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## **РЕЗЮМЕТА НА НАУЧНИ ПУБЛИКАЦИИ НА ДОЦ. ЦВЕТА ГЕРОГИЕВА, ДМ В СПЕЦИАЛИЗИРАНИ НАУЧНИ ИЗДАНИЯ СЛЕД ПРИДОБИВАНЕ НА АКАДЕМИЧНА ДЛЪЖНОСТ «ДОЦЕНТ»**

### **ПУБЛИКАЦИИ В СПИСАНИЯ С ИМПАКТ ФАКТОР**

V. Ilieva, M. Kondeva–Burdina, **Tz. Georgieva**, V. Pavlova, N. Danchev, October 2017, Application of real-time PCR for early detection of toxic cyanobacteria in Bulgarian dam waters, Toxicology Letters 280:S325

DOI10.1016/j.toxlet.2017.08.085 **IF = 3,85**

As a result of blooming, some cyanobacteria (Microcystis, Anabaena, Planktothrix, etc.) produce toxins in concentrations that are high enough to poison and even kill animals and humans. The World Health Organization recommends a maximum allowable value of one of the most dangerous cyanotoxins – microcistins: for drinking water of 1 µg/l and for bathing waters - 20 µg/l. It is a risk factor for humans and the environment, which requires early detection at low concentrations.

According to Annex II of the Framework Directive 2000/60/EC, transformed into the Bulgarian legislation, for the characterization of surface water, but at this stage there are no acceptable limit values for cyanotoxins. Methods for the investigation and monitoring of cyanobacteria and their toxins are under development.

The aim of the study is to selection and application of a constellation of molecular-genetic markers for the early detection of toxic microalgae.

Samples from three Bulgarian dams, which are used for drinking purposes are tested by RealTime PCR. To assess the presence of bacterial DNA, specific primers were selected to demonstrate Cyanobacteria. Genetic markers for identifying the toxic species Microcystis aeruginosa are selected portions of the microcystin genetic cluster mcy: mcyA, mcyB, mcy359.

DNA analyses using RealTime PCR provides a new, important tool for water monitoring in Bulgaria, allows early detection of cyanotoxins contamination and helps assess health risk. The results obtained are the basis for further studies of the mechanism of neurotoxicity and hepatotoxicity through alternative in vitro cell culture methods.

P. Stefanova, G. Blagoeva, V. Gotcheva, **Tz. Georgieva**, Angel Angelov Event-specific PCR quantification of EU-authorized GM soybean events in soybean products IOBC-WPRS Bulletin Vol. 114, 2016, 57-63 Proceedings of the seventh Meeting at Sofia (Bulgaria), June 01 - 03, 2015. Edited by Michael Meissle. ISBN 978-92-9067-297- **IF = 1,98**

The EU has implemented special regulatory requirements for food labeling with regards to genetically modified (GM) materials in order to ensure the informed choice of consumers. All food products containing more than 0.9% of authorized GM materials should be labeled. Therefore, the quantification of GM material in food products is very important to control food labeling. The aim of the present study was to detect and quantify the EU-authorized GM soybean events in 15 soybean products at the Bulgarian market without GM indication on the labels. A multiplex PCR method was applied to screen the products for the presence of genetically modified DNA, targeting three gene-specific (EPSPS, PAT and Cry1Ac genes) and one event-specific (DP 356043) DNA sequences. Positive samples were subjected to event-specific real-time PCR assay for quantification of GM soybean events MON 40-3-2, MON 89788, A2704-12 and A5547-127. Results indicated that 80% of the tested products contained MON 40-3-2, MON 89788 was detected in 13% of the samples and 20% of the tested products contained A2704-12. The amount of MON 40-3-2 was above the threshold of 0.9% in 1 sample, while the percentage of transgenic events MON 89788 and A2704-12 in all tested products was less than 0.9%. The initial screening and real-time PCR analysis showed that none of the tested products contained GM soybean events MON 87701, A5547-127, DP356043 and MON 87701xMON 89788. Therefore, based on the results from the study, only one of the analyzed products was falsely labeled with regards to GM ingredients.

P. Stefanova, G. Angelova, **Tz. Georgieva**, V. Gotcheva, A. Angelov, A novel multiplex PCR method for simultaneous detection of genetically modified soybean events, Food Control Elsevier , *Int. J. Curr. Microbiol. App. Sci.*, 2015, 4 (4), 256-268 - **IF = 2,93**

The increasing number of commercialized GM crops and the growing need for authenticity control of raw materials, feeds and foods set an urgent necessity for the development of sensitive, reliable and cost-effective methods for GMO detection. In the present study, a novel multiplex PCR method for the simultaneous detection of all EU-authorized genetically modified soybean events was developed. The method was based on three gene-specific (EPSPS, PAT and Cry1Ac genes) and one event-specific (DP 356043) DNA sequences. It was characterized with high sensitivity, as the LOD for each sequence was 0.05%. The new method was applied for the screening of 15 soybean products and 36 meat products at the market for the presence of genetically modified soybean events. Results demonstrated that 51% of the tested samples contained EPSPS gene, while PAT gene was detected in 8% of the DNA extracts. In contrast to that, Cry1Ac gene and DP 356043 event-specific sequence were not observed in any of the analyzed products. The data indicated that the proposed method could be used as a

reliable routine screening assay of various food products for the presence of EU-authorized genetically modified soybean events.

Stefanova P., Taseva M., **Georgieva Tz.**, Gotcheva V., Angelov A. A modified CTAB method for DNA extraction from soybean and meat products. Food Biotechnology, 27/2013/3 3803 – 3810 – **OK IF = 0,63**

DNA extraction from different food matrices is a critical step in PCR analysis. The aim of the present study was to explore the efficiency of a modified CTAB method in comparison with three commercial kits for DNA extraction from soybean and meat products. The methods were compared by the yield and purity of the extracted DNA, its fragmentation state and suitability for amplification. The modified CTAB method gave best results for all groups of soybean and meat products - DNA amounts of 114.98 ng/mg product to 314.47 ng/mg product. Compared to the commercial kits, the modified CTAB method was the only one that produced amplifiable DNA from all soybean and meat products tested. Therefore, the modified CTAB method could be the method of choice for DNA extraction from complex and variously processed meat products and from raw and low-processed soybean products in order to ensure a reliable quality and authenticity control in the meat food chain.

T. Panev, B. Lazarov, A. Stanchev, M.Tsoneva, H.kopcheva, E.Mavrodieva, **T.Georgieva**, Assessment of personal exposure to active substances in the production of different pharmaceutical forms, Toxicology Letters, Volume 205, Supplement, 28 August 2011, Page S266 -

<https://doi.org/10.1016/j.toxlet.2011.05.904> **IF = 3,23**

Purpose: The pharmaceutical production is related to the incorporation of different ingredients, their weighing and mixing as following homogenization, drying and compressible take place. Despite state-of-the-art pharmaceutical forms comply with Good Manufacturing Practices requirements, there still exist few operations that rely upon workers' involvement. Workers in these productions are exposed both to different organic dust fractions and several ingredients of the medicinal formulations contained in the dust. Methods: To assess workers' exposure to the incorporation of different ingredients in the production of pharmaceutical forms, personal sampling was performed. The analysis of sampling for toxic substances was performed by using HPLC with photodiode matrix detector and gas chromatography-mass selective detector and flame ionization detector. The assessment of PM 2,5 dust was performed by gravimetric method. Results: The results obtained showed that the concentrations measured in the workplace air of the active substances are in the range of: Codeine phosphate hemihydrate 0.01–0.03 mg/m<sup>3</sup>; Caffein 0.1–0.01 mg/m<sup>3</sup>; Paracetamol 1.04–0.03 mg/m<sup>3</sup>; Metamizole sodium 3.26–0.15 mg/m<sup>3</sup>; Nifedipine 0.1–0.08 mg/m<sup>3</sup>; Ciprofloxacin 0.04–0.24 mg/m<sup>3</sup>; Isosorbide dinitrate 0.09–0.93 mg/m<sup>3</sup>; Enalapril maleate 0.1–0.05 mg/m<sup>3</sup>; Piracetam 0.21–0.05 mg/m<sup>3</sup>; Cinarizin 0.34–0.1 mg/m<sup>3</sup>; Metamizol sodium 0.01–0.07 mg/m<sup>3</sup>; Pitofenon hydrochloride 0.03–0.1 mg/m<sup>3</sup>; Fenpiverinium bromide 0.05–0.11 mg/m<sup>3</sup>; Piracetam 0.1–0.03 mg/m<sup>3</sup>; Paracetamol 0.21–0.01

mg/m<sup>3</sup>; Ethyl alcohol 219–88.7 mg/m<sup>3</sup>, PM 2,5 respirable dust fraction 0.35–5.15 mg/m<sup>3</sup>. The measured concentrations of different active substances incorporated in the pharmaceutical forms are below the limit values.

doi:10.1016/j.toxlet.2011.05.904

**Tz. Georgieva**, T. Panev, B. Dragova, V Nikolova, D. Chohadjieva, T. Popov, S. Garte. "Influence of genetic polymorphism of metabolizing enzymes of polycyclic aromatic hydrocarbons in excretion of 1-hydroxiprene and cotinine by occupational exposure", Toxicology Letters 196S (2010) S37–S351 **IF = 3,58**

Main sources of polycyclic aromatic hydrocarbons (PAHs) are cigarettes smoke, environment, food or occupational exposure. The data presented in this study is a part of a project for examination of DNA damage resulting from PAHs exposure. The aim of the study was assess the effect of polymorphisms of metabolizing enzymes on the biomarkers of exposure to PAHs and cigarettes smoke. The study subjects were 47 traffic policemen, 49 bus drivers and 50 control without professional exposure to PAHs. It was investigated ten polymorphisms of CYP1A1, GSTM1, GSTT1, GSTP, NAT2, EPHX, and CYP1B1. 1-Hydroxypyrene in urine was measured as a biomarker of exposure to PAHs.(benz(a)pyrene). Urinary cotinine, one of the main metabolites of nicotine, has been measured in urine as a biomarker for assessment of direct or passive exposure to cigarette smoke. Effect of polymorphic variation of enzymes involved in biotransformation of PAHs on cotinine and 1-HP was analyzed. High frequency of carriers of 'wild' type in GSTT and CYP1A1 in the study subjects in this study demonstrates the need for new studies and in individuals exposed to other chemical agents. Results of this research fill the scarce data for Bulgarian population and demonstrate the need to extend the research of individual susceptibility by exposure to chemical agents from the environment or working environment. The study of the importance of the variants of enzymes involved in biotransformation of toxicants affecting individual sensitivity will allow an individual prevention among exposed and, accordingly—decrease the health risk.

B Ruseva, D Strashimirov, N Shumkov, **T Georgieva**, A Mihailova, Changes of lipid profile and aortic wall in spontaneously hypertensive rats under diet of different selenium content, Scientific Research Journal of South-West University 2010, 1 (1), 101-104 **IF = 0,29**

Selenium (Se) is an essential trace element that performs its biological role via selenoproteins. They take part in antioxidant protection, redox regulation and energy production. The aim of this study is to investigate the influence of Se intake on the indicators of lipid profile and aortic wall in spontaneously hypertensive rats (SHR). 24 male, 8 months old SHR, were separated into 3 groups, were used: G1 – adequate Se diet, G2 – low Se diet, G3 – supplementation with Se. Serum high density lipoproteins (HDL) cholesterol concentration is lower in G2, than of other groups (p = 0,001), the ratio HDL/LDL (low density lipoproteins) cholesterol is the highest in G3. SHR with low Se intake have several atherosclerotic changes, which tend from thoracic to abdominal aorta. The morphometry of aortic

walls das not denote significant differences in thoracic aorta,, but evaluates higher thinks of abdominal aorta in rats from G2 m than the odder groups (p=0,0008)

Pesatori, A.C., Garte, S., Popov, T., **Georgieva, T.**, Panev, T., Bonzini, M, Consonni, D, Carugno, M, Goldstein, B.D, Taioli, E, Fontana, V, Stagi, E, Bertazzi, P.A., Merlo, D.E.f - Early effects of low benzene exposure on blood cell counts in Bulgarian petrochemical workers, *Med Lav* 2009; 100, 2: 83-90 – **IF =3,58**

Objectives: Only few studies have examined early hematological effects in human populations exposed to low benzene levels and their findings are controversial. We evaluated hematological outcomes (WBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils, RBC, Hb, HCT, MCV, platelets and MPV) in a population of 153 Bulgarian petrochemical workers exposed to benzene (range 0.01-23.9 ppm) and 50 unexposed subjects. Methods: Written informed consent was obtained and a self-administered questionnaire used to collect information on current smoking habits, lifestyle, and occupational activities. Exposure assessment was based on personal monitoring sampling the day before phlebotomy. Urinary trans-trans-muconic acid (t,t-MA) was determined at the beginning and end of the work shift. Based on individual airborne benzene measurements, study subjects were categorized in three exposure categories (referents, <1 and ≥1 ppm). Mean values of each hematologic outcomes in each exposure category were compared with the referent group using a multiple linear regression model adjusted for age, gender, current smoking habits and environmental toluene level. The influence of the CYP2E1 (RsaI and DraI) and NQO1 609C>T genetic polymorphisms on differential hematological parameters was also investigated. Results: No doseresponse effect was observed for most of the examined hematological outcomes (WBC, lymphocytes, neutrophils, monocytes, RBC, Hb, HCT, MCV, platelets and MPV). The eosinophil count was inversely related to benzene exposure only among smokers. Conversely, basophils increased with increasing exposure. No effect on benzene hematotoxicity was found for any of the investigated polymorphisms. Conclusion: In our study we did not find a decline in WBC and lymphocytes related to benzene exposure. A myeloproliferative effect of benzene is highly unlikely to explain the observed reduction in eosinophils and increase in basophils as it would lead to a concordant depression in all granulocyte subpopulations. Whether benzene effects at low doses are present in Caucasian populations remains uncertain, thus warranting further investigations

Seow WJ, A C Pesatori, E Dimont, B Albetti, A S Ettinger, V Bollati, ClBolognesi, P Roggeri, T. I. Panev, **Tz. Georgieva**, D F Merlo, P A Bertazzi, A Baccarelli - Urinary Benzene Biomarkers and DNA Methylation in Bulgarian Petrochemical Workers: Findings and Comparison of Linear and Beta Regression Models for the Methylation Data Analysis PLOSone electronic Journal <http://www.plosone.org/article/related/info%3Adoi%2F10.1371%2Fjournal.pone.0050471>, 2012 **IF = 3,73**

Chronic occupational exposure to benzene is associated with an increased risk of hematological malignancies such as acute myeloid leukemia (AML), but the underlying mechanisms are still unclear. The main objective of this study was to investigate the association between benzene exposure and DNA methylation, both in repeated elements and candidate genes, in a population of 158 Bulgarian petrochemical workers and 50 unexposed office workers. Exposure assessment included personal monitoring of airborne benzene at work and urinary biomarkers of benzene metabolism (Sphenylmercapturic acid [SPMA] and trans,trans-muconic acid [t,t-MA]) at the end of the work-shift. The median levels of airborne benzene, SPMA and t,t-MA in workers were 0.46 ppm, 15.5 mg/L and 711 mg/L respectively, and exposure levels were significantly lower in the controls. Repeated-element DNA methylation was measured in Alu and LINE-1, and genespecific methylation in MAGE and p15. DNA methylation levels were not significantly different between exposed workers and controls (P.0.05). Both ordinary least squares (OLS) and beta-regression models were used to estimate benzenemethylation associations. Beta-regression showed better model specification, as reflected in improved coefficient of determination (pseudo R<sup>2</sup>) and Akaike's information criterion (AIC). In beta-regression, we found statistically significant reductions in LINE-1 (20.15%, P,0.01) and p15 (20.096%, P,0.01) mean methylation levels with each interquartile range (IQR) increase in SPMA. This study showed statistically significant but weak associations of LINE-1 and p15 hypomethylation with SPMA in Bulgarian petrochemical workers. We showed that beta-regression is more appropriate than OLS regression for fitting methylation data.

E Kuzova, **Tz Georgieva**, V. Duleva, Zd. Radionova, V. Birdanova, I. Himcheva, Association Between A Fatty Acid Desaturase 1 Gene Polymorphism And Blood Plasma Cholesterol Biomarkers In Bulgarian Elderlydoi: Scripata Scientifica Pharamaceutica, vol 4, 2017 spl 1 , p 86

<http://dx.doi.org/10.14748/ssp.v4i1.4003>

Introduction: A particular single nucleotide polymorphism (SNP), rs174547, of the fatty acids desaturase (FADS) 1 gene has been significantly associated with low-density lipoproteins (LDL), high-density lipoproteins (HDL), and triglyceride blood plasma concentrations according to published data. FADS1 and FADS2, encoded by FADS1 and FADS2 genes, are membrane-bound key enzymes, rate limiting in the synthesis of long-chain polyunsaturated fatty acids - arachidonic, eicosapentaenoic and docosahexaenoic acid from their dietary precursors linoleic (C18:2 n-6) and  $\alpha$ -linolenic acid (C18:3n-3). Aim: The study aims to detect and determine individual variants of the rs174547 SNP among Bulgarians and analyze a possible association between the allele variants and blood plasma cholesterol levels, being strong indicators for cardiovascular health and lipid metabolism. Materials and Methods: Bulgarian volunteers (N=123) were randomly selected and following standard DNA extraction from buccal swabs, genetically tested for their allele variant (C/T) of the rs174547 SNP in FADS1 gene by real-time polymerase chain reaction. Laboratory assessment for cholesterol profile, triglycerides and blood sugar was

performed, together with bio-impedancemetry for body composition analysis. Dietary information was obtained by a modified diet history methodology combining a Food Frequency Questionnaire, two 24-hour Food Recall Questionnaires and General Lifestyle Questionnaire. Results and Conclusion: Individual genotypes were determined and results were analyzed in order to unveil the magnitude of influence of the given SNP over the studied metabolic pathways. The rs174547 SNP could provide valuable information concerning individual's fatty acid metabolism and may support nutritionists when nutritional requirements for omega-3 and omega-6 fatty acids are estimated. It can also play a predictive role for developing future chronic illnesses and conditions. If so, this molecular biomarker could be successfully integrated in panels of nutrigenetic profiles concerning lipid and cholesterol metabolism and thus enhance the development of personalized nutrition and medicine

## **РЕФЕРИРАНИ И ИНДЕКСИРАНИ БЪЛГАРСКИ И МЕЖДУНАРОДНИ СПИСАНИЯ**

П. Стефанова, Г. Ангелова, **Цв. Георгиева**, Скрининг на месни продукти за откриване на видово-специфична и генно модифицирна последователности чрез SYBR@GREEN PCR метод, *Научни трудове ТОМ LX "хранителна наука, техники, технологии – 2013"*

The aim of the present study was to screen various meat products for the presence of soy taxon-specific lec gene, CaMV 35S promoter, T-nos terminator and CP4-EPSPS gene using SYBR@Green PCR approach. Melting temperature analysis was conducted in order to confirm the specificity of the amplification products. Results demonstrated that all tested meat products were positive for soybean DNA although soy ingredients were not listed on some product labels.

Data from the screening showed that 22% of the samples were positive for CaMV 35S promoter (Ct values = 28.11-38.13), 22% contained T-nos terminator (Ct values = 28.85-38.06) and the CP4-EPSPS gene was present in 69% of the tested samples (Ct values = 27.56-37.78). Melting curve analysis showed that all amplified PCR products generated unique peaks and the Tm values of each peak corresponded to the reference values. In summary, 78% of the screened products gave positive signal for at least one of the genetically modified elements tested.

**Tz. Georgieva**, G. Genova, V. Nikolova, T Panev, T Popov, Health risk associated with genetic polymorphism of Apolipoprotein E in Bulgarian workers exposed to carbon disulfide – *Toxicological Problems*, The NATO Science for Peace and Security Program, 2014 p.345

Apolipoprotein E (APOE) plays a major role in plasma lipoprotein metabolism and is responsible for the transport of lipids in the bodies. APOE gene demonstrate three genetic polymorphic variants-APOE 2, APOE \* 3, APOE \* 4 and the products of these three alleles differ in their functional characteristics. APOE \* 4 allele is a risk factor for a predisposition to

cardiovascular disease and Alzheimer disease. The purpose of this study was to determine if there is a relationship between the presence of mutations in the genes, responsible for Apolipoprotein E synthesis and disturbance of lipid status in individuals exposed to carbon disulphide. Object of study are 90 workers from the Bulgarian production, occupationally exposed to carbon disulfide, distributed into 3 groups based on the level of exposure. The control group included 26, carbon disulphide, unexposed individuals. Individual external and internal exposure was assessed. Biochemical indices were analyzed for illustration the status of lipid metabolism. Explored through RFLP-PCR method polymorphic gene variants of apolipoprotein e genotype results demonstrate the E3/E4 with 18% of the investigated persons and E3/E2 at 11%. The data show that there is an increased health risk for workers with the E3/E4 genotype. The results of this study supplement the meager data about Bulgarian and demonstrate the need for expansion of the research for the study of individual sensitivity to exposure to chemical agents from the environment or working environment.

Стефанова П, М Ташева, **Цв. Георгиева**, А. Ангелов - SYBR®GREEN PCR анализ за откриване на таксон специфични и трансгенни елементи в соеви продукти *Научни трудове ТОМ LX "хранителна наука, техники, технологии – 2013"*

The increasing number of commercialized genetically modified crops set an urgent necessity for the development of sensitive, reliable and cost-effective methods for GMO detection. The most commonly used genetically modified elements in commercial transgenic crops are the CaMV 35S promoter, the T-nos terminator and the CP4-EPSPS gene, conferring the herbicide tolerance. Therefore, the SYBR®Green PCR screening approach applied in the present study was focused on detecting the presence of these three elements together with the soy-specific lectin gene in various soybean products. Results demonstrated that 80% of the screened products gave positive signal for at least one of the genetically modified elements tested. In addition, melting temperature (T<sub>m</sub>) analysis was conducted in order to confirm the specificity of the amplification products. Melting curve analysis showed that all amplified PCR products generated unique peaks and the T<sub>m</sub> values of each peak corresponded to the reference values.

Manolova H, and Petkova, M Naydenova In Nikolova, **Ts. Georgieva** - Optimization and implementation of a procedure for extracting DNA from soya lecithin for the needs of routine practice. „Collection "Science dining - between discussions and evidence," edited by Prof. B. Popov, 2012, стр. 70-76

One of the main raw materials used in the food industry is soy lecithin. About 70% of world soybean production is genetically modified. Application of well developed methods for DNA extraction and screening and quantification of genetically modified organisms (GMOs) in routine practice is crucial. It is known that deoxyribonucleic acid (DNA) is the most stable biochemical molecule. Its isolation from food products is an

important prerequisite for reliable results, since it must be good quality and sufficient quantity for amplification. Lecithin production includes several processes of chemical and thermal processing, preceded by cold pressing. As a result, the ultimate raw material that goes into food can be found a small quantity and poor quality DNA. The purpose of this work is to optimize and implement the needs of routine practice method for extraction of DNA from soy lecithin, which improves the quality and quantity of isolated DNA. Isolation of DNA from soy lecithin is made by four different methods for the presence of soy DNA by reference lektine gene Le1: n-hexane methods (Wurz, 1998); kit ready for the extraction of fatty matrices - Eurofin - DNA extractor; extraction method in ISO 21571 - using STAB (Tsetiltrimetil Ammonium bromide) and n-hexane modified method using guanidine. The results of polymerase chain reaction (PCR) show a better yield of DNA extraction with n-hexane modified method.

П. Стефанова, М. Тасева, **Ц. Георгиева**, А. Ангелов , PCR методи за скрининг на месни продукти за наличие на генетично модифицирана соя, Scientific works UFT, 2012, Volume LIX, 650 – 655;

Since more than one hundred events of genetically modified organisms (GMOs) have been developed and approved for commercialization in the global area, the detection of transgenic crops is one of the most important consumer concerns regarding food safety and quality. On the other hand, the addition of soybean proteins to meat products has significantly increased in recent years due to their functional and nutritional properties. The aim of this study was to explore the presence of genetically modified soybean in various meat products on Bulgarian food market. The extracted DNA was analyzed with conventional PCR for presence of soybean DNA. Further, the positive samples were subjected to GMO screening with primers for detection of 35S promoter sequence and EPSPS gene sequence. The results showed that genetically modified soybean was present in 11.1 % of the examined meat products.

P.Stefanova, **Tz. Georgieva** A.Angelov, GM soy-detection methods and applications guardians products, "Food Science, Engineering and Technology 2010; Volume LVII ,, Volume1 стр 501-506

След одобряването на използване за търговски цели на повече сто генно модифицирани организми в световен мащаб , едно от основните цели на заявителите и контролните органи е да предостави достоверна информация и право на избор на потребителите. Целта на настоящия обзор е да даде основна информация разрешените ГМ линии соя, която може да бъде открита на Европейския пазар. Също така е направен преглед на съществуващите методи за детекция , идентификация и количествено определяне на ГМ соя в различни продукти. Въз основа на направения преглед ще се разработи

стратегия за бърз и с ниска себестойност скрининг за нуждите на контролните лаборатории.

**Ц.Георгиева**, В.Николова – „Национална референтна лаборатория по генно модифицирани организми чества 10 години от създаването си“ - пленарен доклад, Сборник Резюмета на научни доклади по безопасност на храните, Юбилейна конференция 10 години наука за храните в услуга на потребителите“, ноември 2017

Лабораторията започва своята дейност през февруари 2007 г и е създадена със заповед на директора на националния център по опазване на общественото здраве към Министерство на здравеопазването в рамките на проект Twinning PHARE Project BG/2004/IB/EC/01 между Министерство на Здравеопазването на България и Федерална Агенция по Околна Среда на Австрия “Strengthening of health control on chemical substances, preparations and products, novel foods and new food ingredients, GM foods and GM food ingredients, irradiated foods and food components” 2005 – 2007. От октомври 2017 е пълноправен член на Европейската мрежа на ГМО лабораториите (ENGL) Изпълнява аналитична, научно-приложна и обучителна дейност в областта по прилагане на законодателството за ГМО. Участва в научната мрежа за оценка на риска от ГМО към Европейския орган по безопасност на храните (EFSA). Участва във валидирането на официалните метод за контрол на Европейския пазар, като част от регистрацията на нови ГМО. Подпомага лабораториите участващи в официалния контрол в Р България. Ролята на НРЛ се определя от Регламенти Съгласно (ЕС) № 882/2004, № 1829/2004 и № 120/2014 г.

Д. Александрова, Д. Димбарева, С.Арсова, **Цв.Георгиева**, Екстракция на ДНК: микрометод vs макрометод“, Сборник Резюмета на научни доклади по безопасност на храните, Юбилейна конференция 10 години наука за храните в услуга на потребителите“, ноември 2017

Ползването на подходящи стратегии за изолиране на ДНК от различни матрици в важна предостановка за достоверен резултат. В повечето случаи в лабораторни условия се анализират проби от силно преработени матрици, което води до наличите на некестван.

Проведена е екстракция на три различни сорта царевица чрез два относително бързи и евтини метода (микрометод и макрометод). И двата метода включват три основни стъпки: лизис на клетъчната мембрана, екстракция на геномна ДНК и последваща приципитация. Концентрацията на ДНК се определя спектрофотометрично. Резултатите получени от двата метода са съпоставени.

В. Георгиева, **Цв. Георгиева**, Е. Радоилска, Й. Тачев, Г. Анкова, Научен подход при разрешаване на проблеми от практиката чрез прилагане на

микробиологични и рсг методи , *Българско списание за обществено здраве*, том 9 , книжка 1, 3- 15, 01/2017

Провеждайки аналитична дейност, наша основна цел е опазване здравето на хората и предотвратяване разпространяването на болести, източник на които са обекти от околната среда и бита. Във връзка с това са разработени няколко научни проблематики с тясна практическа насоченост в сферата на бутилираните води, храните и утайките от пречиствателни станции за отпадни води. Основната задача на всяка една от тях е на базата на получените резултати от проведените микробиологични анализи да се формулират ясни изводи относно качеството на обследваните обекти, критичните точки при производството им и превенция на крайните потребители – хората.

Изпитваните проби са от бутилирани води, различни групи храни и хранителни продукти, утайки от пречиствателни станции на отпадни води. При всички тези разработки са използвани съвременни стандартизирани методи и са анализирани показатели, залегнали в национални и европейски нормативни документи.

В резултат на проведените обстойни проучвания се достигна до следните важни резултати:

- Установени са биологичните контаминанти при производството на българските бутилирани води
- Създадени са няколко експериментални модели относно експозицията и характеристиката на микробиологичния риск в храните
- Извършена е оценка на риска от патогенни микроорганизми в български храни.
- Разрешени са два много интересни случая от практиката, свързани с приготвянето на детски храни в детска кухня и с производството на прясно пастьоризирано мляко
- Внедрен е PCR –метод за откриване на Ешерихия коли, продуциращи Shiga токсин
- Разработен е метод за обеззаразяване на утайки от ПСОВ чрез използване на варови материали

Заклучение: На базата на получените резултати от проведените проучвания се установиха основните контаминанти и пътищата им на постъпване в готовата продукция на бутилираните води и храните; апробиран е PCR- метод, важен при идентификацията на веротоксигенните E.coli; разработен е метод за третиране на утайки от пречиствателни станции, позволящ достигането на по-добри микробиологични качества за по-кратък период и ускоряване използването им като почвени подобрители.

**Цвета Георгиева**, Оценка на експозицията и токсикологични проучвания на генетично модифицирани храни в изпълнение на Регламент (ЕО) № 513/2013 [http://www.focalpointbg.com/?news\\_item=829](http://www.focalpointbg.com/?news_item=829)

През последните години в ЕС се увеличава значението на оценката здравни рискове от генетично модифицирани (ГМ) култури. Поради краткия исторически път на широката употреба на генетично модифицирани организми (ГМО) и биотехнологични продукти като храни и фуражи, не съществуват достатъчно научни данни за дългосрочните ефекти от употребата им. Малко са научните публикации, които илюстрират въздействието върху човека и животните. Все още няма епидемиологични проучвания, базирани на ГМО, използвани като храна. Предвид повишената обществена чувствителност, породена от наличието ГМ храни на пазара и използването на ГМО като фуражи, процедурите за оценка на безопасността на нови ГМО бяха хармонизирани. Необходимо е да се натрупат достатъчно научни данни, които да доказват необходимостта от провеждане на тестове за токсичност и подходи, които се прилагат при оценката на безопасността на ГМО. Не трябва да се пренебрегва и правото на потребителя да има информиран избор.

В последните няколко години активно се обсъжда ролята и значението на тестове за токсичност върху животни. През 2013 г. е приет нов Регламент № 503/2013 за прилагане на процедури за оценка на риска в съответствие с Регламент № 1829/2004. Възникнаха редица въпроси: необходими ли са по-подробни изследвания на биологичните и дългосрочните ефекти на генетично модифицираните организми върху хората и животните? И как ще се извършва оценка на експозицията на ГМ храни, като част от оценката на риска?

E. Kuzova, **Tz. Georgieva** , V. Duleva Development of methodology for analysis of the FADS1 genetic variants – first pilot study in Bulgaria –*Hrana I Isharana (Beograd)* , *Journal of Serbian nutrition society*, Vol 57, No 1, 23-28,2016

Aim: To develop, adapt and verify methodology for detection and analysis of FADS1 genetic variants (SNPs) in a small scale setting (pilot study), as these SNPs have been associated with the metabolism of fatty acids.

Methods: A particular SNP (rs174547) was identified in the FADS1 gene according to published data, with significant association between its variants and the metabolism of fatty acids. Next, a specific primerset targeted at the SNP region was designed and the reaction was optimized under various conditions: primer concentrations, reaction temperatures, duration and number of amplification cycles, etc.

A pilot group of 10 subjects from Bulgarian volunteers was randomly picked and tested in order that the optimized conditions were verified. Following standard DNA extraction from buccal swabs, a polymerase chain reaction was performed amplifying a 243 bp fragment, with the C/T SNP contained inside. The amplicon was visualized after 40 min electrophoresis on a 2% agarose gel. Products were then run on a capillary

sequencer (Sanger type sequencing), and obtained data were read with the MEGA5 software.

Results: The proposed system for detection and analysis of SNPs in the FADS1 gene was optimized and probated for the first time in Bulgaria. Thus, although a small-scale pilot study, its results represent primary data originated from Bulgarian individuals. It was shown that under the optimized reaction condition our primer systems produced an amplicon with the expected size and indeed contained a fragment from the FADS1 gene, with the analyzed C/T SNP inside.

Conclusions: The introduction of molecular biomarkers would allow an individual assessment of the fatty acid metabolism to be made. Thereby, the developed and probated methodology if is to be applied in a large scale setting, would help to investigate the relationship and influence between genetic predisposition, individual metabolism and food intake.

Е. Кузова, **Цв. Гоергиева** - Значението на FADS1 и FADS2 Гените за усвояването на омега-3-мастните киселини, постъпили с диетата; *Науката за хранене с авторитетно настояще и престижно бъдеще*, Българско Дружество по Хранене и Диететика, 2016, стр.45-49.

Налице са научни доказателства за идентифицирани около 500 единични нуклеотидни полиморфизми (SNPs) на гените FADS1 и FADS2, кодиращи делта-5 и делта-6 десатуразите, основни ензими, участващи в метаболизма на поли ненаситените мастни киселини. Открити са няколко SNPs, които понижават активността на десатуразите и оттам занижени нива на дълговерижните ненаситени мастни киселини (LC-PUFA). Определени генотипове имат по – висока десатуразна активност и като следствие се получава по – голямо съотношение LA/ арахидонова киселина (AA) и алф-линоленова киселина (ALA)/ейкозапентаенова киселина (EPA) или докозахексаенова киселина (DHA).

Въвеждането на молекулни биомаркери позволява индивидуална оценка на пътищата на обмяна на веществата и поведението на основните ензими, отговорни за метаболизирането на хранителните вещества. В последното десетилетие постиженията на генетиката на храненето позволява развитието на хранителната епидемиология и индивидуалния подход при рискови групи.

Проучвания на българска популация, проведени от проф. Ст. Петрова и колектив през 2010/2011 г. доказаха изключително ниския прием на омега 3-мастни киселини, поради слабата консумация на риба. Тези констатации и липсата на данни за българска популация относно генетичните варианти на FADS гените са предпоставка за провеждане на задълбочени проучвания, както на рискови групи, напр. със сърдечно-съдови заболявания или хранителен дефицит, така и при здрави хора.

Д. Гюрова, Л. Мечкуева, Г. Паунова, Д. Станкова, А. Лазарова, С. Младенова, Е. Славкова, **Цв. Гоергиева**, Р. Георгиева – Сравнително проучване на

съдържанието на макронутриенти и микронутриенти в зърно и брашно от лимец и пшеница. „Науката за хранене с авторитетно настояще и престижно бъдеще“, Българско Дружество по Хранене и Диететика, 2016, стр.45-49.

Проучено е съдържанието на макронутриенти (протеини, мазнини, въглехидрати) и микронутриенти (минералите натрий, калий, калций, магнезий, желязо, мед, манган, цинк и фосфор) в зърнени култури (лимец и пшеница) и брашна (от лимец и пълнозърнесто пшенично). Средните стойности на белтък (12%) и въглехидрати (73%) в зърно и брашно от лимец са близки до установените в пшеница (11%) и пълнозърнесто брашно (76%), докато съдържанието на мазнини в зърно и брашно от лимец (2,92 и 4,06%, съотб.) се различава значително от определените в пшеница и пълнозърнесто брашно (1,35% и 2,15%, респ.). Съдържанието на натрий в зърнените култури и брашната е много ниско - около 0,002 %. Установените средни стойности за калий, фосфор, магнезий, манган и цинк в лимец (487, 410, 128, 3,95 и 4,05 mg/100 g съответно) са по-високи в сравнение с нивата на елементите в пшеница (355, 285, 122, 2,85 и 1,90 mg/100 g, респ.). По-високи са нивата на K, P, Mn и Zn (без Mg) и в брашното от лимец спрямо пълнозърнесто пшенично брашно.

Резултатите от това първоначално проучване показват по-високо съдържание на мазнини и минерали в лимец в сравнение с пшеницата: нивата на минералите на K, P, Mn и Zn са по-високи с 4% до 67%, а по отношение на цинка и мазнините разликата е 2 пъти.

Илиева, М. Кондева-Бурдина, В. Павлова, Зл. Братанова, Н. Данчев, **Цв. Георгиева** - Мониторинг на токсични цианобактерии чрез полимеразна верижна реакция в реално време (Real-Time PCR) в проби от български водоеми –Сборник доклади от Годишна университетска научна конференция 20-21 октомври 2016 година, електронно издание, Издателски комплекс на НВУ „Васил Левски“ ISSN 2367-7481, 241-249

Цианотоксините се произвеждат от бактерии, наречени цианобактерии, известни още като синьо-зелени водорасли. Те са част от фитопланктонна и са повсеместно разпространени, като предимно се срещат в езера и океани, в които съществуват условия за тяхното експоненциално размножаване. В резултат на това възникват цъфтежи. При цъфтежа си някои видове цианобактерии (Microcystis, Anabaena, Planktothrix и др.) могат да произвеждат цианотоксини (напр. микроцистин) в концентрации, които са достатъчно високи, за да отровят и дори убият животни и хора. Световната здравна организация препоръчва максимално допустима стойност за питейни води 1 µg/l и 20 µg/l за води за къпане на едни от най-опасните цианотоксини – микроцистини. Той е рисков фактор от околната среда, предизвикващ невродегенеративни заболявания като амиотрофична латерална склероза, паркинсон и алцхаймер. Това налага тяхното ранно откриване при ниски концентрации.

Наличието на цианобактерии в български влажни зони се докладва от края на 19. век, а през втората половина на 20. век за пръв път е съобщено за наличието на вредни водораслови цъфтежи. През 2004г е публикувано първото изследване, което представя резултати от анализи на проби чрез течнохроамтографски анализ за съдържание на микроцистин от 15 водоема в страната (Pavlova et al., 2006) . В България са проведени само няколко проучвания за анализ на цианотоксини.

Според Анекс II от Рамковата Директива 2000/60/ЕС, трансформиран в наредба No 13/2007 за охарактеризиране на повърхностни води, изследванията на количеството, качеството и биомасата на фитопланктона в езерата са абсолютно необходими, но на този етап определени допустими граници за цианотоксини няма. Методите за изследване и мониториране на цианопрокариотите и техните токсини са в процес на разработване.

Целта на дипломната работа е подбор и прилагане на констелация от молекулярно-генетични методи за ранно откриване на токсични микроводорасли.

Изследвани са проби от три язовира „Пчелин“ „Бистрица“ и „Студена“, който се използва за питейни нужди. Пробите са събирани в контърнери от 1,5 л. през период на цъфтеж в две различни години 2013 и 2015. За контролни проби са използвани проби биомаса от Дуранкулак и Шабла, където имат доказано присъствие на токсични цианобактерии чрез микроскопско видово определяне и хорамтографски анализ. Бактериална ДНК е изолирана по два метод: 1. ксантогенатен метод, оптимизиран за целите на настоящата дипломна работа и 2. комерсиален кит. Направен е сравнителен анализ на двата метода за пробоподготовка. Проведен е анализ чрез Полимерзна верижна реакция в реално време чрез TaqMan и SYBRGreen подход. За оценка на присъствие на бактериална ДНК са подбрани специфични праймери за доказване на *Cyanobacteria*. Като генетични маркери за идентификация на токсичния вид *Microcystis aeruginosa* са подбрани участъци от микроцистин генетичния клъстер *mcu*: *mcuA* , *mcuB*, *cu359* .

Оптимизиран е ксантогенатен метод за екстракция на ДНК от водна биомаса. Изолираното количество ДНК е с подходяща чистота и в достатъчно количество за нуждите на последващ PCR анализ, което е доказано със спектрофотометричен анализ и чрез RealTime PCR. Оптимизирани са четири ДНК метода за детекция. Токсичните цианобактерии са особено подходяща мишена за откриване чрез полимеразно верижна реакция в реално време (RealTime PCR), защото тези микроорганизми представляват риск, когато присъстват в концентрация от няколко хиляди клетки на милилитър. Използването на RealTime PCR осигурява нов, важен инструмент за мониторинг на води от Български, позволява ранно откриване на „замърсяване“ с цианотоксини и подпомага оценката на риска за здравето на човека и околната среда.

Ж. Тишкова, В. Христова-Багдасарян, **Ц. Георгиева** Миграция на първични амини от полиамидни кухненски прибори, *Българско списание по общественото здраве*, vol 7, No 1, p87-98, 2015

Материалите, предназначени за контакт с храни, включват както опаковъчни материали, така и прибори за хранене, чинии, контейнери, машини за обработка и т.н. Използването на пластмасови прибори за готвене и пържене (шпатулки, бъркалки, прибори за обръщане на храна, черпаци, лъжици - решетъчни или с други форми) се е увеличило през последните години, тъй като тези предмети са евтини и нечупливи, относително устойчиви са на високи температури и не надраскват повърхностите на съдовете. Повечето са изработени от различни видове найлон, известен с химичното наименование полиамид (РА). Ако при синтеза на полиамидите съотношението между мономерите е добре балансирано и реакцията на химичен синтез е завършена, всички мономерни са включени в образуването на полимерната мрежа. Но при неподходящ баланс между мономерите остават свободни мономерни или изоцианати, които могат да мигрират към храната и да образуват първични ароматни амини. Първичните ароматни амини могат да попаднат в храната като остатъчни мономерни от предмети за контакт с храни, като продукти от хидролизата на изоцианати или като замърсители на азобагрила. Те могат да се формират химически в храните или да мигрират към тях от материали, с които храните са в контакт, ако те не са произведени при спазване на добра производствена практика. Първичните ароматни амини са семейство съединения, класифицирани от Междunarодната агенция за изследване на рака като «възможни канцерогени за човека» и присъствието им в хранителни продукти трябва да се избягва. През последните години в страните-членки на ЕС възникна безпокойство относно получени данни за миграция на първични ароматни амини от полиамидни кухненски прибори, внос от трети страни. Регламент (ЕС) № 284/2011 установява специфични условия и подробни процедури за вноса на тези изделия и посочва максимално допустими граници за специфична миграция. В настоящата работа се публикува валидиран спектروметричен скрининг метод за сумарно определяне на първични ароматни амини, представени като анилин еквивалент, който да удовлетворява изискванията на Регламент 284/2011 г. Методът се основава на диазотиране на първичните ароматни амини с натриев нитрит в кисела среда и следващо неутрализиране на остатъчния нитрозиращ агент с амониев сулфат. Получените нитропроизводни се купелуват с N-(1-нафтил)-етилендиаминов дихидрохлорид до получаване на виолетово оцветен комплекс, който се концентрира чрез твърдофазова екстракция. Измерената екстинкцията при дължина на вълната 550 nm е пропорционална на общото съдържание на първични ароматни амини, изразени в анилин еквиваленти. Границата на откриване LOD е 0,001 mg/kg; границата на количествено определяне LOQ е 0,002 mg/kg, работният обхват на метода е от 0,002 до 0,04 mg/kg. Методът е приложен за проучване на миграцията на първични ароматни амини от

полиамидни кухненски прибори, налични на българския пазар. Получените данни не показват потенциален риск от миграция на тези вещества от полиамидни артикули с етикет, съдържащ информация за произхода, вида на материала, условията и начина на употреба и при спазване от потребителите на посочените в етикета указания. Артикули, за които липсва маркировка или етикет, както и такива, етикетирани с неполезна информация, например, "произведено от безвредна пластмаса", не са обект на настоящото проучване. Широкото разпространение на подобни артикули изисква повишено внимание относно тяхната безопасност за потребителите.

**Tz. Georgieva**, „Next generation transgenic plant and products of synthetic biology–, *EFSA Bulgarian Focal Point Publication*, 2013  
[http://www.focalpointbg.com/?news\\_item=766](http://www.focalpointbg.com/?news_item=766)

Настоящият обзор има за цел да направи преглед на следващо поколение трансгенни растения с подобрен хранителен състав, приложението им и необходимостта от оценка на хранителната им стойност чрез сравняване с техните конвенционални видове. Синтетичната биология е бързо развиваща се научна област, включваща различни дисциплини - инженерни науки, химия, физика, компютърни науки и биоинформатика. Могат ли продуктите на синтетичната биология да се разглеждат като генно модифицирани организми и попадат ли в обхвата на тази дефиниция? Какви са предизвикателствата, свързани с оценката на тяхната безопасност? Повечето от трансгенните култури, получени с техниките на съвременната биотехнология (известен също като генетично модифицирани организми или генетично модифицирани култури) са линии царевица, соя, картофи, памук и рапица, които са модифицирани чрез въвеждане на един или повече гени, кодиращи за характеристики като, устойчивост на болести и насекоми, устойчивост на хербициди, или комбинации на тези характеристики. Генно модифицираните организми намират все по-широко приложение в редица области, различни от селското стопанство. Например, създаване на нови ветеринарно-медицински препарати и препарати за човешка употреба, приложение в областта на научните изследвания, в медицината и др.

В наши дни, интересът на учените е насочен към създаване на ГМ култури с "качествени характеристики", които имат отношение към подобряване здравето на хората или животните. Тези култури (например ориз с повишено съдържание на желязо и провитамин А, царевица и соя с изменен аминокиселинен или мастнокиселинен състав) обикновено се създават чрез модифициране метаболизма на растението, което води до изменение и на състава им.

**Цв. Георгиева** - Генетично модифицирани организми в храни, *издание на НЦООЗ, 2008*

Генетично модифициранте организми са продукт на генното инженерство. Това са организми, чиито генетични характеристики са били променени чрез вмъкването на модифициран ген или гени от друг организъм, използвайки техниките на генетичното инженерство. В директива на Европейската Комисия 2001/18/ЕС в Член 2(2) е посочена следната дефиниция – „генетично модифициран организъм“ (ГМО) означава организъм, с изключение на човешкия организъм, в който генетичният материал е бил променен по начин, който не настъпва естествено при чифтосване и/или естествена рекомбинация” (1 Директивата) . Първата рекомбинантна бактерия е създадена 1973 модификацията представлява експресия на салмонелен ген в E.coli. (Cohen et al, 1973) Масовото отглеждане на генетично модифицирани растения започва през 1995 г. Днес основни производители на ГМ растения и култури са САЩ, Южна Америка, Канада, Китай и др.

Настоящата публикация има за цел да концентрира на едно място най-съществената информация, относно приложението, оценката на безопасността, регистрацията и законодателството в областта на генно модифициранте организми .

**Tz. Georgieva, V.Nikolova** Implementation of European GM legislation in Bulgaria First Global Conference on GMO Analyses, Book of Abstracts, JRC, EUR 23390 EN, 2008 p110

Regulation: As a member of the European Union since 2007, Bulgaria must comply with the rules for placing on the market, control and traceability of GMOs as laid down in EU legislation. The Bulgarian GMO law was adopted in 2005 and amended in April 2008. Competent authority: The Institutions responsible for GMO in Bulgaria are: Ministry of Health (MoH), Ministry of Agriculture and Food Supply (MAFS) and Ministry of Environment (MoE). Control: Based on the national regulation the control of food and feed is divided between both Ministers MoH and MAFA. Bulgarian Ministry of health with 28 Regional Inspectorates (RIPCH) performs the official control of GM food. A yearly plan on the inspections and GMO analysis in food samples is prepared by the MoH and communicated to the RIPCH.

In 2006-2007 under the PHARE Project Twinning Project G/2004/IB/EC/01 "Chemicals and food" between the Bulgarian MoH and Austrian Agency of Environment was organized for inspectors and experts from laboratories trainings on GMO legislation, control/sampling, quality management, application procedures, risk assessment and methods of analysis. Under the PHARE Project BG 2004/016-711.02.03 the Laboratory for GM food analyses within the National Center of Public Health Protection was fully equipped for detection and quantification of GMO. The laboratory is a member of European Network of GMO Laboratory (ENGL) and was nominated to be the National Reference Laboratory.

Risk Assessment: Under the Bulgarian Ministry of Health in February 2008 was established "Risk assessment in food safety council of experts", including GMO group.

П. Стефанова, Г.Благоева, В. Гочева, **Цв. Георгиева**, А. Ангелов. Оценка на ефективността на СТАВ метод за екстракция на днк чрез Real-Time PCR тест за инхибиране на ДНК екстракти от соеви и месни продукти *Научни трудове на Университет по хранителни технологии – ПЛОВДИВ ТОМ LXII 2015 г., стр.482-486*

Food products are complex matrices that might contain a number of PCR inhibitors such as polysaccharides, polyphenols and proteins. The efficiency of the DNA extraction method could be critical for a successful real-time PCR analysis, since there are many compounds that inhibit DNA amplification. The aim of the present study was to evaluate the efficiency of CTAB extraction method with real-time PCR inhibition test of DNA extracts from soybean and meat products. The obtained results showed that the coefficient of correlation ( $R^2$ ) was between 0.997 and 1.000 for all tested products. Moreover, the average difference ( $\Delta Ct$ ) between the measured Ct value and the extrapolated Ct value of the working dilution was less than 0.5. The received data demonstrated the good performance of CTAB extraction method and the absence of inhibitors in the DNA extracts of the analyzed soybean and meat products.

**Tz. Georgieva**, Hr. Kopcheva, T. Panev, Popov, Informative markers of oxidative stress in occupational exposure to carbon disulfide – *Bulgarian Journal Of Public Health*, vol 7, No 1, p87-98, 2015

Предполага се, че повишената експозиция на въглероден дисулфид ( $CS_2$ ) може да доведе до изчерпване на редуцирания глутатион (GSH) и да увеличи образуването на свободни радикали в човешкия организъм. Информацията за въздействието на въглеродния дисулфид върху оксидант-антиоксидантната система на организма върху организма е оскъдна. Изследвани са 77 работника от предприятие за изкуствени влакна, експонирани на различни нива на въглероден дисулфид. Контролната група съставлява 26 лица, без професионална експозиция. Персоналната експозиция е определена чрез персонална дозиметрия. Като маркери за оксидативен стрес са определяни концентрациите на редуциран глутатион (GSH) в еритроцити и нивото на хидропероксидни радикали в капилярна кръв. Не се установява достоверна корелационна зависимост между нивото на експозицията и концентрациите на GSH и хидропероксидните радикали. Регистрира се статистически значима разлика в нивата на GSH и хидропероксидни радикали при сравняване експонираните лица по пол. При жените-работнички, стойностите на GSH са по-ниски, а тези на хидропероксидните радикали статистически достоверно ( $p = 0,018$ ) по-високи. На лице е риск от

оксидативен стрес при жените - работнички, който би могъл да се асоциира с множество фактори – пол, експозицията, фактори различни от професионалната експозиция.

П. Стефанова, **Цв. Георгиева**, А.Ангелов “Event” специфичен RealTime PCR анализ за количествено определяне на разрешени за употреба в ЕС генетично модифицирани соеви линии в месни продукти , *Научни трудове на Русенски университет – 2015, том 54, серия 10.2, 213 – 217*

The quantification of GM material in food products is very important to control food labeling. Therefore, eventspecific PCR quantification of EU-authorized GM soybean events was conducted in 36 meat products at the Bulgarian market without GM indication on the labels. Results indicated that 36.1% of the tested products contained MON 40-3-2, while MON 89788 and A2704-12 were detected in 2.8% of the samples. The amount of MON 40-3-2 was above the threshold of 0.9% in 1 sample, while the percentage of transgenic events MON 89788 and A2704-12 in all tested products was less than 0.9%. None of the tested products contained GM soybean events MON 87701, A5547-127, DP356043 and MON 87701xMON 89788. Based on the results from the study, only one of the analyzed products was falsely labeled with regards to GM ingredients. Key words: Real-time PCR, genetically modified soybean events, meat products, EU legislation.

Ruseva B. K., Strashimirov D., Shumkov N., **Georgieva Tzv.**, Mihailova A. Changes of lipid profile and aortic wall in spontaneously hypertensive rats under diet of different selenium content, *Scientific Research Journal of South-West University*, October 2008, 1 (1): 101-104

The interest on the participation of the free radical processes in physiology and pathophysiology of the organism increases in the last decades of years. Knowledge on the mechanisms of production and reduction of reactive oxygen species (ROS) and factors that may control and modify them will allow the founding of new more efficient modes for prevention and treatment of diseases in pathogenesis they participate. Selenium (Se) is a trace element that performs its biological role mainly as cofactor of enzymes that take action in the antioxidant defense of the organism, in the redox systems and in the metabolism of thyroid hormones. Expression of selenoproteins depends on daily Se intake. The endothelium of the vessels is a dynamic structure that maintains normal functions of cardiovascular system by the production of vasoactive substances. The balance between production of vasoconstrictors and vasodilators is important in the control of the vessel tone. The glutathione peroxidases (GPx) and thioredoxine reductases are the main selenoproteins, expressed in the

endothelial cells. ROS and the products of lipid peroxidation are the main cause for injury of endothelium. GPx-1 is an antioxidant enzyme that catalyzes reduction of the hydrogen hydroperoxide and the other organic hydroperoxides. The deficit of GPx-1 may induce the increase of oxidative stress, leading to endothelial dysfunction. The aim of this study is to investigate the effects of the different Se intake on oxidative status and their impact to the lipid profile and the state of the vessel wall in normotensive Wistar-Kyoto rats and spontaneously hypertensive rats (SHR). The different selenium content diets (low, adequate and high) were applied for 8 weeks on the experimental rats in two periods of their life (pubertal and adult). Our results showed that the low Se intake worsens antioxidant status and leads to severe degenerative changes and thickening of the aortic wall of WKY and SHR that may be a prerequisite for disturbance of the perfusion of the important organs. Increased Se intake did not cause significant influence on the state of the vessel walls in young WKY, but in adults it slowed down the development of the degenerative changes in the aortic wall and increased index HDL- /LDL- cholesterol by improvement of redox state. Selenium supplementation had a positive effect on SHR in both investigated periods on the antioxidant status, on the lipid metabolism, on the elastin synthesis and slowed down the development of the pathological processes in the aortic wall and the coronary arteries. In conclusion we speculate that normotensive rats are more sensitive to Se deficit, whereas hypertensive rats are more sensitive to Se supplementation. The presented study suggests an idea for the implementation of more clinical investigations on the use of selenium supplementation in treatment and prevention of cardiovascular diseases.

### 3. СПИСЪК С РЪКОВОДНИ ДОКУМЕНТИ

Corbisier P., Barbante A., Berben G., Broothaerts W., De Loose M., Emons H., **Georgieva Tz**, Lievens A., Mazzara M., Papazova N., Perri E., Sowa S., Stebih D., Terzi V., Trapmann S, Recommendation for the unit of measurement and the measuring system to report traceable and comparable results expressing GM content in accordance with EU legislation, ISBN 978-92-79-66971-2 ISSN 1831-9424 doi: 10.2760/177516, 2017 <http://gmo-crl.jrc.ec.europa.eu/ENGL/docs/WG-UoM-Final-Report.pdf>

It is important to guarantee that results expressing the GM content are reliable, comparable and fulfil the requirements of existing EU legislation. The use of different measurement units to express a GM content, the appearance of new analytical methods that do not require a calibrant and the composite EU legislation on GMOs have triggered the need for a document to clarify how to obtain reliable and comparable results. For this guidance document, past and current EU legislations have been reviewed with a special emphasis on what is meant by 'GM percentage' in the different

legal texts. The metrological traceability of measurement results and the currently available guidance are explained and summarised. The particular case of botanical impurities and the genetic constitution of GM seeds are described and illustrated to better understand the complexity hidden behind this type of analysis. An overview of the different analytical methods based on DNA measurements and used for the expression of quantitative GM content results is provided, including the use of new techniques based on digital PCR (dPCR). A measuring system that allows for comparing results by making them traceable to the same reference system has been elaborated in detail. Needs and tools are described and a solution has been proposed to convert results expressing GM content to the required measurement unit, whenever this is needed. By following these recommendations, results obtained in GM copy number per haploid genome equivalent (cp/HGE) by dPCR can be converted into mass fraction percentage and compared to the results obtained by quantitative PCR (qPCR) either with a calibrant certified for its GM mass fraction or with a calibrant certified for its GM purity. The general principle is to relate a measurement result to a GM quantity embedded in a specified certified reference material (CRM) either directly or via one single conversion factor (CF) per event. This conversion factor and its related uncertainty need to be determined precisely for each CRM batch, preferably on the pure GM CRM (100 %), using, for example, dPCR. The estimated uncertainty associated with this conversion factor must be integrated into the measurement uncertainty of the final results expressed in GM mass fraction. CF are currently not yet established for most CRMs. CF values have been recently reported in a few pioneer dPCR studies. However, such proof of concept studies remain incomplete. Therefore, to avoid a gap between new technologies and current EU regulation, the working group recommends to launch a dedicated study to determine CF values. Such a study should involve a limited number of competent laboratories with a proven experience in dPCR. The study could be coordinated by the EURL-GMFF.

**Tz.Georgieva.** Guidelines for GMO analysis (legislation, methods for detection, identification and quantification; practical protocols) FAO Project TCP/SEC/3502 "Safety assessment of Food Derived from Biotechnology" *This guidelines document is prepared in the framework of the FAO Project TCP/SEC/3502 "Safety assessment of Food Derived from Biotechnology". It has been drafted for project internal purposes and is not intended for publication.*2017

This guidelines document is prepared in the framework of the FAO Project TCP/SEC/3502 "Safety assessment of Food Derived from Biotechnology". It has been drafted for project internal purposes and is not intended for publication.

This document is intended to provide background information and information on methodologies and protocols currently used. The subject matter covers a variety of techniques for DNA extraction and detection, identification, characterisation, and quantification of GMO's for the needs of the trainees of the GMO detection training course - Subregional training course in detection, identification and quantification of Genetically Modified

Organisms (GMO) and laboratory accreditation according to EN/ISO IEC 17025:2005 , September 2017, BAKU, Azerbaijan

This manual aims to complement existing information in the specialised literature and to provide basic information for GMO analysis. In no way it can substitute textbooks, where the basic information on molecular techniques such as the principles of PCR, hybridisation or electrophoresis can be found. For the daily work in enforcement laboratories it is essential to get up to date information from sources such as the internet, scientific publications and scientific meetings.

ISO standards on the detection of GMOs in foodstuffs (see “References”) where the basic principles on quality control and requirements for GMO analysis are given should be used as a basis for the establishment of a GM analytical system. Some methods, including their validation data are also part of these standards.

#### **4. СПИСЪК С ТЕХНИЧЕСКИ ДОКЛАДИ**

Annual report of the EFSA Scientific Network for Risk Assessment of GMO s for 2017 European Food Safety Authority (EFSA), I Olaru, E Waigmann, EFSA Supporting Publications 15 (2), 2018 1372E

EFSA endeavours to develop networking and stronger cooperation with the Member States, and to strengthen its relationship with institutional partners (European Union and international) and stakeholders, as recommended by EFSA’s Management Board. In accordance with EFSA’s strategy for cooperating with Member States, the EFSA Scientific Network for Risk Assessment of GMOs (hereafter referred to as “the GMO Network”) was established in 2010. Since its inaugural meeting in November 2010, the GMO Network has met once per year. The overall goals of the GMO Network are to improve dialogue among members, build mutual understanding of risk assessment principles, enhance knowledge and confidence in the scientific assessments carried out in the EU, and increase the transparency of the process among Member States and EFSA. It aims to raise the level of harmonisation of the risk assessments developed in the EU. Currently 28 Member States and Norway are members of the GMO Network. Switzerland, Turkey and Bosnia-Herzegovina are invited to the GMO Network as observers. Each country was allowed to nominate two Member Organisations: one with competence in molecular characterisation and food/feed safety (MC/FF) and one with competence in environmental risk assessment (ERA). These Member Organisations have appointed in total over 60 selected scientific experts to attend the annual meetings in the light of the topics on the agenda. A maximum of two experts per country are invited to each meeting.

The 2017 annual meeting of the GMO Network, held on 23-24 May, was attended by 43 scientific experts from 25 Member States and Norway, one observer from Switzerland, one from BosniaHerzegovina and one from Turkey, two representatives of the European Commission (Directorate General for Health and Consumers – DG SANTE), two EFSA GMO Panel

members, a hearing expert, and 16 EFSA scientific staff members from the GMO Unit. At the 2017 meeting of the GMO Network, the appointed experts were informed about active mandates of the GMO Panel (including GMO applications), risk assessment guideline development, requests for scientific advice, and procurement contracts. This was followed by discussions on the explanatory note on literature searching conducted in the context of GMO applications, the draft guidance for the risk assessment of the presence at low level of genetically modified plant material in imported food and feed under Regulation (EC) No 1829/2003, limits of concern in environmental risk assessment, baseline information to support the risk assessment of RNAi-based GM plants, omics technologies used to identify potential unintended effects in GM plants, and living modified organisms and synthetic biology. Two breakout sessions were organised according to the expertise of the two groups of experts, to allow in-depth discussion of specific topics. The experts in the field of MC/FF discussed the supplementary guidelines for the allergenicity assessment of GM plants and the explanatory note on Next Generation Sequencing for the characterisation of GM plants. The experts in the ERA field discussed the assessment of representativeness of sites used for the agronomic/phenotypic and compositional characterisation of GM plants, the EFSA GMO Panel opinion on the 2015 annual PMEM report for maize MON810, and the EFSA report on the impact of teosintes. In 2017, GMO Network members and other experts from Member State Competent Authorities in the area of GMO risk assessment (Article 36 Member Organisations) contributed to the development of a GMO Panel draft guidance documents, namely the guidance for the risk assessment of the presence at low level of genetically modified plant material in imported food and feed under Regulation (EC) No 1829/2003. GMO Network members also participated to the 118th GMO Panel open plenary meeting and to the webinar “Presentation of the guidance on allergenicity assessment of genetically modified plants”

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scientific assessments carried out in the EU, and increase the transparency of the process among Member States and EFSA. It aims to raise the level of harmonisation of the risk assessments developed in the EU. Currently 28 Member States and Norway are members of the GMO Network. Switzerland, Turkey and Bosnia-Herzegovina are invited to the GMO Network as observers. Each country was allowed to nominate two Member Organisations: one with competence in molecular characterisation and food/feed safety (MC/FF) and one with competence in environmental risk assessment (ERA). These Member Organisations have appointed in total over 60 selected scientific experts to attend the annual meetings in the light of the topics on the agenda. A maximum of two experts per country are invited to each meeting. The 2016 annual meeting of the GMO Network, held on 31 May – 1 June, was attended by 39 scientific experts from 22 Member States and Norway, one observer from Switzerland, one representative of the European Commission (Directorate General for Health and Consumers – DG SANTE), three EFSA GMO Panel members, two members of the GMO Panel's Working Groups, and 13 EFSA scientific staff members from the GMO and Scientific Committee and Emerging Risks (SCER) Units. At the 2016 meeting of the GMO Network, the appointed experts were informed about follow-up activities to the discussions held at the 2015 meeting of the GMO Network, active mandates of the GMO Panel (including GMO applications), risk assessment guideline development, requests for scientific advice, and procurement contracts. This was followed by discussions on the draft guidelines on possible derogation to existing requirements for applications of GM food and feed at low levels submitted under Regulation (EC) No 1829/2003, the Scientific Committee's guidance on specific protection goals, Next Generation Sequencing in the risk assessment of GMOs, and gene drive and potential implications for environmental safety. Two breakout sessions were organised according to the expertise of the two groups of experts, to allow in-depth discussion of specific topics. The experts in the field of MC/FF discussed the development of the allergenicity guidance and the role of sampling in the risk assessment of GM plants. The experts in the ERA field discussed EFSA's recommendations on resistance monitoring for corn borers and the potential exposure of NT lepidopteran larvae to Btmaize pollen deposited on their host plants. In 2016, GMO Network members and experts from Member State Competent Authorities in the area of GMO risk assessment (Article 36 Member Organisations) contributed to the development of two GMO Panel draft guidance documents. One was the supplementary guidance for allergenicity assessment of GM plants, on which GMO Network members commented in the context of the public consultation; EFSA organised an Info session to address the received public comments, and several GMO Network experts attended and provided further input. The other one was the guidance for the risk assessment of low level presence of genetically modified food and feed submitted under Regulation (EC) No 1829/2003; for this guidance, EFSA organised a dedicated commenting period exclusively for Member States. This consultation was facilitated by the EFSA Focal Points. This was the first time EFSA organised a dedicated commenting period for Member States, and the experience was found very

useful by both sides: for EFSA it was important to take into account the input from Member States, as key partners in risk assessment, before launching the guidance for public consultation, while Member States welcomed the opportunity to contribute to the development of the guidance at that stage of the process. GMO Network members also participated to the 110th GMO Panel open plenary meeting. During this meeting, several applications for authorisation of GM plants submitted under Regulation (EC) No 1829/2003 were discussed. GMO Network members attended this meeting as observers.

#### Annual report of the EFSA Scientific Network for Risk Assessment of GMOs for 2015 European Food Safety Authority (EFSA), EFSA-Q-2015-00751

EFSA endeavours to develop networking and stronger cooperation with the Member States, and to strengthen its relationship with institutional partners (European Union and international) and stakeholders, as recommended by EFSA's Management Board. In accordance with EFSA's strategy for cooperating with Member States, the EFSA Scientific Network for Risk Assessment of GMOs (hereafter referred to as "the GMO Network") was established in 2010. Since its inaugural meeting in November 2010, the GMO Network has met once per year.

The overall goals of the GMO Network are to improve dialogue among members, build mutual understanding of risk assessment principles, enhance knowledge and confidence in the scientific assessments carried out in the EU, and increase the transparency of the process among Member States and EFSA. It aims to raise the level of harmonisation of the risk assessments developed in the EU.

Currently 27 Member States and Norway are members of the GMO Network. Switzerland is invited to the GMO Network as observer. Each country was allowed to nominate two Member Organisations: one with competence in molecular characterisation and food-feed safety (MC/FF) and one with competence in environmental risk assessment (ERA). These Member Organisations have appointed in total over 60 selected scientific experts to attend the yearly meetings in the light of the topics on the agenda. A maximum of two experts per country are invited to each meeting.

The sixth meeting of the GMO Network, held in May 2015, was attended by 42 scientific experts from 25 Member States and Norway, one observer from Switzerland, two hearing experts invited as speakers, one representative of the European Commission (Directorate General for Health and Consumers – DG SANTE), five EFSA GMO Panel members, and 14 EFSA scientific staff members from the GMO and Evidence Management (DATA) Units.

At the sixth meeting of the GMO Network, the appointed experts were informed about follow-up activities to the discussions held at the fifth meeting of the GMO Network, active mandates of the EFSA GMO Panel, including GMO applications, risk assessment guideline development, requests for scientific advice, and procurement contracts. This was followed by discussions on the draft guidance on agronomic and phenotypic characterisation of GM plants and the draft guidance document for the risk assessment of the renewal of GM plant products authorized under Regulation (EC) No 1829/2003. Two breakout sessions were organised according to the expertise of the two groups of experts, to allow in-depth discussion of specific topics. The experts in the field of MC/FF discussed the use of EFSA Comprehensive European Food Consumption Database for estimating dietary exposure to GM foods. The experts in the ERA field discussed EFSA's self-task activity to supplement its previous risk mitigation measures reducing exposure of non-target Lepidoptera to maize MON 810, Bt11 or 1507 pollen. At the following joint plenary session, the GMO Network experts discussed with the invited speakers risk assessment considerations for plants developed by new plant breeding techniques or synthetic biology, and for second generation GMOs respectively. This was followed by a discussion on the use of negative segregants in the comparative assessment of GMOs. EFSA also presented its Document Management System and shared information on upcoming scientific events.

In 2015, GMO Network experts participated in two EFSA meetings relevant for the risk assessment of GMOs. The first one was the 96th GMO Panel plenary meeting, which was held in Brussels on 4-5 March 2015. During this meeting, the draft guidance document on agronomic and phenotypic characterisation of GM plants and the draft guidance for the risk assessment of the renewal of GM plant products authorised under Regulation (EC) No 1829/2003 were discussed. GMO Network members expressed their views and asked questions related to these draft documents, in their quality as observers to this meeting. The second one was the 'Workshop on allergenicity assessment of GM plants', held in the context of guidance development in Brussels, on 17 June 2015. The objective of this workshop was to involve stakeholders at an early stage of the guidance development and to enhance their participation in EFSA scientific work. GMO Network members actively participated to the discussions held at this workshop and provided valuable input.