ivanova

ТОКСИКОХИМИЧЕН АНАЛИЗ

MERCURY CONTENT IN BULGARIAN COSMETIC PRODUCTS

Daniela Stankova, Ginka Paunova, Lydia Metchkueva, Rossitsa Georgieva

EXTRACTION OF MICROCYSTINS FROM WATER

Vera Pavlova

ВОЕННА ТОКСИКОЛОГИЯ

CONTEMPORARY APPROACHES IN DESIGN OF NEW REACTIVATORS OF CHOLINESTERASE AS ANTIDOTES AGAINST POISONING WITH ORGANOPHOSPHORUS COMPOUNDS (NERVE AGENTS AND PESTICIDES)

Ahmed Nedzhib, SilviyaStoykova, Ivan Samnaliev

NEUROMUSCULAR TRANSMISSION – IN VIVO RAT MODEL

Yana Goranova, Evelina Odiseeva, Vasil Atanasov, Ivan Samnaliev

14:00 – 15:00

ГОДИШНА СРЕЩА НА ДРУЖЕСТВОТО ПО ТОКСИКОЛОГИЯ

ПЛЕНАРЕН ДОКЛАД

EUROPEAN REGISTRATION OF TOXICOLOGISTS IN BULGARIA

Christophor Dishovsky, MD, PhD, DSc, ERT
President of Bulgarian ERT Commission
President of Bulgarian Toxicological Society

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The European Register of Toxicologists is a service of EUROTOX established in 1994. It constitutes a list of toxicologists who excel by high standards of education, skills, experience, and professional standing.

Individuals who want to be registered and are found to comply with the requirements defined by EUROTOX and National Societies of Toxicology, and are accepted, are qualified to use the title EUROPEAN REGISTERED TOXICOLOGIST, ERT, with their name.

Registration is performed by a two-step procedure. First, National Registration boards in Europe evaluate applications of candidates according to a consensual process described in the ERT Guidelines for Registration, and admit successful applicants to the national register. Second, upon request, EUROTOX will certificate these individuals as ERT.

EUROTOX lists almost 1900 toxicologists as ERT. The Bulgarian Register of Toxicologists was launched in 2011 and is a part of the European Register. The requirements for becoming ERT are summarized as follows: an academic degree in a science linked to toxicology, further theoretical training, practical training and expertise in toxicology (overall at least 5 years), current active professional participation in the field of Toxicology. They are described in the Guidelines for Registration.

Inclusion, exclusion or removal from the Register is decided by a Registration Panel, consisting of 7 members appointed for 5 years, (including representative of EUROTOX Registration Sub-Committee). There are 13 European Registered Toxicologists (ERT) in Bulgaria.

Certification as an ERT is widely appreciated by national and international agencies and authorities and by companies. Recognition of professionals in scientific disciplines such as toxicology is becoming more important than ever in our increasingly globalised environment.

Qualified toxicologists recognized as ERT are now working in a wide array of different fields (drug, nutritional, chemical and environmental safety, risk assessment and risk management, basic research, clinical and occupational toxicology, etc).

The EUROTOX system for recognizing the professional qualifications and experience of toxicologists will improve harmonisation among national registration systems.

Application details for registration and re-registration are based on Guidelines for Registration, sections A and D, and can be obtained from national registries. (<http://www.bgts.tasatbg.com>).

The next Bulgarian ERT Panel meeting to review new applications will be at December 2016!

Key words: EUROTOX, European Registered Toxicologists, ERT

ДОКАЛДИ

МОНИТОРИНГ, ДИАГНОСТИКА И ПРОГНОЗА НА ИНТОКСИКАЦИИТЕ

ETIOLOGY OF FATAL ACUTE EXOGENOUS POISONING - EPIDEMIOLOGICAL STUDY OF TWO YEARS PERIOD

Radenkova-Saeva J.

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Objective: To investigate the etiology of acute exogenous intoxication with fatal outcome in patients, admitted for treatment at the Clinic of Toxicology, Department for Adults, UMHATEM "N.I. Pirogov", Sofia for a period of two years.

Methods: A retrospective study of severe fatal poisonings, between 01.10.2014 - 01.10.2016 was performed. The following criteria were taken into consideration: etiology, age, type of poisoning, motives for the poisoning.

Results: 36 deaths were recorded for the studied period. Patients were aged from 26 to 93 years. From a total deaths, the most common cause was multidrug poisoning - 7 cases (19.44%) and poisoning with psychoactive substances - heroin, methadone - 7 cases (19.44%), followed by poisoning with corrosive substances - 6 cases (16.66%), methanol - 6 cases (16.66%); ethylene glycol - 2 cases (5.55%); pesticides - 2 cases (5.55%); Amanita phalloides mushroom poisoning - 1 case (2.77%), others - detergents, disinfectants, lactic acidosis of unknown etiology - 5 cases (13.88%). The majority of fatal intoxications occurred in the age group 40-60 years. Poisoning was intentional in 15 persons (41.66%).

Conclusion: Acute multidrug poisoning and poisoning with psychoactive drugs - heroin and methadone had the highest frequency in the etiology of poisoning deaths in the studied period.

Key words: etiology, multidrug poisoning, psychoactive drugs, fatal outcome

ТОКСИКОЛОГИЯ НА БИОЛОГИЧНО АКТИВНИ ВЕЩЕСТВА ОТ ПРИРОДЕН И СИНТЕТИЧЕН ПРОИЗХОД

COMPARING THE INDUCTIVE CAPABILITY OF DIOSGENIN AND PHENOBARBITAL IN RATS

Kondeva-Burdina M.¹, Mitcheva M.¹, Nikolov S.², Krasteva I.²

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Diosgenin is a steroid sapogenin, which revealed different pharmacological effects – anti-inflammatory, spasmolytic, antihypercholesterolemic and antioxidant. In this experiment, we used Diosgenin, isolated from *Asparagus officinalis*, which was identified by physical and spectral data, comparing with literature. In our previous studies, we found good *in vitro* cytoprotective and antioxidant activity of Diosgenin on carbon tetrachloride-induced toxicity model on isolated rat hepatocytes and good hepatoprotective effects *in vivo* on rats. It's known, that carbon tetrachloride is bio-activated to reactive metabolites by different isoforms of Cytochrome P450, one of them is CYP3A.

According to the literature data about the increased expression of CYP3A by synthetic Diosgenin in mice, we exam in rats the possible inducible effects of Diosgenin, on the activity of Ethylmorphine N-demethylase (EMND) (marker for CYP3A), the total quantity of Cytochrome P450 and the expression of isoform CYP3A (by Western blot analysis), administered *in vivo* alone and comparable

with Phenobarbital at equimolar concentrations.

Diosgenin and Phenobarbital, administered alone, increased statistically significant, compared to the control, the total amount of Cytochrome P450 – with 36 % and with 85 % and the activity of EMND – with 58 % and with 184 %, respectively. Western blot analysis showed that Diosgenin increased the expression of CYP3A isoform, similar to Phenobarbital.

According to the literature data, about the influence of synthetic Diosgenin on PXR receptor, which play role in the expression of CYP3A, and the mechanism of Phenobarbital induction, we can conclude that the possible mechanism of the inductive effect of Diosgenin, isolated from *Asparagus officinalis*, is similar to the mechanism of Phenobarbital induction.

Key words:

Induction, Diosgenin, Ethylmorphine N-demethylase, Cytochrome P450, CYP3A

TOXICITY EVALUATION OF NANO-SIZED CARRIERS FOR DRUG DELIVERY

Virginia Tzankova*, Magdalena Kondeva-Burdina*, Denitsa Aluani*, Yordan Yordanov*, Borislav Tzankov**, Krassimira Yoncheva**, Nikolay Danchev*

* Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia, Bulgaria ** Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University of Sofia, Bulgaria

Nanotechnology is being utilized in the field of medicine and pharmacy as diagnostic and therapeutic tools to better understand, detect and treat human diseases. Cell specific targeting, enhanced therapeutic efficacy and improved safety are the main goals of the new drug – delivery systems development. Recently, different types of nano-sized drug carriers (polymers, liposomes, dendrimers, silicon or carbon materials, and magnetic nanoparticles) have been developed to improve the drug delivery and to overcome some drug - dependent limitations, as water insolubility, instability in biological environment or poor inter- and intramembrane transport. The great challenge is to develop and introduce in the practice therapeutically efficient, non-toxic, biocompatible, biodegradable, and safe nano-sized drug delivery systems. The use of nanoparticles as drug carriers involves intentional contact to the biological system, so that the understanding of the effect of the nanoparticles on the body before their clinical use is very important. Nanotoxicology is a new scientific direction of Laboratory of Toxicology and Drug metabolism in the Faculty of Pharmacy, Medical University-Sofia. Our special attention is focused on the complex *in vitro* and *in vivo* toxicity evaluation of mesoporous silica nanoparticles and polymeric nanoparticles as drug carriers.

Key words: silica nanoparticles, polymeric micelles, nanotoxicity

IN VITRO AND IN VIVO EVALUATION OF PROTECTIVE EFFECTS OF FREE AND NANOENCAPSULATED QUERCETIN

Denitsa Aluani*, Yordan Yordanov*, Vladislav Batchev*, Virginia Tzankova*, Magdalena Kondeva-Burdina*, Krassimira Yoncheva**

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** Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University of Sofia, Bulgaria

Quercetin is the most common dietary flavonoid and it was reported to prevent a number of chronic diseases, including cancer and cardiovascular diseases. However, the clinical use of quercetin is limited due to its poor absorption, low oral bioavailability, very low tissue distribution, rapid metabolism and instability under physiological conditions. Nano-scaled drug carriers are promising candidates for overcoming these problems. Many of the beneficial effects of quercetin in different pathologies have been attributed to its antioxidant activity. It acts as a free radical scavenger and protects the cells from oxidative stress generated as an outcome of reactive oxygen species. The

present study aims to explore the protective effects of quercetin (free or loaded into polysaccharide nanoparticles) in different models *in vitro* and *in vivo*. The nanoparticles were prepared varying different ratios of chitosan and sodium alginate. *In vitro* antioxidant effects of free and encapsulated quercetin were investigated in two cell culture models (human hepatoma cell line Hep G2 and freshly isolated rat hepatocytes) and in isolated liver microsomes and isolated brain synaptosomes. The *in vivo* effects were examined after repeated treatment (12 mg/kg, p.o., 7 days) in male Wistar rats. The results showed that neither empty, nor quercetin loaded nanoparticles induced toxicity changes, e.g. changes in hematology and blood biochemistry parameters and liver tissue damages.

Key words: quercetin, antioxidant effects, nanoparticles

EFFECTS OF PURE AND MICELLAR PROPOLIS ON CYTOTOXICITY AND PROTECTION AGAINST H₂O₂-INDUCED OXIDATIVE STRESS IN VITRO

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** Department of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, Medical University, Sofia, Bulgaria

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Abstract: Propolis is a complex natural product containing bioactive compounds. Its composition depends mainly on the geographical features of its origin. Propolis exerts antioxidant properties most probably related to its flavonoid contents. A contemporary approach to overcome its poor water solubility is the inclusion of propolis in polymer nanoparticles.

The aim of this work was to study the impact of propolis loading in poly(ethylene oxide)-block-poly(n-butyl acrylate) diblock copolymer micelles on the extent of cytotoxic effects on hepatocellular carcinoma, fibroblastoma and neuroblastoma cell lines (HepG2, L929 and SH-SY5Y). The neuroprotective effects of pure and micelle-loaded propolis were also assayed by means of an oxidative stress model with the neuroblastoma cell line SH-SY5Y.

The cytotoxicity of cultures of the three cell lines, treated with pure and loaded propolis in the concentration interval 25-500 µg/ml was evaluated by MTT assay. To evaluate the neuroprotective effects, a model of hydrogen peroxide-induced damage on SH-SY5Y was implemented.

Experimental results reveal that loading of propolis in micelles decreases its cytotoxic effects while neuroprotective effects are preserved. Pre- and post-treatment of the SH-SY5Y oxidative stress model with micelle-loaded propolis leads to an increase of the neuroprotective effects in comparison with pure propolis treatment. This tendency is mostly pronounced and statistically significant after pre- and post-treatment with the highest concentration evaluated (500 µg/ml).

Key words: propolis, polymer micelles, cytotoxicity, neuroprotection

IN VITRO/IN VIVO EXPERIMENTAL STUDY ON SOME METABOLIC INTERACTIONS WITH AMPHETAMINE – POSSIBLE MECHANISMS

Vessela Vitcheva, Rumyana Simeonova, Magdalena Kondeva-Burdina, Mitka Mitcheva

Laboratory of Drug Metabolism and Drug Toxicity, Department of pharmacology, pharmacotherapy and toxicology, Faculty of Pharmacy, Medical University – Sofia

The objectives of this study were to investigate the influence of induction and inhibition on amphetamine hepatotoxicity *in vivo/in vitro* and to elucidate a possible involvement of CYP 3A and CYP 2D in the toxic mechanisms. Male Wistar rats were treated with nifedipine (5 mg.kg⁻¹, i.p., 5 days), a substrate and inducer of CYP3A, alone and along with amphetamine (5 mg.kg⁻¹, i.p., 5 days). Amphetamine induced increase MDA production and GSH depletion and led to significant decrease

of P 450 quantity and EMND activity, without changing AH activity. In combination with nifedipine, however, EMND and AH activities were increased by 34% and 21%, versus control. For the *in vitro* part of the study, hepatocytes isolated from control and induced with nifedipine rats were incubated with amphetamine (100 $\mu\text{mol L}^{-1}$ for one hour) alone and along with amiodarone (14 μM) - CYP 3A inhibitor and quinidine (75 μM) - CYP2D inhibitor. Nifedipine potentiated amphetamine cytotoxicity, judged by decreased cell viability, GSH levels, increased LDH activity and MDA production. Pre-incubation of nifedipine-treated hepatocytes with either amiodarone or quinidine significantly lowered amphetamine cytotoxicity. Using and inducer and inhibitor of CYP3A and CYP2D proved their involvement in amphetamine-mediated liver damage.

Key words: amphetamine toxicity, nifedipine, isolated hepatocytes, amiodarone, quinidine

IN VITRO EFFECTS OF NEWLY SYNTHESIZED DERIVATIVES OF CAFFEINE-8-THIOGLYCOLIC ACID ON ISOLATED RAT SUBCELLULAR FRACTIONS

Alexandra Kasabova¹, Magdalena Kondeva-Burdina¹, Javor Mitkov², Maya Georgieva², Virginia Tzankova¹, Alexander Zlatkov²

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Oxidative stress is implicated in the pathogenesis of many diseases, such as neurodegenerative, cancer and others. The increased production of reactive oxygen species (ROS) lead to damage of number of biomolecules such as lipids, proteins and DNA. Caffeine is the most commonly used psychostimulant, which revealed protective effects in conditions of chronic liver and neurodegenerative diseases.

The aim of the study was to evaluate the effects of newly synthesized derivatives of caffeine-8-thioglycolic acid JTA 1-13 (100 μM) on isolated rat liver microsomes. The possible neuroprotective effects of the compounds with antihypoxic effect, JTA-1 and JTA-2 (100 μM), in a model of 6-hydroxydopamine-induced oxidative stress was also investigated on isolated rat synaptosomes.

The derivatives of caffeine-8-thioglycolic acid (100 μM) didn't show any statistically significant pro-oxidant toxic effects on isolated rat liver microsomes, compared to the control (non-treated microsomes). In an *in vitro* model of 6-hydroxydopamine-induced oxidative stress, the compounds JTA-1 and JTA-2 preserved significantly the synaptosomal viability and the level of reduced glutathione, thus showing neuroprotective effects on isolated rat synaptosomes. The possible mechanism of neuroprotection might be due to the potential competition between the 6-hydroxydopamine and JTA-1 and JTA-2 for binding to the dopamine receptor.

Key words: oxidative stress, caffeine-8-thioglycolic, neuroprotective effects

PHARMACOGENOMICS OF ADVERSE DRUG REACTIONS: IMPLEMENTING PERSONALIZED MEDICINE

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The accumulating knowledge of human genomic variation is being used for the development of personalized medicine, with the aims of increasing the efficacy of drug treatment and decreasing the number of adverse drug reactions (ADRs). There is clinical evidence that polymorphisms in genes encoding drug-metabolizing enzymes, drug transporters, drug targets (e.g. receptors & enzymes) and human-leukocyte antigen (HLA) can lead to the occurrence of ADRs. In addition, mutations of certain genes can precipitate ADRs. Over the past years, genome-wide association studies (GWAS) have identified a number of common and rare variants that are associated with increased risk of ADRs. As affordable and reliable genetic testing tools become available to physicians, pharmacogenomics looks promising to facilitate individualization of drug therapy and as a result, this will maximize the therapeutic efficacy of drugs in patients while minimizing the occurrence of ADRs. In this short review, we present some new data on pharmacogenomics of ADRs, especially on genes

coding for drug metabolizing enzymes, drug transporters and HLAs, as well as the potential use of these genomic biomarkers in clinical practice for dose adjustment and for preventing drug toxicity.

CYP 2D6 gene variability reflected in the different consequences and clinical behavior regarding the use of *codeine* and *tamoxifen*: in ultrarapid metabolizers with potential risk of increased toxicity and in poor metabolizers with lack of effectiveness. The presence of **CYP 2C19** polymorphism (intermediate and poor metabolizers) in patients with acute coronary syndromes, who are undergoing percutaneous coronary intervention, hampered the usage of antiplatelet drug *clopidogrel* as it needs metabolic activation.

The percent of patients with adverse reactions to **coumarin anticoagulants** vary widely, within 5-40%. The main reasons for this large inter-individual variability are well known: polymorphic character of main coumarin metabolic pathway (**CYP2C9**) and high genetic polymorphisms of coumarin pharmacological target – vitamin K epoxide reductase complex subunit 1 (**VKORC1**). Three single nucleotide polymorphisms (SNPs), two in the *CYP2C9* gene and one in the *VKORC1* gene, have been found to play key roles in determining the effect of coumarine drug therapy on coagulation. A genetic polymorphism on the *VKORC1* gene results in a patient having less available VKORC enzyme to complete this reaction thus lower warfarin doses will be needed to achieve anticoagulation. The prevalence of these variants also varies by race, with 37% of Caucasians and 14% of Africans carrying the A allele. Two alternatively spliced transcripts encoding different isoforms have also been described. These isoforms result in acenocoumarol **resistance** (requiring higher doses) in humans and rats, because the amount and effectiveness of the VKORC enzyme has not changed, but the ability of acenocoumarol to exert its effect (antagonize the enzyme) has changed. These isoform mutations are rare except in Ethiopian and certain Jewish populations.

The efflux **transporter** ATP-binding cassette genetic variants (**ABCB1**), encoding p-glycoprotein (Pgp) associated with multiple drug resistance, may account for a difference of 25% in the renal clearance of *cyclosporine* thus leading to higher nephrotoxicity as well as for decrease effectiveness of chemotherapeutic action of *paclitaxel*. FDA has recommended **HLA-B*1502** genetic screening before prescribing *carbamazepine* and *phenytoin* to reduce the risk of Stevens–Johnson syndrome and toxic epidermal necrolysis and **HLA-B*5701** testing to avoid *abacavir*-induced hypersensitivity, in patients with ancestry from areas in which those HLA-B alleles are prevalent.

In conclusion there are two general goals in the study of pharmacogenetics and these include, the ability to predict those patients at high risk of toxicity (and hence a lower dose or change in therapy would be advised) and the ability to predict those patients who are more likely to obtain the desired therapeutic efficacy from the drug. Through prediction of how certain patients will respond to a drug, it is likely to avoid the unwanted ADRs.

Key words: pharmacogenomics, adverse drug reactions, personalized medicine

METAL [ZN(II), CU(II), CO(II), NI(II)] COMPLEXES OF MELOXICAM AND ISOXICAM: TOXIC EFFECTS IN CULTURED HUMAN AND ANIMAL CELLS

Lora Dyakova¹, Tanya Zhivkova², Milena Georgieva³, Robert Khairallah^{2,4}, Pencho Beykov^{2,5}, Georgi Miloshev³, Gabriela Marinescu⁶, Daniela-Cristina Culita⁶, Luminita Patron⁶, Radostina Alexandrova¹

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Meloxicam and isoxicam are well known non-steroidal anti-inflammatory drugs used in the treatment of various pathological conditions. Zinc, copper and cobalt are essential elements with broad spectrum of biological activities. The aim of our study was to evaluate the influence of Zn(II), Cu(II), Co(II) and Ni(II) complexes of meloxicam and isoxicam on viability and proliferation of cultured human and animal cells. The following permanent cell lines were used as model systems: LSCC-SF-Mc29 (chicken liver cancer), LSR-SF-SR (rat sarcoma), HeLa (human cervical carcinoma), 8MGBA (human glioblastoma multiforme), A549 (human non-small cell lung cancer), Lep3 (human embryo fibroblasts). The investigations were carried out by MTT test, neutral red uptake cytotoxicity assay,

crystal violet staining, double staining with acridine orange and propidium iodide, Comet assay, FACS, colony-forming method. The results obtained revealed that applied at concentrations of 5– 500 µg/ml the compounds examined decrease in a time- and concentration-dependent manner viability and proliferation of the treated cells. Metal complexes were found to be more pronounced cytotoxic/cytostatic agents as compared to the independently administered meloxicam and isoxicam. *Supported by: Grant № ДФНИ Б 02 30 from 12.12.2014, Fund “Scientific Research”, Bulgarian Ministry of Education and Science; Grant ДФНП-74 А-1, Program “Support of Young Scientists at the Bulgarian Academy of Sciences”; a bilateral project between Bulgarian Academy of Sciences and Romanian Academy. R. Khairallah and P. Beykov were “volunteers in the Laboratory”.*

CU(II) AND CO(II) COMPLEXES WITH THE SAME SCHIFF BASES EXPRESS DIFFERENT RATE OF CYTOTOXICITY

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Various Schiff bases and their metal complexes have been reported to possess promising biological, including anticancer and antimicrobial, properties. In our study Cu(II) and Co(II) complexes with Schiff bases obtained by a condensation reaction of *o*-Vanillin with amino acids (Tyrosine, Threonine, Tryptophan and Serine) were prepared. Their cytotoxic activity was studied in short-term (24-72 h, with monolayer cultures) and long-term (40-45 days, with 3D cell colonies) experiments using methods with different molecular/cellular targets and mechanisms of action: MTT test, Neutral red uptake cytotoxicity assay, Crystal violet staining, Propidium iodide/Acridine orange double staining, Comet assay at neutral pH, Annexin V/FITC and colony-forming technique. Permanent cell lines established from human breast cancer (MDA-MB-231, MCF-7) and cervical carcinoma (HeLa) as well as bovine kidney cells (MDBK) were used as model systems in our study. The results obtained reveal that applied at a concentration range of 1 to 200 µg/ml Cu(II) complexes significantly decrease percent of the viable treated cells in a time- and concentration-dependent manner and are more pronounced cytotoxic and cytostatic agents as compared to the Co(II) complexes of the same ligands.

Supported by Grant № DFNI Б 02 30 from 12.12.2014, Fund “Scientific Research”, Bulgarian Ministry of Education and Science.

Key words: Schiff bases, Metal complexes, Copper, Cobalt, Cell lines, Cytotoxicity

11. ADVERSE EFFECTS OF E280 ON CNS AND PROTECTIVE EFFECT OF ELLAGIC ACID ON RAT’S BEHAVIOR

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Introduction: The common food additive E280 – (propionic acid) becomes recognized as environmental factor for several neurodevelopmental diseases like autism spectrum disorders (ASD). A limited data was reported for improving effect of Ellagic acid (EA) on verbal memory in humans and on cognition of animals (Tancheva et al. 2014) as well as preventive action in several pathologies (cancer, neurodegeneration, Alzheimer’s disease etc.).

Aim: To evaluate neuroprotective effect of EA on the cognition, motor and social activity of rats with ASD model.

Methods: Experimental model of ASD was produced on male Wistar rats at two different ages (21 days and 90 days old) by oral E280 administration (250 mg kg⁻¹, *i.p.*, 3 days). The controls were given phosphate buffered saline. EA-treatment (200 mg kg⁻¹, *i.p.*, 3 days) started together with E280. On the 4th days after the last treatment some standard tests were used for evaluation of: learning and memory (Step-trough test); motor coordination (Rot-a-Rod test) and social behavioral interactions (open field). Experimental data are processed by t-test of Student-Fisher.

Results: All cognitive and motor tests performances were worsened by E280-treatment, especially in young animals. EA-treatment improved significantly learning and memory and motor coordination of treated animals as compared to E280-treated controls. EA impacted best the social interactions and reduced significantly the atypical and aggressive behavioral elements characteristic for ASD social interactions.

Conclusion: The EA has promising age dependent neuroprotective effect on E280-induced model of ASD. EA preventive effect is better in young animals as compared to adult rats.

Key words: ASD model; autism; EA, neurotoxicity; short fatty acid.

ПРОФЕСИОНАЛНА ТОКСИКОЛОГИЯ

RISK MANAGEMENT OF THE CHEMICAL EXPOSURE AT PORT VARNA WEST

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Introduction: Ports are characterized by variety of jobs, work tasks, workplaces and health risk factors.

Aim of the study was to analyze the working conditions and the health of the workers at port Varna West as well as to develop a risk management program.

Methods: Documentary method : protocols of the measurement of the concentrations of the chemical substances used at all workplaces at the port; analyses of the temporary disability reports for 2 years period.

Results: Risk assessment of the health status of 850 workers at port Varna West was provided. The measurement of the chemical substances revealed exposure to Ammonia, Ammonium hydroxide, Carbon Oxide and Carbon Dioxide, Hydrogen sulfite, Sodium sulfide and coal dust, Phosphorite, Copper, Lead. The concentrations exceeded the total limit values for coal dust and ammonium at numerous workplaces.

The analysis of the health status of workers revealed the predominant diseases which caused temporary disability. The highest prevalence was registered for Cancer (62% of all cases of temporary disability), Psychological disturbances (42%), Traumas and Accidents at work (30,3%), Cardio-vascular diseases (21%), Eye problems (17,6%).

Conclusion: Risk management program was implemented at the highest risk workplaces. The main steps consisted of: Monitoring of the magnitude of the exposure to the risk factors: unhealthy microclimate, dust concentration, noise, vibrations, chemical substances, shift work; Monitoring of the health status of the workers through pre- and periodic check-up exams; Monitoring of the health status of workers with percentage of disability; Training pf workers and information about the adverse health effects due to exposure to chemical substances, Health promotion programs.

Key words: port workers, exposure assessment, health analysis, risk management

13 FOURTH LIST OF INDICATIVE OCCUPATIONAL EXPOSURE LIMIT VALUES PURSUANT TO COUNCIL DIRECTIVE 98/24/EC, AND AMENDING COMMISSION DIRECTIVES 91/322/EEC, 2000/39/EC AND 2009/161/EU

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In September 2016 a draft Commission Directive establishing a 4th list of Indicative Occupational Exposure Limit Values (IOELVs) was accepted by the Technical Progress Committee.

An overview of the 4th list of IOELVs and its contribution to the improvement of the safety levels of occupational exposure to chemical hazards is presented in the report. It draws attention to the methodology for the derivation of IOELVs and on the new scientific data, on which are based some of the exposure limit values, which have been summed up in the reports of the Scientific Committee on Occupational Exposure Limits (SCOEL) for the respective chemical agents.

The main aspects of the legislative policy in the area of monitoring and protecting the workers from the effects of chemical agents at the workplace, and the amendments in the Bulgarian legislation, which would be needed after the adoption of the 4th list of IOELVs, are also discussed in the report.

Key words: indicative occupational exposure limit values, toxicological studies, health and safety legislation

14 SIGNIFICANCE OF TOXICOLOGICAL STUDIES IN THE SAFETY ASSESSMENT OF GMO FOOD IN LINE OF REGULATION № 503/2013

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In recent years in EU is increasing the importance of assessing health risks of GMO crops. Because of the short historical time of widespread use of GMO crops and products of bio-technology as a food and feed there is no sufficient scientific data on long-term effects of their use. Few are scientific publications in the literature, illustrating the impact on the human and animals. There are no epidemiological studies based on GMO used as a food. Given the increased sensitivity on food consumption and use of feed containing GMOs requirements for the safety assessment of any new GMOs for registration are harmonized. It is necessary to accumulate enough scientific data to prove the necessity of conducting toxicity tests and approaches to be applied in the safety assessment of GMOs. Should not be overlooked and the consumer's right to have an informed choice.

In last year's actively debated the role and importance of toxicology tests on animals. In 2013 new Regulation № 503/2013 for implementation of risk assessment procedures in line of Regulation № 1829/ 2004 was adopted. Questions raised are whether a need for more detailed studies to study biological and long-term effects of GMOs. How to assess exposure of human and animals to GM food and feed? Will support updated scarce scientific data the safety assessment of foods containing GMOs?

Key words:

safety assessment, toxicological studies, Genetically modified organisms (GMO), food

15. STUDY OF TOXICOLOGICAL CHARACTERISTICS OF CERTAIN FOOD COLORS

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The issue of the safety of food color agents is very topical. They are widely used by many manufacturers, but there are no conclusive data on the toxicological properties. Some sources even mention the risk of carcinogenicity in use, but it is not confirmed. This led us to the aim of this study: To explore toxicological characteristics of certain food colors (E122 and E133).

The study included two popular coloring agents: E122 (azorubine) and E133 (brilliant blue). They are widely found in various colored and instant drinks. Studied dyes were applied to male and female adult Wistar rats. They were given like their oral form of colored drinks with strawberry and blueberry taste. After 10 daily treatment were taken biological samples for analysis.

The results of the experiment indicate that the tested substances azorubine and brilliant blue have some neurotoxicity. In the brains of test animals are observed perivascular mikrohematomas in the brain parenchyma. Were established and nephrotoxicity. There is proteinuria in all tested animals.

This suggests further research to establish the full toxicological profile of the substances.

ПОСТЕРНИ ПРЕЗЕНТАЦИИ

МОНИТОРИНГ, ДИАГНОСТИКА И ПРОГНОЗА НА ИНТОКСИКАЦИИТЕ

STUDY OF ACUTE POISONING WITH CHLOPHAZOLIN FOR A PERIOD OF ONE YEAR

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Clofazolinat is imzidazol derivative, which is widely used for the treatment of severe hypertension and hypertensive crises. Objective: To study the acute chlophazolin poisoning in patients over 18 years, requiring a hospitalization. Methods: Retrospective epidemiological study covers a period of one year. All cases of chlophazolin poisoning analyzed in terms of gender, age, clinical course, treatment and outcome. Results: During the period from 01.01. 2015 to 31.12. 2015 in the Clinic of Toxicology, Department for adults, УМНАТЕМ "NI Pirogov" were treated 8 patients with acute chlophazolin intoxication. The patients were between the ages of 20 and 65, 2 of them were male and 6 female. The severity of poisoning vary from moderate to severe. Conclusion: Patients with acute poisoning with chlophazolin should be carefully monitored concerning their basic vital signs that can quickly and dramatically worsen. Acute poisoning with chlophazolin are a serious challenge for the physician - toxicologist.

Keywords: acute poisoning, chlophazolin

ТОКСИКОЛОГИЯ НА БИОЛОГИЧНО АКТИВНИ ВЕЩЕСТВА ОТ ПРИРОДЕН И СИНТЕТИЧЕН ПРОИЗХОД

ISOTHERMAL TITRATION CALORIMETRY (ITC) AND DIFFERENTIAL SCANNING CALORIMETRY (DSC) FOR INVESTIGATION OF INTERACTION OF DEXAMETHASONE WITH BLOOD PLASMA PROTEINS

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Introduction

Characterization of the thermodynamics of drug binding with blood plasma (BP) proteins is of essential importance for a better understanding of drug absorption, distribution and turnover in the circulation. Human serum albumin (HSA), the most prominent protein in plasma, plays a fundamental role in the transport of drugs.

Dexamethasone is a medication, corticosteroid, used to treat various endocrine, rheumatic, dermatologic diseases and allergic states, etc. Corticosteroids, including dexamethasone, are involved in a wide range of physiological processes such as immune response, regulation of inflammation, blood electrolyte levels, etc.

In this work, we used Isothermal Titration Calorimetry (ITC) and Nano Differential Scanning Calorimetry (DSC) to investigate the energetics of dexamethasone binding to BP proteins.

Isothermal titration calorimetry (ITC) is a thermodynamic technique for directly measuring the heat change of the molecular interactions.

The Nano DSC has the versatility and precision for characterizing molecular stability, determining high affinity ligand binding and deconvoluting multi-domain structures.

The aim of this study is to characterize the mechanism of dexamethasone interactions with BP proteins, in particular, with HSA.

Materials and Methods

The thermal effects of dexamethasone binding to human blood plasma from healthy volunteers and to HSA (Sigma Aldrich) was examined by ITC (Nano ITC, TA Instruments). ITC measurements were conducted in 0.05mM PBS (pH 7.4). The 1 mL sample cell was filled with 10 μ M HSA, and a

human blood serum, respectively. The 250 μ l injection syringe was loaded with 0.5 mM solution of dexamethasone. The dexamethasone solution was injected 25 times in 10 μ l increments with 400 s intervals into the isothermal cell. The titration process was computer-controlled. The stirring speed was set as 1000 rpm and the cell temperature was kept at 37°C. The experiment results were computed using the calorimeter software.

Immediately after ITC measurement the samples were degassed for 5-10 min. PB solution (0.05mM, pH 7.4) was used for reference cell. After degassing, the sample and reference were loaded into the cells of a Nano DSC (Nano DSC, TA Instruments), equipped with 300 μ l measuring cell volume. Scans were performed immediately with a temperature increase from 20 °C to 110°C at a scanning rate of 1°C/min, under a pressure of 3 atm.

Results

The ITC measurements demonstrate high binding affinity of dexamethasone to BP proteins and to HSA as evidenced by the exothermic thermal effects. The differences, recorded by DSC, between the thermal denaturation of the proteins in control blood plasma and HSA, as well as the thermograms of the drug-protein complexes suggest preferential binding of dexamethasone by albumin.

Conclusion

The present study helps to better understand the binding mechanism of dexamethasone with major BP proteins and suggests preferential binding of dexamethasone to albumin.

Key words: ITC, DSC, human blood plasma, albumin, dexamethasone

PROTECTANT DRUG EFFICACY AGAINST SCOPOLAMINE-INDUCED DEMENTIA IN MICE, A DSC APPROACH

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In this study, we employed a new approach based on differential scanning calorimetry (DSC) to detect and characterize at a molecular level the changes in brain tissues associated with drug-induced neurodegenerative disorder of Alzheimer's disease (AD) type and to evaluate the efficacy of preventive treatments with various biologically active compounds expected to hinder the AD development. We used an experimental animal (mouse) model of scopolamine-induced dementia. The DSC measurements performed on supernatants of brain tissue homogenates revealed large differences between the heat capacity profiles for healthy animals and for mice with scopolamine-induced dementia. The heat capacity profiles of brain tissue supernatants from healthy animals displayed well expressed low-temperature exothermic transitions peaking in the range 35-45°C, thus preceding in temperature the endothermic denaturational transitions. The exothermic transitions were only observed in supernatants of brain tissue homogenates, and not in other samples from the same animals, e.g., centrifugation sediments of brain tissue homogenates, liver homogenates, blood plasma. Remarkably, the low-temperature exotherms were completely eliminated by the scopolamine treatment and replaced with high-temperature exothermic transitions. Preventive treatments with various substances (myrtenal, ellagic acid, lipoic acid) and their combinations (including also ascorbic acid and galantamine), applied simultaneously with the scopolamine treatment, were found to neutralize the scopolamine effect, i.e., to result in partial or complete preservation of the low-temperature exothermic transitions. In principle, exothermic transitions might result from processes of protein aggregation or fibrillization, or from reversal of protein cold denaturation processes. The enthalpy (area) of the exothermic transitions is similar in magnitude to that of the endothermic denaturational transitions, thus suggesting that a substantial portion of the brain proteins are involved in the exothermic processes. These experiments demonstrate that DSC is an appropriate method with great potential for detection and characterization of brain proteome changes taking place in brain tissues affected by neurodegeneration.

Key words: differential scanning calorimetry (DSC); Alzheimer's disease (AD); animal (mouse) model; of scopolamine-induced dementia; heat capacity profiles; brain tissue supernatants; neurodegeneration; exothermic transitions

BEHAVIORAL EFFECTS OF NEW NEUROTENSIN ANALOGUES ON EXPERIMENTAL MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is neurodegenerative disorder with high social significance. It is result of a progressive loss of nigrostriatal dopaminergic (DA) neurons. The decrease in striatal DA-ergic innervation due to this loss is responsible for motor disturbances characteristic of the disease such as akinesia, muscular rigidity and tremor.

Neurotensin (NT) has been shown to modulate DA release in some brain regions including striatum (Hetier et al., 1988). The antiparkinsonian properties of NT in 6-OHDA-lesioned animals have also been reported (Jolicœur et al., 1991; Rivest et al., 1991). However, neurotensin is rapidly metabolized in plasma by endogenous peptidases, terminating its biologic effect under physiologic conditions. New NT analogues (with codes NT2 and NT4) were synthesized with the goal to avoid their fast biodegradation (Pajpanova et al., 2013).

Aim of the present work is to study the neuroprotective effects of the new NT- analogues on the behaviour of rats with experimental model of PD.

PD model was induced in male Wistar rats via unilateral and stereotaxic injection of 6-hydroxydopamine (6-OHDA) in right striatum. Animals were treated for 5 days daily with 10 mg/kg intraperitoneally with NT2 and NT4, as well as with NT as referent or saline for sham operated controls. Verification of PD model was evaluated by apomorphine-induced rotations and changes in muscular coordination (Rot-a-Rod test) and in the cognition (step-trough latency- STL) - Passive avoidance learning test. Behavioural tests were performed on the first and on the second weeks post the lesion.

Treatment with NT4 analogue significantly reduced the number of contralateral rotations on the first (82.36%) and on the second (80.41%) weeks post lesion in compare to untreated 6-OHDA group. Gradual improve in the motor performance (Rot-a-rod test) in NT2 and NT4 treated groups also was established. NT- treated groups showed a significant decrease in number of falls/min on the first week (NT2 by 91.71% and NT4 by 98.82%) and also on the second week (NT2 and NT4 by 98.31%) in compare to untreated 6-OHDA group. Treatment of PD rats with new NT analogues and NT as referent significantly improved STL in passive avoidance learning test. On the first week post lesion retention latency increased by 112.06%, 139.19 % and 129 % in 6-OHDA groups treated with NT2, NT4 and NT respectively, compared to untreated 6-OHDA group. On the second week post lesion only NT4 treated group showed increased retention latency with 36.92% in compare to untreated 6-OHDA group.

The two newly synthesized NT analogues are biologically active compounds with promising preventing effects on PD development.

Key words: neurotensin analogues, Parkinson's disease, 6-OHDA, prevention

DIFFERENTIAL SCANNING CALORIMETRY - METHOD FOR DETECTION OF NEURODEGENERATIVE DISEASES

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Introduction: Differential scanning calorimetry (DSC) is a method for thermal analysis used to characterize the stability of biomolecules in their native forms. By changing the temperature, DSC

measures the heat quantity, which is evolved or absorbed by the sample on the basis of a temperature difference between the sample and the reference material. It is a highly sensitive method which is able to detect compositional changes in the molecular and supramolecular profiles of the affected by the disease tissues. Drug-induced type of dementia can be caused to experimental animals (mice) by using namely scopolamine. Neurotensin (NT) is a neuropeptide and putative neurotransmitter which is expected to hold up the development of some neurodegenerative diseases (NDD), such as Alzheimer's disease (AD).

Aim of this study is to assess the role of DSC as a new method for detection of NDD by evaluating NT's impact on scopolamine treated (AD) animals.

Methods: Experimental model of dementia from AD type was produced on male Albino mice via central neuronal/neurotransmitter pathways manipulation (scopolamine 1 mg/kg, i.p., 11 days) and was verified by cognitive and biochemical tests. NT was applied for 11 days simultaneously with Scopolamine. On the 24th hour after the last treatment the DSC measurements were performed on brain tissue homogenates of healthy, dement and NT treated animals using a Nano DSC.

Results: Distinctive differences between thermograms of healthy and dement animals are reported from the DSC measurements. The scans of NT treated mice differ significantly from those infused with scopolamine and have come near to the control, healthy animals. In result the differences in thermograms between the three groups of mice can be correlated to the cognitive changes they have undergone.

Conclusion: DSC is an useful method for obtaining information on the disease mechanisms at molecular level and for diagnostic of different NDD. It is also appropriate for detection and characterization of compositional changes taking place in AD brain tissues and in future can be helpful for further studies of these diseases.

Key words: DSC, Neurotensin, Scopolamine, Neurodegenerative diseases, Alzheimer's disease

ПРОФЕСИОНАЛНА ТОКСИКОЛОГИЯ

ASSESSMENT OF ASBESTOS CONTAMINATION IN AREAS OF FORMER PRODUCTIONS AND ASBESTOS-CONTAINING EQUIPMENT IN THE COUNTRY

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After abandoning the use of proven carcinogen asbestos since 2005 actions undertaken to prevent health risks of exposure to asbestos fibers have been directed towards securing activities to eliminate asbestos materials invested in the past in buildings and facilities. They are accompanied by generating a large amount of asbestos wastes that in inefficient management create conditions for contamination of adjacent areas and soils.

In accordance with a Regulation № 1272/2008 the contamination of soils and industrial minerals with carcinogenic (category 1A) asbestos fibers is significant in asbestos content more than 0,1 mass%.

The aim of the study is to assess the asbestos contamination in areas of former production of asbestos products and equipment after removing the asbestos insulation.

Forty seven samples of wastes and soils were tested in areas of two former asbestos plants and two facilities after removing industrial asbestos insulations.

To identify and quantify low contents of asbestos fibers is implemented PLM-PCM-methodology. Asbestos content higher than 2 mass% is determined by IR-spectrometric methods. Data for asbestos content higher than 0.1% are established (maximum 43-45%) for the pollution in the regions of former asbestos industries and certain locations in the areas of industrial facilities. This creates a prerequisite for air contamination, too, and reinforces attention to the need to take adequate measures to prevent pollution.

Key words: asbestos, contamination of soils, identification and quantification.

7. HYGIENIC CHARACTERISTIC OF INSULATION WOOLS FROM MAN-MADE MINERAL FIBERS

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Man-made mineral fibers (MMMF) have been established as the most effective asbestos substitutes because of their similarity to asbestos according to technical and physicochemical properties. Based on data from "in vivo" and "in vitro" studies of the biological effects of fibers criteria are established for classifying the MMMF in the categories of carcinogens: length weighted geometric mean diameter of the fibers and the content of alkali and alkaline-earth oxides. The aim of the study is to explore a hygienic assessment on these indicators of insulation wools from MMMF offered currently on the Bulgarian market. There have been studied 3 types of insulation wools produced by different manufacturers. The results obtained show that for all three types of tested fibers the length weighted geometric mean diameter is less than 6 μm , and the total content of alkali and alkaline-earth oxides is higher than 18 weight %. According to the classification criteria of MMMF in the categories of carcinogens, the studied types of materials refer to category 2 (suspected human carcinogens - the classification is based on data resulting from studies of human and/or animal studies that are not sufficiently convincing for classifying the substance in category 1A or 1B).

Key words: man-made mineral fibers, length weighted geometric mean diameter, content of alkali and alkaline-earth oxides, category 2 carcinogens, asbestos substitutes

STUDY ON THE AIR POLLUTANTS IN THE WORKING ENVIRONMENT WITH LASER CUTTING OF PAPER AND PLASTIC PRODUCTS

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The study aims to collect data on air pollutants in areas where people do laser cutting of technical thermoplastics and paper.

An analysis on the composition of emissions has been done in order to identify volatile and semi-volatile organic compounds emitted during laser cutting. Quantitative measurements of aromatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), phenols, carbon monoxide and carbon dioxide are made.

The air is investigated for these indicators in workplaces of thirty sites, where CO₂ laser cutting of sheets made of polycarbonate and paper has been performed.

Samples of air in the work environment are taken for one shift; each object has been studied in seven days randomly for a period of five years.

Identification of the organic compounds is made by using gas chromatograph with a Mass Selective Detector, by comparing the mass spectra of the analyzed components to those of a library containing 1,230,100 spectra of various compounds. Quantitative analyses of chemical pollutants in the air - aromatic hydrocarbons and PAHs are performed on a gas chromatograph with a flame ionization detector by comparing the peak areas with those of certified standard materials. Measurements of the concentration of CO and CO₂ are done by automatic gas analyzer.

This study presents assessment of emissions in the air of the working environment with CO₂ laser cutting of polycarbonate and paper sheets. The concentrations of 16 PAHs, benzene, toluene, xylenes, phenols, carbon monoxide and carbon dioxide are determined, and more than 100 volatile organic compounds in the air of the working environment are identified.

Key words: polycyclic aromatic hydrocarbons (PAHs); aromatic hydrocarbons; carbon monoxide; carbon dioxide; emissions; CO₂ laser cutting; polycarbonate; paper; air of the working environment

SYNOPSIS OF LEGISLATION IN THE REPUBLIC OF BULGARIA IN THE FIELD OF MONITORING AND PROTECTING THE WORKERS FROM THE EFFECTS OF CHEMICAL FACTORS IN THE WORKPLACE

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The paper is about EU policy in the area of monitoring and protecting the workers from the effects of chemical factors in the workplace and relevant Bulgarian legislation. Draws attention to ways to collect reliable statistical information about the chemical environment factors and related occupational diseases and future opportunities for the development of methodologies and legislative changes.

Key words: occupational medicine, chemical factors in the working environment, health and safety legislative framework, biological monitoring to chemical factors

ТОКСИКОХИМИЧЕН АНАЛИЗ

MERCURY CONTENT IN BULGARIAN COSMETIC PRODUCTS

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Mercury is considered by the World Health Organization as one of the ten most toxic chemicals that are particularly dangerous for human health (WHO, 2013). Mercury and its compounds are related to the prohibited by Regulation (EC) 1223/2009 substances in cosmetic products, excluding special cases in Appendix 5 - preservatives thiomersal and phenyl mercury salts allowed for use in cosmetic products for the eyes. The maximum permissible level in the final product is stated at 0,007 % (as Hg), corresponding to 70 mg/kg. Mercury content in various Bulgarian cosmetic products (n=823), divided in three groups: hair care products (n=237), cosmetic products for face (n=234) and cosmetic products for body (n=352) was studied. Determination of mercury was performed by means of DMA 80 (direct mercury analyzer), Milestone. In the most of the tested products the Hg concentrations were below the limit of quantification of the analytical procedure (LOQ 0,008 mg/kg). Mercury was detected only in 24 (2,9%) of the tested products. The concentrations observed vary between 0,0083 mg/kg and 0,040 mg/kg; almost all samples of henna (10/11) contain Hg, although at very low concentrations (0,013 ÷ 0,028) mg/kg.

The results of the study indicate that the tested cosmetic products, made in Bulgaria, comply with the European legislation relating mercury content. Concentrations of Hg above 1,0 mg/kg, the permissible level as a technical pollution, according to Guide „Indicators and the levels of microbiological and chemical purity for cosmetic products and methods for checking compliance with these indicators” were also not established.

Key words: mercury, cosmetic products

EXTRACTION OF MICROCYSTINS FROM WATER

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Eutrophication of freshwaters and appearance of cyanobacterial blooms connected with hepatotoxins microcystins, have become a worldwide problem. The maximal permissible level for the most dangerous microcystin-LR is 1 µg/dm³ for drinking and 20 µg/dm³ for bathing water. The usage of cartridges and extraction disks for investigation of spiked water samples allows achieving a sufficient recovery of microcystins for analysis purposes (67-99 %, 79-110 %, 74-85 % for cartridges and 87-93 % for disks respectively). Shorter extraction time per sample, a possibility for second and third usage of extraction disks and their commensurate price with that of cartridges makes them more appropriate for use.

Key words: cyanotoxins, cyanobacteria, microcystins

12 CONTEMPORARY APPROACHES IN DESIGN OF NEW REACTIVATORS OF CHOLINESTERASE AS ANTIDOTES AGAINST POISONING WITH ORGANOPHOSPHORUS COMPOUNDS (NERVE AGENTS AND PESTICIDES)

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Organophosphorus compounds irreversibly inhibit the enzyme acetylcholinesterase (AChE), which leads to many complications in living organisms and ultimately to death. Chemical warfare agents are a class of organophosphorus compounds with pronounced neurotoxicity and currently represent a threat to modern civilization. Representatives are tabun, sarin, soman, VX and others. Abuse of this type of compounds along with organophosphorus pesticides (eg. malathion, paraoxon, chlorpyrifos, etc.) focus the attention to find more effective antidotes in case of poisoning by nerve agents. The main medical approach to this type of intoxications is based on immediate implementation of cholinolytic, anticonvulsant and acetylcholinesterase reactivators, which currently can represent by mono- and bis-pyridinium aldoxime. However, these antidotes were not sufficient to ensure the efficacy of treatment by 100%, even when they are applied immediately after intoxication and in general they have several drawbacks. In this connection a brief overview is made of current efforts of various scientific groups leading to the developing of newly synthesized reactivators, of promising strategies to design new and effective antidotes for the purpose of military toxicology.

Key words: Organophosphorus compounds, pesticides, acetylcholinesterase, reactivators, tabun, sarin, soman, VX

13. NEUROMUSCULAR TRANSMISSION – *IN VIVO* RAT MODEL

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Electrophysiological study of neuromuscular transmission is a fundamental method used for characterization of compounds with neurotoxic effects. Experimental research requires implementation of complex *in vivo* model involving skeletal muscle preparation from rats and isolation of *n. ischiadicus*. Changes in neuromuscular transmission can be monitored by recording the amplitude of the muscle (*extensor digitorum longus*) twitch. After induction of anesthesia and mechanical ventilation according to the experimental group, all rats were injected intraperitoneally with neurotoxins, classified according to their site of action: pre- or post-synaptic and produce neuromuscular blockade by several mechanisms. Received electrophysiological mechanograms were used as standard models of two main types blockade- presynaptic and postsynaptic block.

The results obtained from the current study makes possible characterization of newly isolated substances with neurotoxic effects.

Key words: neuromuscular transmission, rats, neurotoxins