eemgs EUROPEAN ENVIRONMENTAL MUTAGENESIS & GENOMICS SOCIETY

ICAW

Joint EEMGS meeting & International Comet Assay Workshop




May 23rd – 26th 2022

Maastricht, The Netherlands

Including discussion forums, young scientist sessions, allocated poster sessions and informal gatherings on the riverbanks of the Maas.

Hosted by Maastricht University's departments of Pharmacology & Toxicology and Toxicogenomics, and financially supported by the Limburg University Fund/SWOL

Maastricht University

Universiteitsfonds Limburg | SWOL |

CONFERENCE GUIDE

Welcome at the
14th International Comet Assay Workshop (ICAW) &
50th meeting of the European Environmental Mutagen and Genomics Society (EEMGS)

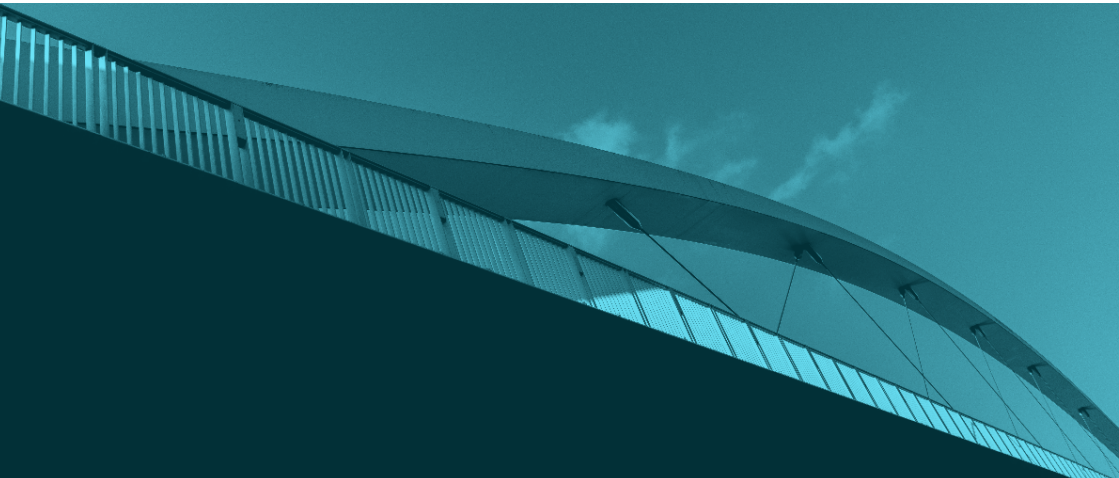
The International Comet Assay Workshops are a series of scientific conferences dealing with practical and theoretical aspects of the Comet Assay, aimed at both experienced and new users of this technique. The Workshop has always been rather informal. It began as a satellite meeting to the 2nd International Conference on Environmental Mutagens (ICEM) in 1995, but continued every 2 years after that. Unfortunately, the meeting was postponed in 2021 due to the covid19 pandemic. Also the annual meetings of other societies/ interest groups were postponed and we are therefore happy that we can now join up here in Maastricht. Therefore, this meeting is a joined meeting of ICAW, the Dutch Environmental Mutagen Society (DEMS), the Belgian Environmental Mutagen Society (BEMS) and the European Environmental Mutagen & Genomics Societies (EEMGS).

In 2020, former ICAW organizers, together with hCOMET COST-Action (CA15132) members, created the International Comet Assay Working Group (ICAWG). This group became an affiliated working group of the European Environmental Mutagenesis & Genomics Society (EEMGS) since 2020. ICAWG exists to unite those scientists working in the field of genetic toxicology, DNA damage and DNA repair with interest in the comet assay (a.k.a. single cell gel electrophoresis). It encompasses research into the mode-of-action of genotoxic agents and associations of genome instability markers with disease outcomes, and applications of this knowledge in the field of regulatory toxicology, biomonitoring studies, (nutritional) interventions as well as clinical studies. Studies into the impact of genotoxic agents on genome stability in organisms other than man – a field known as ecogenotoxicology – is also gaining attention.

CONFERENCE GUIDE

The 14th ICAW, joint with the 50th EEMGS meeting, will be hosted by the Department of Pharmacology and Toxicology, from the University of Maastricht, from May 23rd until 26th 2022 at the Crown Plaza hotel. The programme includes oral and poster presentations depicting the highlights of the past, the present and especially the future of the (regulatory) genotoxicity testing; and discussing the latest advances in the development and application of the Comet Assay. A dedicated EEMGS New Investigators (EEMGS NI) session, allows early career scientists to chair their results and receive expert input. The BEMS/DEMS session will focus on "New approaches to assess genotoxicity of poorly soluble materials", since the genotoxic effects of nanomaterials/nanoplastics on humans and the environment is a topic of growing interest. The programme also includes an open discussion session to exchange thoughts about practical issues and the latest innovations. This will encourage the exchange of information between specialists from both the academic and industrial sectors.

We wish you a wonderful meeting here in Maastricht.



The Team

Co-Chairs of ICAW/EEMGS 2022

Sabine Langie

Roger Godschalk

Simone van Breda

Scientific committee

Amaya Azqueta *(Spain)*
Elisa Boutet-Robinet *(France)*
Paul Fowler *(UK)*
Roland Frötschl *(Germany)*
Goran Gajski *(Croatia)*
Marko Gerić *(Croatia)*
Roger Godschalk *(The Netherlands)*
George Johnson *(UK)*
Marcin Kruszewski *(Poland)*
Sabine Langie *(Belgium)*
Matjaz Novak *(Slovenia)*
Bertrand Pourrut *(France)*
Marie Vasquez *(USA)*
Sona Vodenkova *(Czech Republic)*

Instructors of the hands-on training session

Amaya Azqueta and Miguel Collía Martín
University of Navarra, Spain

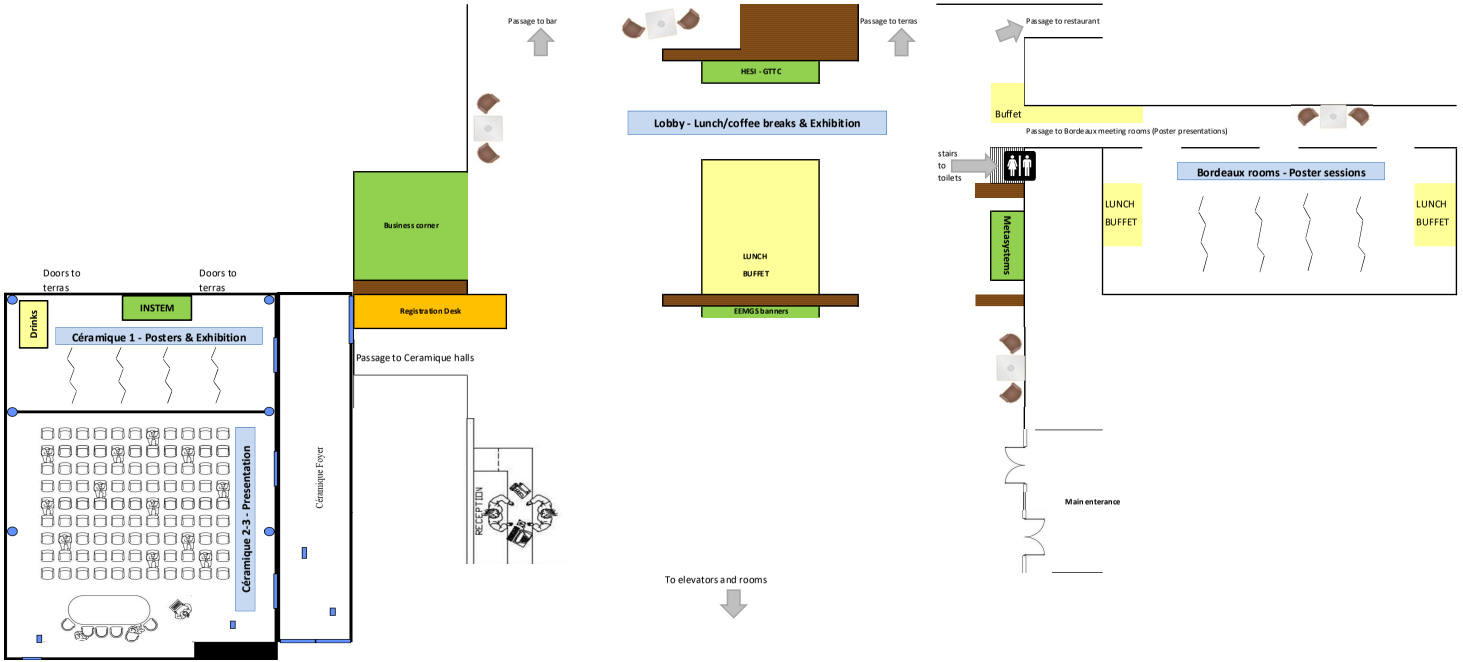
Martina Stampar and Matjaž Novak
National Institute of Biology, Slovenia

Sabine Langie, Roger Godschalk and Shan Wang
Maastricht University, The Netherlands

Programme Overview

	Hands-on training course			EEMGS/ICAW 2022			
Time/Day	**Saturday 21st May**	**Sunday 22nd May**	**Time/Day**	**Monday 23rd May**	**Tuesday 24th May**	**Wednesday 25th May**	**Thursday 26th May**
8:30			8:30		REGISTRATION		
9:00	REGISTRATION		9:00		**EEMGS NI session**	**Session 3 - part 1:** Progress in genotoxicity testing	**Session 4:** Ecotoxicology
9:30	Welcome & Introduction 3D models	Introduction high througput comet assays	9:30				
10:00			10:00	Pre-conference AOP workshop & Lunch			
10:30	coffee break	coffee break	10:30		coffee break	coffee break	coffee break
11:00	Preparation of spheroids	Training with CometChip part 1	11:00		**BEMS/DEMS session** New approaches to assess genotoxicity of poorly soluble materials	**Open disussion session** Factors that influence comet data interpretation	**Session 5:** Techincal innovations of the comet assay
11:30			11:30				
12:00	3D skin comet part 1		12:00			**Session 3 - part 2:** Genotoxicity testing	
12:30			12:30				**CLOSING**
13:00	Lunch 13h - 14h	Lunch 13h - 14h	13:00	REGISTRATION	Lunch 13h - 14h	Lunch 13h - 14h	
13:30			13:30	Welcome & Keynote lecture			
14:00	3D skin comet part 2	Training with CometChip part 2	14:00		**EEMGS/ICAWG GA**	Social programme 14h - 18:30h	
14:30			14:30	**Session 1:** Human biomonitoring	**Poster session 2**		
15:00			15:00				
15:30	coffee break		15:30	coffee break	coffee break		
16:00	Processing spheroids for comet assay use	coffee break	16:00	**EEMGS award session**	**Session 2:** Nutrition & neutraceuticals		
16:30		Comet viewing data analysis Q&A	16:30				
17:00			17:00	*Check-in/refresh*			
17:30			17:30	**Poster session 1** & Welcome reception			
18:00			18:00				
18:30			18:30		Conference dinner 18:30h - 00:00h		
19:00		*Pub quiz organized by NIEEMGS*	19:00				
19:30			19:30				
20:00			20:00				

Venue


Passage to bar
Business corner
Doors to terras
Doors to terras
INSTEM
Drinks
Céramique 1 - Posters & Exhibition
Registration Desk
Passage to Ceramique halls
Céramique 2-3 - Presentation
Céramique Foyer
RECEPTIEN
Passage to terras
HESI - GTTC
Lobby - Lunch/coffee breaks & Exhibition
LUNCH
BUFFET
EEMGS banners
To elevators and rooms
Passage to restaurant
Buffet
Passage to Bordeaux meeting rooms (Poster presentations)
stairs to toilets
Bordeaux rooms - Poster sessions
LUNCH BUFFET
LUNCH BUFFET
Metasystems
Main enterance

Advanced comet assay training course

The use of 3D models and high throughput comet chip.

Saturday 21st May - Sunday 22nd May, 2022

Preceding the joint ICAW and EEMGS meeting there will be the opportunity to take part in a hands-on training session with novel applications of the comet assay. You will get the opportunity to work with 3D skin models (provided by Henkel - Phenion) and HepG2 liver spheroids (training by National Institute of Biology, Slovenia) on the first day of the training. On Sunday you can try out the 12 gel/slide system and the CometChip (kindly provided by Bevin Engelward, MIT Dept. of Biological Engineering, & the commercial ones by Bio-Techne").

Hands-on training course sponsors

Detailed programme

Monday

AM

PM

09:00-09:30	Registration AOP workshop
10:00-12:30	**Pre-conference AOP workshop** Trainers: Dalma Martinovic-Weigelt *(University of St. Thomas, USA)* and Sivakumar Murugadoss *(Sciensano, Belgium)*
12:30-13:30	Lunch for AOP workshop

12:30-13:30	Registration EEMGS/ICAW

13:30-14:30 - Welcome & Keynote ***Chair: S. Langie***	
13:30-13:45	Welcome by EEMGS (Sabine Langie) and ICAWG (Goran Gajski)
13:45-14:30	**Keynote speaker:** **O1 "Four decades of genotoxicity testing: innovations on the horizon"** *- Birgit Mertens* *(Sciensano, Belgium)*

Meet the Keynote Speaker

Birgit Mertens obtained her Master in Pharmaceutical Sciences, and PhD in Neuropharmacology, from the Vrije Universiteit Brussel in Belgium. She joined Sciensano in 2010 and is currently working as senior toxicologist and leader of the Experimental Toxicology Unit in the Risk and Health Impact Assessment service.

She coordinates and participates in multiple (inter)national research projects and activities on the genotoxicity of physical and chemical agents, with a particular focus on food contaminants and the development and application of new approach methodologies. She has published over 40 peer-reviewed scientific papers and contributed to multiple (inter)national scientific advices. She is National Coordinator of the Test Methods Programme both at the European and OECD level and the Belgian contact point for the Preliminary Assessment of the Regulatory Relevance of Alternative Methods (PARERE) network. She is a member of the OECD genotoxicity expert group and has co-led the OECD project on the miniaturized versions of the bacterial reverse gene mutation test.

She participates in different (inter)national working groups and scientific committees on hazard and risk assessment and is the current president of the Belgian Environmental Mutagenesis Society. She is also guest lecturer in the toxicology courses of different Belgian universities and was associated with the Universiteit Antwerpen from 2018 until 2022.

14:30-15:45 - Session 1: Human biomonitoring ***Chairs: G. Gajski and A. Opattova***	
14:35-15:00	**O2 "Carcinogenic exposure biomarkers in human populations"** *- Nancy B Hopf (Centre universitaire de médecine générale et santé publique, Switzerland)*
15:00-15:15	**O3 "Ten-year DNA damage evolution of French female farm workers and link with cancer development."** *- Poppy Evenden* *(INSERM & Université de Caen Normandie, France)*
15:15-15:30	**O4 "The Comet Assay as a Human Biomonitoring Tool: effect of cryopreservation and blood cell type"** *- Ezgi Eyluel Bankoglu* *(University of Wuerzburg, Germany)*
15:30-15:45	**O5 "Occupational exposure to antineoplastic drugs - What are the gaps?"** *- Susana Viegas* *(Public Health Research Centre, Portugal)*

15:45-16:15	Coffee break

16:15-17:15	**EEMGS award lectures *(Chair: A. Azqueta)***

17:30-19:00	Poster presentation session 1 & Welcome reception

Tuesday

08:30-09:00	Registration
09:00-10:30 - New Investigators EEMGS session *Chairs:* **S. Vodenkova** ***and M. Novak***	
09:00-09:15	**O6 "The role of dietary AhR ligands in the development of inflammation bowel disease and subsequent risk of colitis-associated cancer"** *- Shan Wang (Maastricht Univeristy, The Netherlands)*
09:15-09:30	**O7 "In vitro mutagenicity assessment of fried meat-based food and French fries from mass catering companies"** *- Julen Sanz-Serrano (University of Navarra, Spain)*
09:30-09:45	**O8 "Mechanistic investigations of Mn-induced oxidative stress, DNA damage, DNA repair, and neurodegeneration in two different model systems"** *- Merle M Nicolai (University of Wuppertal, Germany)*
09:45-10:00	**O9 "Does air pollution have an impact on our DNA? Measuring DNA damage by the comet assay and BTEX exposure in human blood cells"** *- Katarina Matkovic (Institute for Medical Research and Occupational Health, Croatia)*
10:00-10:15	**O10 "Influence of antioxidants resveratrol and melatonin on telomere dynamics and DNA damage in liver and kidney of the Wistar rat model of aging"** *- Lucia Nanić (Ruđer Bošković Institute, Croatia)*
10:15-10:30	**Pitch presentations:** **P39 "Genotoxicity of European chub (*Squalius cephalus L. 1758*) erythrocytes as an effective indicator in monitoring of water bodies under different pollution pressure"** - Karolina Sunjog (University of Belgrade, Serbia) **P6 "Comparison of biological activity of thymol and newly synthesized derivative on colorectal cancer cells".** *- Michaela Blažíčková (Slovak Academy of Sciences, Slovakia)*

10:30-11:00	Coffee break

11:00-13:00 - BEMS/DEMS session: New approaches to assess genotoxicity of poorly soluble materials ***Chairs: B. Mertens ans S. Wang***	
11:00-11:10	Welcome by BEMS/DEMS and overall intro of session
11:10-11:40	**O11 "Arsenolipids: lessons learned from cells, worms, flies and mice."** *- Tanja Schwerdtle (University of Potsdam & German Federal Institute for Risk Assessment (BfR), Germany)*
11:40-12:10	**O12 "A tiered testing approach to predict genotoxicity and carcinogenicity of poorly soluble cobalt compounds"** *- Vanessa Viegas (Cobalt Institute, UK)*
12:10-12:40	**O13 "Adaptation of the in vitro Micronucleus Assay for nanomaterial testing: A status Update"** *- Naveed Honarvar (BASF SE, Germany)*
12:40-13:00	**Pitch presentations:** **P5 "Biosafety determination of innovative nanocomposites with potential application in regenerative medicine"** *- Lucia Bálintová (Biomedical Research Center of the Slovak Academy of Science, Slovakia)* **P9 "Graphene oxide: DNA damage induction in HEK 293T cells"** *- Tamara Cetkovic (University of Sarajevo, Bosnia and Herzegovina)* **P3 Analysis of in vitro genotoxicity of different nanoparticles in human blood lymphocytes by comet assay -** *Milda Babonaitė (Institute of Biosciences, Lithuania)*

13:00-14:00	Lunch

14:00-15:00	**EEMGS/ICAWG GA** *All members and interested researchers are welcome to attend*
14:00-15:30	Poster session 2

AM
PM

15:30-16:00	Coffee break

16:00-17:30 - Session 2: Nutrition & neutraceuticals ***Chairs: H. Stopper and M. Gerić***	
16:05-16:30	**O14 "Critical interplay between gut microbiome and mycotoxins with concequences for genotoxicity"** *- Doris Marko (Vienna, Austria)*
16:30-16:50	**O15 "DNA damage, oxidative and inflammatory status in vegetarians compared to omnivores"** *- Goran Gaijski (Institute for Medical Research and Occupational Health, Croatia)*
16:50-17:05	**O16 "Health effects of red meat products containing phytochemicals and reduced levels of nitrite: the PHYTOME project"** *- Simone van Breda (Maastricht University, The Netherlands)*
17:05-17:20	**O17 "The protective effects of phytochemicals against DNA damage"** *- Julia DeBenedictis (Maastricht University, The Netherlands)*
17:20-17:30	**Pitch presentations:** **P18 "Heat killed paraprobiotic *Lactiplantibacillus plantarum* S1 reduces DNA damage in induced oxidative stress"** **-** *Deni Kostelac (Zagreb University, Croatia)* **P25 "Genotoxicity assessment of some industrially processed meat products in the human Caco-2 cell line using the alkaline comet assay"** **-** *Khallef Messaouda (Université Guelma, Algeria)*

18:30-00:00	**Conference dinner & party** *Location: Grand Café Soiron, Vrijthof*

Wednesday

09:00-10:30 - Session 3: Recent progresses in the field of genotoxicity testing - Part 1
Chairs: R. Godschalk and S. Kolarević

09:05-09:30	**O18 "Hazard assessment of nanomaterials with the comet assay; what properties determine genotoxicity?"** *- Maria Dusinska (NILU, Norway)*
09:30-09:45	**O19 "Genotoxicity of pyrrolizidine alkaloids in HepG2 cells in vitro"** *- Helga Stopper (University of Wuerzburg, Germany)*
09:45-10:00	**O20 "Assessing testicular germ cell DNA damage in the comet assay; introduction of a proof-of-concept"** *- Yvette Dirven (Norwegian Institute of Public Health, Norway)*
10:00-10:15	**O21 "Use of advanced in vitro systems for risk assessment of xenobiotics"** *- Monika Sramkova (Slovak Academy of Sciences, Slovakia)*
10:15-10:30	**O22 "Statistical consideration for analysing negative and positive control in vivo alkaline comet assay data"** *- Timur Tug (TU Dortmund University, Germany)*

10:30-11:00	Coffee break

11:00-11:50	**Open discussion: Factors that influence comet data interpretation** *(Moderated by R. Godschalk and A. Collins)*

AM
PM

11:55-13:00 - **Session 3: Recent progresses in the field of genotoxicity testing - Part 2**
Chairs: A. Collins and E. E. Bankoglu

11:55-12:10	**Pitch presentations:** **P30 "DNA repair in ovarian cancer therapy response: The role of MRE11"** *- Alena Opatova (Czech Academy of Sciences & Charles University, Czech Republic)* **P34 "Comet Assay for human health purposes"** *- NI Ryabokon*
12:10-12:35	**O23 "Quantitative Interpretation of In Vivo Mutagenicity Dose Response Data for Regulatory Decision-making: Recent Progress and Persistent Challenges"** *- Paul White (Health Canada, Canada)*
12:35-13:00	**O24 "HESI GTTC Comet Project - An update"** *- Marie Vasquez (Helix3, USA)*

13:00-14:00 Lunch

14:00-18:30 Social programme *(start at the venue)*

Thursday

09:00-10:30 - Session 4: Applications of the comet assay in ecotoxicology *Chairs: B. Pourrut and T. Ćetković*	
09:05-09:30	**O25 "Application of comet assay in aquatic organisms – a summary and lessons learned in past 10 years of field research"** *- Stoimir Kolarević (University of Belgrade, Serbia)*
09:30-09:50	**O26 "Heavy metals: a grand tour"** *- Marko Gerić (Institute for Medical Research and Occupational Health, Croatia)*
09:50-10:05	**027 "Recent advances in the development of the comet assay on the hemocytes of zebra mussels and its application in field studies for evaluating environmental genotoxicity."** *- Marc Bonnard (Université de Reims Champagne-Ardenne, France)*
10:05-10:20	**O28 "Measuring DNA Strand Breaks in Plant Models"** *- John Einset (University of Oslo, Norway)*
10:20-10:30	**Pitch presentations:** **P23 "The comet assay in Pieris brassicae (Lepidoptera, Pieridae): a useful tool to evaluate genotoxic effects induced by particulate matter (PM10)"** *- Manuela Macrì (University of Torino, Italy)* **P12 "Could DNA damage in Ligustrum vulgare L. leaves also indicate soil pollution"** *- Mujo Hasanović (University of Sarajevo, Bosnia and Herzegovina)*

10:30-11:00	Coffee break

AM

PM

11:00-12:30 - Session 5:
Technical innovations of the comet assay
Chairs: A. Azqueta and D. Ozkan Vardar

11:05-11:30	**O29 "Spheroids – a 3D cell based system for genotoxicity assessment"** *- Bojana Zegura (National Institute of Biology, Slovenia)*
11:30-11:55	**O30 "Comet assay from a new perspective introducing the Flash-comet – from hairpins to low conductive medium"** *- Erik Bivehed (Uppsala University, Sweden)*
11:55-12:15	**O31 "HepaCometChip: A Cell-Based Platform to Replace Animal Studies of Liver Genotoxicity"** *- Bevin P. Engelward (MIT Dept. of Biological Engineering, USA)*
12:15-12:30	**O32 "Electrophoresis with vertically-oriented comet slides: advantages and disadvantages"** *- Aliy Zhanataev (Research Zakusov Institute of Pharmacology, Russia)*

12:30-13:00 Closing - award giving and next meetings

O1 - Four decades of genotoxicity testing: innovations on the horizon

Birgit Mertens

SD Chemical and Physical Health Risks, Sciensano, Brussels, Belgium

Since the introduction of the OECD Test Guidelines (TGs) for genotoxicity in the eighties, more knowledge on the underlying mechanisms for genotoxicity and the strengths and limitations of the different tests have been obtained. Moreover, significant economic changes have increased the number of substances that need to be tested, triggering the need for more efficient, faster and cheaper tests. At the same time, ethical and scientific concerns have strengthened the need to reduce or even replace the use of animals in toxicology testing. These new insights resulted in an update of the OECD genetic toxicology TGs which was completed in 2015. The update included the development of new and revised TGs as well as the deletion of some TGs that were considered obsolete. With respect to the *in vitro* genotoxicity tests, modifications were made to reduce the high number of misleading positive results, without affecting the sensitivity of the tests. In parallel, scientists developed new *in vitro* assays with improved predictive capacity such as the comet and micronucleus assay in 3D reconstructed skin models and tests that detect changes at the molecular level providing insights in our understanding on compound toxicity. For example, several methods evaluating changes in gene expression either at the level of a single gene, the whole transcriptome or a selection of genes (e.g. ToxTracker and transcriptomics-based biomarkers) have become available. Modifications to existing tests to collect additional mechanistic information (e.g. modified versions of the comet assay) or to extend their application (e.g. miniaturized versions of the Ames test requiring less test compound) have been made as well. Now it is time to reflect on how to deal with the new knowledge and assays: is it sufficient to just integrate them in the existing testing strategies or should we rethink the way we are currently evaluating genotoxicity?

O2 - Carcinogenic exposure biomarkers in human populations

Nancy B. Hopf

Center for Primary Care and Public Health (Unisanté), University of Lausanne, Switzerland.

Human populations are often exposed to a mixture of chemicals and consequently, the health risks from these exposures are multifactorial. Biomonitoring provides unequivocal evidence that both exposure and uptake of a chemical have occurred. It also integrates exposures from all routes (ex. inhalation, skin penetration and ingestion). Biomonitoring of populations at risk is necessary to reduce the probability of disease. In addition, biomonitoring can help in identifying unintentional and unexpected exposures and assess the effectiveness of existing risk- management measures, especially in occupational settings. Biomonitoring using effect biomarkers as opposed to exposure biomarkers are the only tool to address risks from exposures to unknown chemical mixtures as well as the integrated effect of the chemical mixture. Despite all these well-recognized advantages, using effect biomarkers such as comet assay and micronuclei in biomonitoring is lacking. Reasons could be that an explanation of the mode of action (MoA) is missing as well as the corresponding effect biomarkers. Work is ongoing to associate mechanistic knowledge to key characteristics of carcinogens. There are also difficulties in interpreting the effect biomarker values in terms of disease risk, and this is especially true for cancer. Work is ongoing to understand the distribution of effect biomarkers in the general population and in populations with cancer using systematic reviews. OECD in collaboration with stakeholders in many countries are working towards a guidance document on biomonitoring. Effect biomarkers that have a strong link to the Adverse Outcome Pathway (AOP) knowledge are preferred. A systematic understanding of both relevance and interpretation of effect biomarker data will lead to increased protection for the human population, and especially for workers who often have greater exposures.

O3 - Ten-year DNA damage evolution of French female farm workers and link with cancer development.

Evenden P[1,2], Lecluse Y[1,2,3], Lacauve AS[1,2,3], Niez E[1,2,3], Perrier S[1,2,3], Perdry H[4], Boutet-Robinet E[5], Tual S[1,2,3], Boulanger M[1,2,3], Delépée R[1,2,3], Lebailly P[1,2,3], Meryet-Figuière M[1,2,3].

[1] Institut National de la Santé et de la Recherche Médicale (INSERM), Unité Mixte de Recherche (UMR) 1086 ANTICIPE, Caen, France ;
[2] Université de Caen Normandie, Caen, France ;
[3] Centre de Lutte Contre le Cancer François Baclesse, Caen, France ;
[4] Univ Paris-Saclay & INSERM CESP, Villejuif, France
[5] Toxalim (Research Centre in Food Toxicology), Université de Toulouse, INRAE, ENVT, INP-Purpan, UPS, Toulouse, France.

Many studies have shown links between pesticide exposure and increased DNA damage. However, longitudinal studies exploring changes in DNA damage and associations between DNA damage measured by the comet assay and cancer development are scarce. The aim of this study was, for females included in a French agricultural cohort (cattle breeders and open field farming), to quantify the level of DNA damage at two time points and investigate the potential link between DNA damage evolution and cancer development.
Ninety-nine female participants gave blood samples in 1997 and in 2007. Using the comet assay, with a standardization control at each experiment, DNA damage levels were quantified using a 4-class visual scoring system on 2x100 nuclei per sample. Score difference between follow-up and enrolment was defined as positive if superior to the range of scores of the control, negative if inferior and stable if comprised within. Incident cancers were identified thanks to a population-based cancer registries.
At enrolment, mean age was of 44 years and 92% were never-smokers. Fifty-two percent of females had a « normal » BMI, 10% increased their BMI at follow-up. DNA damage score was significantly higher at follow-up than enrolment (p=0.0003). Ageing was not associated with score difference (p=0.64). A decrease in BMI class at follow-up tended to be associated with less damage increase (p=0.17). Females who milked cows had less damage increase (p=0.04), whereas pest control use on cattle shown an increase in damage (p=0.05). Ten females were diagnosed with cancer between enrollment and 2018. Increase in raw damage score difference tended to be associated with cancer development (HR=1.80, p=0.10).
The occupational exposure that females are exposed to could be reflected by the DNA damage levels. Furthermore, an increase in damage could potentially be a marker of cancer development.

O4 - The Comet Assay as a Human Biomonitoring Tool: effect of cryopreservation and blood cell type

Ezgi Eyluel Bankoglu[1], Carolin Schuele[1], Franzisca Stipp[1], Tilman Kuehn[2], Rudolf Kaaks[2] and Helga Stopper[1]

[1]Institute of Pharmacology and Toxicology, University of Wuerzburg, Wuerzburg, Germany
[2]Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany

The comet assay is a very sensitive method for detection of DNA damage. With the addition of Fpg, it allows an assessment of the oxidative DNA damage. The comet assay is applied in human biomonitoring, usually for analysing DNA damage using whole blood or peripheral blood mononuclear cells (PBMCs). Ideally, all samples should be analysed within a limited period. For larger studies or if samples become available at different times/ locations, this can only be achieved by storing samples, but there is much less experience regarding analysis of long-term cryopreserved samples compared to fresh samples in the comet assay.
We have now compared DNA damage in fresh and cryopreserved samples in whole blood, buffy coat and PBMCs with and without Fpg. While all sample types could be used fresh or cryopreserved, PBMCs yielded a slightly higher basal damage than the other two sample types. The use of Fpg detected increased damage in all samples, either fresh or cryopreserved. Next, we analysed a group of long-term cryopreserved samples from the Heidelberg-EPIC cohort consisting of PBMCs and buffy coat preparations from different times. We had buffy coat samples from baseline and at re-invitation approximately after 10 years (t_1) together with PBMCs from the same individuals at first re-invitation (t_1) and at second re-invitation (t_2) one year later. Our results showed a good correlation for PBMCs from the same donor between the t_1 and t_2, whereas buffy coat samples from the same donor between baseline and t_1 showed weak correlation. Furthermore, there was no correlation between the buffy coat preparations and PBMCs from same donor at t_1.
As conclusion, we were able to conduct Fpg comet assay with decade old cryopreserved samples and PBMCs are promising for use in human biomonitoring, whereas conducting comet assay with cryopreserved buffy coat preparations requires more assessment.

O5 - Occupational exposure to antineoplastic drugs - What are the gaps?

Susana Viegas[1,2]

[1)]NOVA National School of Public Health, Public Health Research Centre, Universidade Nova de Lisboa; [2)]Comprehensive Health Research Center (CHRC)

Antineoplastic drugs (ADs), also known as chemotherapy or cytotoxic drugs, include compounds with various mechanisms of action that are used to treat cancer, preventing or disrupting cell division of neoplastic cells. However, their action on malignant cells is only partially selective and normal ones may also be affected, leading to significant toxic side effects. This aspect makes ADs dangerous not only for patients, but also for exposed workers. Workplace exposure may occur in manufacturing, distribution, receipt, storage, transport, compounding, and administration, as well as during waste handling and care of treated patients. This happens not only in human heath but also in animal health services since the use for treating cancer in animals is growing.
Recent reports stated that the potential exposure to ADs cannot be completely eliminated and exposure is still happening even when several risk management measures are already in place. This situation and the current lack of harmonized standards for the prevention of exposure makes this issue an occupational health priority. What is missing to address this issue? How we can guarantee that ADs continue to be a suitable therapeutic option without damaging workers health? Do we need a more tailored risk assessment and risk management measures? Do we need better surveillance of workers exposure and health effects? Which methods should be prioritized to evaluate exposure and early health effects? Can biomonitoring have an important role in exposure and risk assessment? These questions will be answered during a brief presentation that intends to promote knowledge sharing and discussion.

O6 - The Role of Diet in Genotoxicity of Fecal Water Derived from IBD Patients and Healthy Controls

Shan Wang[1], Roger Godschalk[1], Corinne Spooren[2,3], Marlijne de Graaf[2,3], Daisy Jonkers[2,3], Frederik-Jan van Schooten[1]

[1] Department of Pharmacology and Toxicology, School of Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University, Maastricht, The Netherlands.
[2] Department of Internal Medicine, School of Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University, Maastricht, The Netherlands.
[3] Division of Gastroenterology-Hepatology, Maastricht University Medical Centre, Maastricht, The Netherlands.

Patients with inflammatory bowel disease (IBD) are at increased risk of developing colitis-associated cancer (CAC). Certain dietary factors with anti-inflammatory and/or anti-cancer properties would be a promising preventive strategy for IBD patients against CAC. The aim of this study was to examine the effect of diet on the genotoxicity of fecal water (FW) derived from IBD patients and healthy controls (HCs).
FW was obtained from propensity score matched 20 HCs and 80 IBD patients. The comet assay was applied to determine the DNA damage induced by FW, and the protective potential of FW against hydrogen peroxide (H_2O_2) induced DNA damage in human colonic epithelial cells (Caco-2). Information on diet was obtained via food frequency questionnaires. Principal component analysis was performed to identify dietary patterns. Multiple linear regression models were applied to explore the genotoxicity-related dietary factors.
FW from IBD patients, especially patients with flares, induced higher levels of direct DNA damage in Caco-2 cells and showed less protection against H_2O_2-induced DNA damage, when compared to HCs. The DNA damage induced by FW was positively associated with consumption of processed meat and sugary foods, and nutrient intakes including heme iron and added sugars, whereas negatively correlated to intakes of soy products, and a dietary pattern characterized by high consumption of potatoes, white meat, nuts and seeds, eggs, legumes and soy products. FW from subjects with high coffee consumption protected against H_2O_2-induced DNA damage.
FW from IBD patients increased the genotoxic potential of Caco-2 cells. Specific dietary factors including food groups, nutrients and dietary patterns were found to be associated with the genotoxicity of FW. These results can help to develop potential preventive strategies for IBD patients to reduce the CAC risk.

O7 - *In vitro* mutagenicity assessment of fried meat-based food and French fries from mass catering companies

Julen Sanz-Serrano[1] , Roncesvalles Garayoa[2] , Ana Isabel Vitas[3,4] , Adela López de Cerain[3,5] and Amaya Azqueta[3,5]

[1]Vrije Universiteit Brussel, Department of Pharmaceutical and Pharmacological Sciences, Entity of In Vitro Toxicology and Dermato-Cosmetology, Laarbeeklaan 103, 1090 Brussels, Belgium.
2 Universidad de Navarra, School of Pharmacy and Nutrition, Department of Nutrition, Food Science and Physiology, Irunlarrea 1, 31008, Pamplona, Spain.
[3]IdiSNA, Navarra Institute for Health Research, Irunlarrea 3, 31008, Pamplona, Spain.
[4]Universidad de Navarra, School of Medicine, Department of Microbiology and Parasitology, Irunlarrea 1, 31008, Pamplona, Spain.
[5]Universidad de Navarra, School of Pharmacy and Nutrition, Department of Pharmacology and Toxicology, Irunlarrea 1, 31008, Pamplona, Spain.

Two major events related to process contaminants in food have had a great impact in society in the last years. These are the classification of the IARC of red meat as probably carcinogenic to humans, and the detection of acrylamide, a known in vivo carcinogen, in food. On the one hand, the IARC statement led to renew the interest in providing information on the molecular mechanisms, such as mutagenicity, in order to sustain the epidemiological evidence between red meat consumption and cancer. On the other hand, the detection of acrylamide in food as a consequence of heating processes led to the extensive assessment of the genotoxicity of the compounds formed, but not of the food as a whole. The evidence gathered in this matter, although obtained from few and limited studies, presumes that French fries have none or weak in vitro mutagenic activity. The current article aimed to evaluate the in vitro mutagenicity of: a) ten extracts of fried meat-based food by the 6-well-miniaturized Ames test in Salmonella typhimurium TA98, and the micronucleus test (OECD TG 487), and b) ten extracts of French fries by the micronucleus test (OECD TG 487). Each sample was obtained from a different mass catering company from Navarra (Spain). None of the ten extracts of fried meat-based food induced gene mutations in S. typhimurium TA98 with or without metabolic activation, but five induced chromosomal aberrations after 24h treatment. Eight out of ten samples from mass catering companies induced chromosomal aberrations. Moreover, French fries deep-fried in the laboratory at different times (0, 3, 5, 10, 20, 30 min) showed an increase in the level of micronuclei in all the frying times from 3 min on. This study shows the need for more studies evaluating the mutagenicity of French fries and fried meat-based food, in order to further explore the biological relevance of these outcomes

O8 - Mechanistic investigations of Mn-induced oxidative stress, DNA damage, DNA repair, and neurodegeneration in two different model systems

Merle Marie Nicolai[1], Barbara Witt[2], Sharleen Friese[2,3], Nicola Winkelbeiner[2,3], Ann-Kathrin Weishaupt[1,3], Franziska Ebert[2], Andrea Hartwig[4], Tanja Schwerdtle[2,3,5], and Julia Bornhorst[1,3]

[1]Food Chemistry, Faculty of Mathematics and Natural Sciences, University of Wuppertal, Wuppertal, Germany
[2]Department of Food Chemistry, Institute of Nutritional Science, University of Potsdam, Nuthetal, Germany
[3]TraceAge – DFG Research Unit on Interactions of Essential Trace Elements in Healthy and Diseased Elderly (FOR 2558), Berlin-Potsdam-Jena-Wuppertal, Germany
[4]Karlsruhe Institute of Technology (KIT), Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe, Germany
[5]German Federal Institute for Risk Assessment (BfR), Berlin, Germany

Manganese (Mn) is an essential trace element, but (chronic) overexposure has been associated with various adverse neurological effects. While oxidative stress has been identified as one of the main pathways in Mn toxicity, the specific underlying mechanisms of Mn-induced neurodegeneration are not yet fully understood.
Using the multicellular model organism *Caenorhabditis elegans* (*C. elegans*) and dopaminergic-like differentiated Lund human mesencephalic (LUHMES) neuronal cells, we investigated the link between oxidative stress and neurotoxicity after Mn overexposure, focussing hereby on oxidative DNA damage, DNA damage response, and DNA repair.
The nematode has gained increasing recognition in various research areas, but methods for specifically investigating genotoxicity are still scarce. By developing and utilizing novel methods for assessing oxidative stress (cardiolipin oxidation) and genomic integrity (alkaline unwinding) in *C. elegans*, we were able to utilize this model organism for genotoxicity assessment in the niche between *in vitro* and *in vivo* studies. In contrast to *C. elegans*, using LUHMES cells allowed *in vitro* assessment of the genomic integrity in post-mitotic dopaminergic-like neuronal cells, directing the research specifically on Mn-induced neurodegeneration.
Studies of the Mn bioavailability show a time- and concentration-dependent Mn uptake in both model systems that inversely correlates with the survival rate/ cytotoxicity. Quantification of DNA strand breaks (alkaline unwinding) and formation of oxidative DNA damage (8oxodG) indicate a decrease of the genomic integrity at respective subtoxic and toxic Mn concentrations. While investigations of PARylation imply an initiation of the DNA damage response after Mn exposure, results regarding DNA repair (mainly base excision repair) are still inconclusive. Lastly, βIII-tubulin staining in LUHMES cells indicates a significant adverse effect of Mn overexposure on the neurite network.
Altogether, these studies help to elucidate the role of Mn-induced oxidative stress and genomic integrity in Mn toxicity; globally in a multicellular organism and in post-mitotic dopaminergic-like neurons.

O9 - Does air pollution have an impact on our DNA? Measuring DNA damage by the comet assay and BTEX exposure in human blood cells

Katarina Matković[1], Andreja Jurič[1], Ivana Jakovljević[1], Silvije Davila[1], Mirta Milić[1], Vilena Kašuba[1], Gordana Pehnec[1], Irena Brčić Karačonji[1], Goran Gajski[1], Marko Gerić[1]

[1]*Institute for Medical Research and Occupational Health, Zagreb, Croatia*

Outdoor air pollution in urban and rural areas causes millions of premature deaths worldwide. Cities are more exposed to higher concentrations of pollutants because of the urban agglomeration, traffic, and industrial activities. Additionally, the emission of BTEX (benzene, toluene, ethylbenzene, and xylene) compounds becomes higher, mainly due to intensive industrialization and urbanization. Hence, we aimed to investigate possible effects of air pollution and BTEX exposure on DNA damage detected by the alkaline comet assay in human peripheral blood cells. The study was conducted during the colder period of the year 2021 and involved 60 healthy subjects (34 females and 26 males), aged 36.4±9.6 years and BMI<30 kg/m2 living in Zagreb (Croatia). To evaluate potential impact of air pollution on comet assay results, association was made using different time windows of measured air pollution parameters prior to blood sampling while BTEX were analysed in whole blood using headspace solid-phase micro extraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS). All measured outdoor air pollution parameters were bellow regulatory limit except for benzo[a]pyrene bound to PM10 particle fraction (particles with aerodynamic diameter less than 10 μm), that exceeded regulatory annual limit level. Toluene with average blood concentration 146.3 ng/L and m-/p-xylene, with average concentration 171.7 ng/L, were the most dominant among the BTEX compounds. Average tail intensity was 1.2±0.5%, which is in accordance with our previous data for healthy general population in Croatia. There was no statistically significant Spearman's correlation between the measured biomarkers of exposure and DNA damage. Linear regression modelling revealed that PM10 (particles with aerodynamic diameter less than 10 μm) was the most significant feature and has a significant positive linear relation with the tail intensity. Future research will include the analysis of the same study cohort in the warmer period of the year, as well as two other Croatian cities, where we expect exposure to different set of air pollutants.

Supported by the Croatian Science Foundation #1192 (HUMNap)

O10 - **Influence of antioxidants resveratrol and melatonin on telomere dynamics and DNA damage in liver and kidney of the Wistar rat model of aging**

Lucia Nanić[1], Marko Gerić[2], Goran Gajski[2], Jasna Jurasović[,2], Dean Karaica[,2], Marija Ljubojević[,2], Vedran Micek[,2], Ivana Novak Jovanović[,2], Tatjana Orct[,2], Maja Peraica[,2], Dubravka Rašić[,2], Ivana Vrhovac Madunić[,2], Ivan Sabolić[,2], Davorka Breljak[,2], Ivica Rubelj[1]

[1]Ruđer Bošković Institute, Zagreb, Croatia
[2]Institute for Medical Research and Occupational Health, Zagreb, Croatia

Aging is the major risk factor for the emergence of numerous pathological conditions leading to the onset of age-related diseases. Large body of evidence suggests that telomere shortening and DNA damage in cells may lead to the progression of senescence and aging. Oxidative stress also plays an important role in telomere dynamics. Various strategies for the neutralization of oxidants are used. Most widespread among them involves the use of antioxidant supplements, which are commonly considered healthy. Today, frequently used antioxidants are resveratrol and melatonin, both for experimental purposes and in various diets. To test whether oxidative stress could be ameliorated by antioxidants, we treated male and female Wistar rats for 9 or 21 months with melatonin and resveratrol (daily dose 300−400 μg/kg b.m.). We investigated their effect on telomere attrition rate in the liver and different zones of the kidney. Telomere length was assessed by Southern blotting of genomic DNA. We also investigated the baseline DNA damage using the comet assay in kidneys and liver of treated animals. Significant reduction in telomere loss in the liver of female Wistar rats after 21-month long resveratrol treatment has been observed, while other tissues did not show significant differences. Contrary to expected beneficial properties of tested antioxidants, in our experimental system following long-term treatment with melatonin and resveratrol in both Wistar male and female rats, these effects have not been observed. These findings call for more cautious use of these antioxidants as anti-aging supplements in the human population.

Supported by the Croatian Science Foundation projects IP-2018-01-8654 (REPROSKIN) and IP-11-2013-1481 (AGEMETAR).

O11 Arsenolipids: lessons learned from cells, worms, flies and mice

Tanja Schwerdtle [1, 2]

[1]*German Federal Institute for Risk Assessment (BfR), Berlin, Germany*
[2]*University of Potsdam, Institute of Nutritional Science, Nuthetal, Germany*

Organic lipid-soluble arsenic species, so called arsenolipids, belong to the emerging contaminants in fish and seafood. Among others, arsenic-containing fatty acids (AsFAs), arsenic containing, hydrocarbons (AsHCs), arsenic-containing triacylglycerides (AsTAGs) and arsenic-containing phosphatidylcholines (AsPCs) represent four major arsenolipid classes, the latter three being poorly soluble in aqueous solutions. The toxicity of arsenolipids has yet to be systematically studied, whereas the mode of genotoxic and carcinogenic action of inorganic arsenic is well characterized. Overall, the studied endpoints so far show that the four arsenolipid classes exert a different mode of toxic action as compared to inorganic arsenic. In cultured human cells AsHCs exterted highest cellular toxicity, but no genotoxicity. The toxicity of AsPCs might be largely governed by their arsenic fatty acid content. A reliable toxicological characterisation of AsTAGs was not possible *in vitro*. Taken also into account the investigations *in C. elegans*, *Drosophila melanogaster* and mice, AsHCs were the most potent toxic compounds. AsHCs massively disturbed the neuronal network *in vitro*, and accumulated in the brain of *Drosophila melanogaster* and *mice*.
Overall, these studies indicate the need for a full hazard identification of the subclasses of arsenolipids to assess whether they pose a risk to human health.

O12 - A tiered testing approach to predict genotoxicity and carcinogenicity of poorly soluble cobalt compounds

V. Viegas and R. Danzeisen

Cobalt Institute, Guildford, United Kingdom

Cobalt and cobalt substances are utilised in critical societal functions, however these substances have inherent hazard profiles that require robust risk assessment. There are over 20 cobalt substances that differ in physico-chemical properties, in which the majority of the available data address the highly soluble, bioavailable and reactive cobalt substances. To determine the genotoxic and carcinogenic properties of the less bioavailable, poorly soluble substances via inhalation exposure, a read-across approach was developed to reduce the number of tests conducted and adhere to the 3Rs in animal testing. The read-across was based on the toxic moiety of interest (the Co^{2+} ion) and addressed the hazard of chronic inhalation toxicity by utilising the cobalt adverse outcome pathway (AOP) to define several tiers of testing. The lower tiers of the approach consisted of release of Co^{2+} in artificial lung fluids and evaluation of specific in vitro biomarkers. The approach is refined by considering an in vivo short-term inflammatory response and/or upper respiratory tract effect using histopathological markers in an acute test. The higher-level tiers consist of repeated-dose inhalation toxicity tests. The cobalt substances were grouped using a recognisable pattern in the tiered information generated. Two read-across groups are proposed for chronic inhalation toxicity: (1) highly bioavailable and reactive Co substances which released a significant amount of Co^{2+} in neutral and acidic media, activated oxidative stress and hypoxia biomarkers, induced a 'persistent' inflammatory response and longer-term lung effects; whereas (2) poorly soluble, poorly bioavailable cobalt substances had little release of Co^{2+} in the relevant fluids, lacked upregulation of specific biomarkers, did not induce 'persistent' inflammation but did contribute to infiltration of polymorphonuclear neutrophils and lung burden. In conclusion, by applying the read-across and grouping approach, an informed prediction can be made regarding cobalt-specific inhalation hazards, leading to the application of appropriate risk assessment.

O13 - Status Update: Adaptation of the *in vitro* Micronucleus Assay Nanomaterial Testing

Honarvar, N.[1], Burgum, M.[2], Ulrich, C[1], Partosa, [1]; Gomes, C[1]; Berit-Seiffert, S[3]; Landsiedel,R[1], Doak, S.[2]

[1]BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany
[2]University of Swansea, Experimental Toxicology and Ecology, Ludwigshafen, Germany
[3]BASF SE, Material Physics, Analytics & Formulation, Ludwigshafen, Germany

Current OECD guidelines for *in vitro* genotoxicity testing may not be directly applicable for the assessment of the mutagenic potential of nanoparticles. In a joint effort organized by the EU Joint Research Centre (JRC) a protocol was developed for the testing of nanoparticles using the *in vitro* micronucleus assay. The adapted protocol was applied using the (adherent) cell lines V79 and HepG2 as well as cells grown in suspension, namely primary human lymphocytes and TK6 cells. Further characteristics of the protocol modifications were treatment period corresponding to 1 cell cycle followed by a 24 h Cytochalasin B treatment, formulation of the nanoparticles according to the "Nanogentox" protocol, testing only in the absence of S9 mix as well as limiting the top concentration to 100 µg/mL.
The mutagenic potential of five nanoparticles ($BaSO_4$, CeO_2, Au_{5nm}, Au_{30nm} and SiO_2) was analysed using this protocol. Concurrently, Tungstencarbide Cobalt (WCa-Co) nanoparticles were used as a potential particulate positive control and Ethylmethane sulphonate (for V79 cells), Mitomycin C (for TK6, HepG2 and human lymphocytes) and Colcemide (for human lymphocytes and V79 cells) as chemical positive controls. The cellular dose of nanoparticles was quantified by the Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) method after the treatment period.
The tested nanoparticles induced different (partially contradictory) levels of micronuclei depending on the cell type used. The putative particulate positive control WCa-Co, however, increased the micronucleus frequencies in all used cell types except V79 cells. LA-ICP-MS data using V79 and lymphocytes showed concentration related increases of particle mass associated with the target cells.
In conclusion the data show that nanomaterials can be tested for their genotoxic potential in vitro using the indicated modifications of the OECD 487 protocol. In general, the cells grown in suspension were better adapted for nanomaterial testing as the adherent cells used. The particle burden of the cells can be quantified by LA-ICP-MS and WCa-Co can be used as a particulate positive control for these nanomaterial genotoxicity tests.

O14 Critical interplay between gut microbiome and mycotoxins with consequences for genotoxicity

Doris Marko

Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Vienna, Austria

Actual estimations speculate that up to 90% of the global food crop are contaminated with mycotoxins, at least at trace levels. In addition to the few regulated and monitored mycotoxins nowadays numerous additional secondary metabolites can be detected. Species belonging to the genus *Alternaria* produce a spectrum of secondary metabolites, some of which are speculated to pose a threat for human health. These "emerging" mycotoxins might occur in a multitude of different plant-based food commodities, including apples, tomatoes, wheat or sunflower seeds. *Alternaria* mycotoxins are rarely found as single contaminants but more often in complex mixtures with alternariol (AOH), its monomethyl ether (AME), tenuazonic acid and tentoxin (TEN) representing the most studied so far. Several *Alternaria* toxins have been identified as genotoxicants. Despite the apparent frequent occurrence, little is known so far on the fate of *Alternaria* mycotoxins in the gastrointestinal tract. In this context, the possible role of the gut microbiota in modulating the toxic effects of this class of mycotoxins and, vice versa, the ability of the latter to target the gut microbiota were recently investigated.

Short-term anaerobic fecal incubations substantially decreased the *in vitro* DNA-damaging effects of a complex extract of *Alternaria* mycotoxins (containing eleven chemically characterized mycotoxins). Thereby, not only fermentive but also absorptive effects appear to contribute. In addition, the presence of *Alternaria* toxins were found to affect human gut bacterial strains belonging to five of the most dominant human gut microbial phyla, in a differential way, ranging from inhibition of proliferation to growth promotion.

Taken together, these findings highlight the potential role of mycotoxins in impacting the gut microbial community, as well as the importance of the latter in modifying genotoxicity and the systemic bioavailability of this class of food contaminants.

O15 - DNA damage, oxidative and inflammatory status in vegetarians compared to omnivores

Goran Gajski[1], Marko Gerić[1], Marijana Vučić Lovrenčić[2], Sandra Božićević[2], Ivica Rubelj[3], Lucia Nanić[3], Nikolina Škrobot Vidaček[3], Laila Bendix[4], Maja Peraica[1], Dubravka Rašić[1], Ana-Marija Domijan[5], Valentina Gluščić[1], Jasna Jurasović[1], Tatjana Orct[1], Selma Cvijetić Avdagić[1], Gordana Jurak[6], Jasna Bošnir[6], Vera Garaj-Vrhovac[1]

[1]Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2]Merkur University Hospital, Zagreb, Croatia
[3]Ruđer Bošković Institute, Zagreb, Croatia
[4]Danish Aging Research Center, University of Southern Denmark, Odense, Denmark
[5]Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia
[6]Andrija Štampar Teaching Institute of Public Health, Zagreb, Croatia

The health benefits of the vegetarian diet are still under debate because there is evidence that it may result in a higher intake of some micronutrients, while others may be reduced, thus influencing various metabolic pathways and health-related biomarkers. Hence, we aimed to investigate the differences in those biomarkers comparing vegetarians and omnivores. Our study was performed in a group of healthy adult vegetarians and matched omnivores practicing a traditional mixed diet. Two groups were analysed for different DNA damage, oxidative and inflammatory biomarkers. Apart from those, we analysed their haematological and biochemical status, telomere length, bone mineral density, nutrient and toxic elements as well as pesticides and mycotoxins. Results revealed differences in various biomarkers that were in favour of a traditional mixed diet, rich in fruit and vegetables in this particular case, indicating that vegetarians have a lower nutritional status of some nutrients (Ca, Cu, Zn, vitamins B_{12} and D) accompanied with a lower antioxidant defense system (glutathione) and higher homocysteine and genome damage (micronuclei frequency and DNA strand breaks), along with shorter telomeres. This suggests that the supplementation of animal derived nutrients to this particular dietary group would be beneficial for the improvement of some measured health-related biomarkers. In case of inflammation, when comparing high-sensitivity C-reactive protein (hs-CRP) values of vegetarians with those of omnivores there was no significant difference between these two groups. However, the level of certain toxic metals (As and Hg) was higher in omnivores. This multi-biomarker approach offers complex insight into the differences of selected biomarkers related to specific dietary preferences and health outcomes, which could directly benefit clinicians and nutritionists in patient counselling about nutrition and dietetics. Besides, further research in well-defined and sufficiently sized cohorts is needed to provide more evidence.

O16 - Health effects of red meat products containing phytochemicals and reduced levels of nitrite: the PHYTOME project

Simone G. Van Breda* and Theo M. de Kok on behalf of the PHYTOME consortium

[1]*Department of Toxicogenomics, GROW School for Oncology and Reproduction, Maastricht University Medical Center, Maastricht, The Netherlands*

Endogenously formed N-nitroso compounds (NOCs) are partly responsible for the link between red meat consumption and colorectal cancer (CRC) risk. As nitrite has been indicated as one of the critical factors in the formation of endogenous NOCs, it is of high importance to reduce the nitrite levels in meat. Therefore, the PHYTOME project was initiated (www.phytome.eu EU-FP7 grant no. 315683), aiming to develop innovative meat products in which the food additive nitrite has been replaced by natural compounds originating from fruits and vegetables.A human dietary intervention study was conducted in which healthy subjects (n = 63) consumed 300 grams of meat for two weeks, in subsequent order: normal processed red meat, white meat, and red processed meat with normal or reduced levels of nitrite and added phytochemicals. Before and after each intervention period, colorectal biopsies, urine and faeces were collected. Consumption of standard-nitrite PHYTOME meat products leads to a significant reduction in Apparent Total N-nitroso Compounds (ATNC) levels in faecal water, a surrogate marker of endogenously formed NOCs. A reduction of nitrite in the PHYTOME meat lowered these levels even further. DNA strand breaks induced in *ex-vivo* faecal water exposed Caco-2 cells and O^6-methyl-guanine adducts levels in colonic DNA were significantly higher after consumption of normal processed red meat as compared to white meat. PHYTOME meat intake resulted in reduced levels of these genotoxic markers; however, these were not statistically significant. Whole genome gene expression analyses identified differentially expressed genes associated with ATNC, which are related to molecular pathways which may explain cancer risk initiation after intake of processed red meat and cancer risk prevention after intake of the PHYTOME meat. Together these results indicate that addition of natural extracts to conventional processed red meat products may contribute to a reduced risk of CRC.

O17 - The protective effects of phytochemicals against DNA damage (The MiBlend Study)

Julia N. DeBenedictis, Simone G.J. van Breda, Theo M.C.M. de Kok

Department of Toxicogenomics, GROW school of Oncology and Reproduction, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands.

Sufficient consumption of fruit and vegetables (F&V) reduces the risk of developing chronic diseases. The protective impact of F&V is largely attributed to their phytochemical content. Phytochemicals are bioactive compounds which can neutralize free radicals and may target and regulate expression of genes involved in antioxidant mechanisms. Although mostly studied in isolation, recent research suggests that phytochemicals consumed in their whole-food form and in complex combinations can affect their physiological impact. The MiBlend Study is a human dietary intervention developed to test the impact of increasing F&V intake and phytochemical complexity on genotypic and phenotypic parameters, including protection from *ex-vivo*-induced DNA damage measured by comet assay. After two-weeks of a diet low in F&V and a baseline test day (TD), participants were randomly assigned to two of the 9 unique F&V blends to consume for 2 weeks separated by a 1-week wash-out period with each intervention phase ending in a TD when blood samples were collected and alkaline comet assay performed. Lymphocytes were isolated and exposed to 25uM H_2O_2 for 1 hour at 37C. Exposed and unexposed samples were mounted to slides in triplicate then stored at 4C in lysis buffer. Slides were unwound for 40 min then electrophoresed at 1V/cm for 20 min. Slides were stained by ethidium bromide and imaged by BIO-TEK Cytation 3. Comets were scored with Comet IV software. Results were analyzed via linear mixed model and corrected for age, BMI, and sex. Data from 25 of 145 participants so far have been scored and analyzed. % Tail DNA significantly decreased by 6.9% at TD2 and especially after intervention #2 (9.0% decrease), a blend high in flavonoids (anthocyanins, catechins, and quercetin). Increased daily consumption of F&V, especially those with high antioxidant properties, could protect DNA against oxidative stress. Transcriptome analysis will be performed to underpin mechanisms.

O18 Hazard assessment of nanomaterials with the comet assay; what properties determine genotoxicity?

Maria Dusinska[a], Naouale El Yamani[a], Espen Mariussen[a,b] , Maciej Gromelski[c], Ewelina Wyrzykowska[c], Dawid Grabarek[c], Tomasz Puzyn[c,], Elise Rundén-Pran[a]

[a]Health Effects Laboratory, Department of Environmental Chemistry, NILU- Norwegian Institute for Air Research, 2007 Kjeller, Norway
[b] Norwegian Institute of Public Health, Department of Environmental Health, section of_air quality and noise, PO box 222 Skøyen, Norway.
[c]University of Gdańsk, Wita Stwosza 63, 80-308 Gdańsk, Poland

Hazard assessment of the huge variety of engineered nanomaterials (NMs), calls for robust *in vitro* toxicity screening tests specifically validated for NMs, as standard protocols for toxicity testing of chemicals might not be fully compatible with the special characteristics and translocation properties of nanosized particles. The most used method to assess genotoxicity of NMs *in vitro* is the comet assay, which detects transient DNA breaks and altered bases. While there is no OECD test guideline for the *in vitro* comet assay, there have been several efforts to validate the assay for NMs. Within the H2020 RiskGONE project, a series of interlaboratory studies has been performed, and an application for formal validation by the OECD is planned.
The toxicity of NMs is closely connected with their physico-chemical properties, such as size, shape, surface coating and charge. Thus*, in vitro* toxicity screening combined with *in silico* models to identify physico-chemical descriptors responsible for genotoxicity can allow grouping of NMs with similar properties to predict human hazards from NMs.
In this work cytotoxicity and genotoxicity of seventeen NMs from the JRC repository, derived from titanium dioxide, zinc oxide, silver and silica, were tested in human lung alveolar epithelial cells A549. Cytotoxicity was assessed with the Alamar Blue (AB) and colony forming efficiency (CFE) assays, and genotoxicity by the enzyme-linked version of the comet assay. Nanoparticle tracking analysis (NTA) was applied to measure the size of the NMs in stock dispersion and in medium at different time points. Categorization and ranking of cytotoxic and genotoxic potential were performed. Descriptors for prediction of NM toxicity were identified by quantitative structure-activity relationship (QSAR) analysis.
Our results showed that ZnO NMs (NM-110 and NM-111), and Ag NMs (NM-300K and NM-302) were cytotoxic, while the TiO_2 and SiO_2 NMs were non-cytotoxic. Regarding genotoxicity, TiO_2 (NM-100), ZnO (NM-110), SiO_2 (NM-203) and Ag (NM-300K) were categorized as positive.
Cheminformatics modelling identified electron properties and overall chemical reactivity as important for cytotoxic potential; HOMO-LUMO energy parameter, ionization potential and pristine size for the NMs´ genotoxic potential; and presence of surface coating for induction of oxidized base lesions. Thus we have demonstrated the value of the comet assay for genotoxicity testing of NMs – and, in combination with *in silico* approaches, providing data for grouping of NMs and hazard prediction.

Supported by the EC FP7 NANoREG [grant agreement NMP4-LA-2013-310584, 2013]; the Research Council of Norway, project NorNANoREG [239199/O70, 2015]; the EC Horizon 2020 projects NanoREG2 [646221, 2015]; RiskGONE [814425, 2019]; and NanosolveIT [814572, 2019].

O19 - Genotoxicity of pyrrolizidine alkaloids in HepG2 cells *in vitro*

Naji Said Aboud Hadi [1, 2], Ezgi Eyluel Bankoglu [1] and Helga Stopper [1]

[1] *Institute of Pharmacology and Toxicology, University of Wuerzburg, Wuerzburg, Germany.*
[2] *School of Health and Human Sciences, Pwani University, Kilifi, Kenya.*

Hundreds of different pyrrolizidine alkaloids (PAs) have been discovered in thousands of plants as natural constituents. Certain PAs are hepatotoxic and genotoxic and are potentially harmful to humans via food, food supplements and herbs/spices. PAs can cause hepatic sinusoidal obstruction syndrome and are carcinogenic in animals.
Here we investigated the genotoxicity of PAs of different esterification types, such as europine, retrorsine and lasiocarpine, in HepG2 cells using the cytokinesis-block micronucleus (CBMN) assay and the comet assay. DNA-crosslinking activity was investigated using a modified comet assay. Chemical inhibitors were used to investigate the role of metabolic activation and of in- and outward trans-membrane transport.
An increase in micronucleus formation was found with all tested PAs of different chemical structures in the micromolar range. In the comet assay, PAs did not cause increased DNA damage after 24 hours treatment, but in the modified crosslink comet assay, the diester type PAs reduced tail formation after hydrogen peroxide treatment, while an equimolar concentration of the monoester europine did not significantly reduce DNA migration. In addition, micronucleus induction by lasiocarpine was abolished after pre-treatment with the cytochrome P450 inhibitor ketoconazole and reduced by the addition of inward transporter inhibitors, while addition of outward transporter inhibitors slightly increased the genotoxic damage.
In conclusion, PAs are genotoxic and the extent and quality of DNA-damage is influenced by their ester-type. Metabolic activation plays a crucial role in PAs genotoxicity, while trans-membrane transporters only affect it to a limited extend.

O20 - Assessing testicular germ cell DNA damage in the comet assay; introduction of a proof-of-concept

Yvette Dirven[1,2], Dag Markus Eide[1,2], Erika Witasp Henriksson[3,4,5], Rune Hjorth[6], Anoop Sharma[7], Anne Graupner[1,2], Gunnar Brunborg [1,2], Jarle Ballangby[1,2], Anne Mette Zenner Boisen[7], Stellan Swedmark[4,5], Kristine Bjerve Gützkow[1,2], Ann-Karin Olsen[1,2,*]

[1]Norwegian Institute of Public Health, Division of Environment and Health, Oslo, Norway
[2]Centre for Environmental Radioactivity (CERAD, Centre of Excellence of the Norwegian Research Council), Oslo, Norway.
[3]Swedish Chemicals Agency, Department of Development of Legislation and Other Instruments, Unit of Proposals for Classification and Restriction, Sundbyberg, Sweden.
[4]Swedish Chemicals Agency, Department of Development of Legislation and Other Instruments, Unit of Evaluation of Substances, Sundbyberg, Sweden.
[6]The Danish Environmental Protection Agency, Odense, Denmark.
[7]Technical University of Denmark, National Food Institute, Lyngby, Denmark.

The *in vivo* comet assay is a widely used genotoxicity assay. An adapted approach for specific assessment of testicular germ cell within the comet assay is highly warranted, especially for regulatory purposes. Currently, OECD TG 489 it is not considered appropriate to obtain testicular germ cell specific data, since testicular cell suspensions consist of a mixture of somatic and germ cells. Here we provide a proof-of-concept to selectively analyse primary spermatocytes and round spermatids, distinguishing them from other cells of the testicle. An adaption of the comet assay to include analysis of testicular germ cells adds a versatile, sensitive, rapid and resource effective assay to the currently limited toolbox for regulatory germ cell genotoxicity assessment. We utilize the comet assay's ability to record both DNA damage (% tail intensity) and DNA content (total fluorescence intensity) of individual comets and propose a framework for distinguishing testicular cell populations based on their different DNA content/ploidy. The different cell populations are identified through 1) visual discrimination of DNA content distributions, 2) setting DNA content thresholds, and 3) fitting a normal three mixture distribution function. We also describe an approach to distinguish primary spermatocytes during comet scoring due to their large physical size and high DNA content. Both somatic and testicular germ cell comets can be obtained from the same animal. This provides valuable information regarding the distribution of a chemical and its ability to induce pre-mutagenic DNA damage in various tissues. Considering the increasing global production of and exposure to potentially hazardous chemicals, new and easily implementable methods to provide germ cell genotoxicity data are urgently needed. We propose to adapt the current OECD TG 489 *in vivo* comet assay to include our proposed approach to provide specific testicular germ cell comet data, thereby contributing to the assessment of male germ cell mutagenicity.

[5]This paper represents the opinions of the author and is the product of professional research. It is not meant to represent the position or opinions of the Swedish Chemicals Agency.

O21 - Use of advanced *in vitro* systems for risk assessment of xenobiotics

Eva Celkova, Lucia Balintova, Monika Mesarosova, Monika Sramkova

Cancer Research Institute, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

The effort to reduce the use of animal models in preclinical and toxicity tests is closely linked to the development of innovative test systems that will allow better prediction of the *in vivo* response.
In vivo, the liver is the major organ in which xenobiotics are metabolized and transformed, and the kidneys are subsequently responsible for their uptake, concentration, and elimination from the body. Thus, both organs play a key role in the detoxification and elimination of xenobiotics and their metabolites and represent the two primary targets of the toxic effect of xenobiotics.
The studied xenobiotics were chosen based on their effect: aflatoxin B1 (AFB1) - a very potent genotoxic hepatocarcinogen, ifosfamide (IFO) - a synthetic analog of cyclophosphamide that has a nephrotoxic effect. The cytotoxic and genotoxic effects of AFB1 and IFO were studied on stable human cell lines HepG2 (liver) and TH-1 (kidney), in various *in vitro* conditions (monolayer, 3D spheroids, co-culture).
Our data showed that IFO had a stronger cytotoxic effect after 2 h in TH-1 cells than HepG2 cells (IC50 35 mM vs. 75 mM in HepG2). After 24 h, IC50 was reached at 25 mM for both cell lines. AFB1 did not show cytotoxicity after 2 h using selected concentrations (0.5-100 μM), after 24 h HepG2 cells showed higher sensitivity (IC50 60 μM vs. 100 μM in TH-1 cells). Differences in IC50 were observed in different culture conditions (spheroids, co-culture). Both chemicals were able to significantly increase the level of DNA breaks as detected by comet assay and the percentage of micronuclei in both cell lines cultivated in monolayers.
As expected, our results showed different cell responses upon AFB1 and IFO treatment, confirming the differences between cell lines along with the culture conditions.

This study is based upon work from COST Action CA 17140 "Cancer Nanomedicine from the Bench to the Bedside" supported by COST, VEGA grant 2/0121/21, and project VISION (Strategies to strengthen scientific excellence and innovation capacity for early diagnosis of gastrointestinal cancers) No. 857381.

O22 - Statistical consideration for analysing negative and positive control *in vivo* alkaline comet assay data

Timur Tug[1]*, Julia Duda[1]*, Frank Bringezu[2], Steffi Chang[3], Martina Dammann[4], Roland Frötschl[5], Volker Harm[6], Katja Ickstadt[1], Bernd-Wolfgang Igl[7], Marco Jarzombek[8], Rupert Kellner[9], Fabian Kriegel[8], Jasmin Lott[7], Stefan Pfuhler[10], Ulla Plappert-Helbig[11], Jörg Rahnenführer[1], Markus Schulz[3], Lea Vaas[6], Marie Vasquez[12], Verena Ziegler[13] and Christina Ziemann[2]

[1]Department of Statistics, TU Dortmund University, Dortmund, Germany, [2]Merck KGaA, Biopharma and Non-Clinical Safety, Darmstadt, Germany; [3]ICCR-Roßdorf GmbH, Rossdorf, Germany, [4]BASF SE, Ludwigshafen am Rhein, Germany; [5]Federal Institute for Drugs and Medical Devices (BfArM), Bonn, Germany; [7]Bayer AG, Berlin, Germany; [7]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany; [8]NUVISAN ICB GmbH, Preclinical Compound Profiling,Germany; [9]Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany; [10]Procter & Gamble, Cincinnati, Ohio, USA, [11]Lörrach, Germany, [12]Helix3 Inc, Morrisville, NC, USA; [13]Bayer AG, Wuppertal, Germany.
**: presenting authors: tug@statistik.tu-dortmund.de, duda@statistik.tu-dortmund.de*

DNA damage and repair can be determined *in vivo* on single cell level using the alkaline comet assay (OECD 489) or single cell gel electrophoresis. The assay is part of several regulatory frameworks and is widely used, because it is a fast and sensitive method to detect DNA-strand breaks. In 2015 nonclinical statisticians and genotoxicologists from academia, industry, and one regulatory body established the interdisciplinary working group "Statistics" within the GUM. The goal of this interdisciplinary research project is to deepen statistical analysis of comet assay data, using single cell data from more than 200 experiments (data from liver, duodenum, stomach, blood and lung) from different companies.
In the present project, we aim at statistically analysing the test itself as well as the associated data processing techniques using descriptive statistics and hierarchical mixed effect models. We give a general overview of the data pool and the effects of different summarizing methods on data transformation from the cell to slide or slide to animal level, which may significantly affect the outcome of the test. In addition, we have investigated distribution, zero handling, the relationship between negative and positive control data, and the involvement of historical control data.
The analyses allowed us to confirm with a large data set the zero-value handling, as proposed by OECD 489, and supported popular choices of summarizing measures. Further, we offer an orientation on how to check the validity of studies by comparing negative and positive control values. Variation decomposition analysis offers interesting insights on the sources of noise (cell, slide or animal level), suggesting a discussion on more slides per animal.

O23 - Quantitative Interpretation of *In Vivo* Mutagenicity Dose Response Data for Chemical Prioritization and Risk Assessment: Recent Progress and Persistent Challenges

Paul A. White

Environmental Health Science and Research Bureau, Health Canada, Ottawa, Canada

Interpretations of genotoxicity test results have traditionally been restricted to hazard identification (i.e., dichotomous screen and bin). However, this approach is predicated on three false assumptions: that genotoxicants are rare, that genotoxicity dose–response functions do not contain a low-dose region mechanistically characterized by zero-order kinetics, and that genotoxicity is not a *bona fide* toxicological endpoint. Consequently, there is a need to develop and implement quantitative methods to interpret genotoxicity dose–response data for chemical prioritization and risk assessment. Standardized methods to analyze dose-response data have been established; the most robust approach calculates the BMD (Benchmark Dose). However, although 5–10% is often used as the critical effect size (CES) for BMD determination, values for genotoxicity endpoints have not been established. The use of BMDs to determine health-based guidance values (HBGVs) commonly requires uncertainty factors (UFs) to account for interspecies differences and variability in human sensitivity. Analyses of published dose-response data scrutinising the effects of compensatory pathway deficiency indicate that a default UF of 10 for sensitivity differences might be appropriate. Published dose-response data can also be used to evaluate the default UF commonly employed to adjust for less-than-chronic treatment durations. Despite a paucity of chronic treatment data for genotoxicity endpoints, results to date suggest that a default UF of 10 may not be sufficient. Future work should explore the application of IVIVE (*in vitro*-to-*in vivo*) to support the use of *in vitro* dose-response data for prioritization and risk assessment; moreover, the use of probabilistic methods for determination of mutagenicity HBGVs.

O24 - HESI GTTC Comet Project – An Update

Marie Z. Vasquez

Helix3 Inc. Morrisville, United States

Since the release of OECD TG 489 in 2014, regulatory requirements for the *in vivo* comet assay have increased. But many recommendations and criteria in TG489 were based on limited experience and data generated mostly from non-regulated studies with well-characterized compounds (e.g. EMS). This has led to some confusion and difficulty applying and interpreting regulated comet assay studies in safety testing scenarios with unknown compounds, particularly for non-pharmaceutical industries and test materials unintended for human consumption. The HESI GTTC Comet Assay Project was initiated to make practical recommendations for updating OECD TG 489 based on GLP study data generated for or under regulatory submission conditions. Scientists from various industries (e.g. pharmaceutical, industrial chemical, consumer products, food additives) and their associated international regulatory agencies (e.g. FDA, BfArM, ECHA, EFSA, EMA) have provided input and are actively collaborating on this project to ensure that any recommendations made will address practical concerns of both industry and regulatory reviewers. An overview of this project objectives, the status, preliminary data, and future plans will be presented.

O25 - Application of comet assay in aquatic organisms – summary and lessons learned in past 10 years of field research

Stoimir Kolarević[1], Margareta Kračun-Kolarević[1], Jovana Jovanović Marić[1], Jelena Đorđević[2], Momir Paunović[1], Jovana Kostić-Vuković[2], Karolina Sunjog[2], Zoran Gačić[2], Branka Vuković-Gačić[3]

[1]University of Belgrade, Institute for Biological Research ¨Siniša Stanković¨, National Institute of Republic of Serbia, Department of Hydroecology and Water protection, Bulevar despota Stefana 142, 11000 Belgrade, Serbia
[2]University of Belgrade, Institute for Multidisciplinary Research, Kneza Višeslava 1, 11000 Belgrade, Serbia
3University of Belgrade, Faculty of Biology, Chair of Microbiology, Center for Genotoxicology and Ecogenotoxicology, Studentski trg 16, 11000 Belgrade, Serbia

Within the past decade, our research group placed great effort in exploration of the impact of treated/untreated wastewaters on aquatic ecosystems as less than 13 % of collected municipal wastewaters are processed before their release to receiving waters in Serbia. Having in mind that more than 90% of the rivers within the territory of Serbia belong to the Danube River Basin, our major focus is naturally on the large transnational waters such as the Danube and Sava rivers. Depended on the concept of the study and hydro-morphological characteristics of the sites, various approaches have been employed for in situ assessment of eco/geno-toxicity which use different organisms in passive and active biomonitoring. Aquatic organisms of different trophic levels have diverse life strategies, metabolism pathways, and consequently, they have a different response to pollutant pressure. In eco/geno-toxicological studies we have successfully employed several species of freshwater mussels (Unio tumidus, Unio pictorum and Sinanodonta woodiana) as well as few economically important fish species (common carp (Cyprinus carpio), freshwater bream (*Abramis brama), bleak (Alburnus alburnus, chub (Squalius cephalus)).Comet assay is our primary choice for the assessment of DNA damage considering its sensitivity and cost effectiveness. Selection of certain types of tissues for the assessment (such as blood or haemolymph), which do not require sacrificing of the animals or additional manipulation in preparation of cell suspension coupled with mini-gel format of the slides enables high-throughput screening of the genotoxic potential in situ. Additionally, cryopreservation of the blood samples directly onsite expands the research area as it overcomes issues related to safe transportation of the samples to the laboratory. Data obtained so far indicated that* the genotoxic response in the studied indicator organisms significantly differ which is understandable considering the difference in uptake, accumulation and physiological responses.

O26 - Heavy metals: a grand tour

Marko Gerić[1], Goran Gajski[1], Višnja Oreščanin[2], Robert Kollar[3], Damir Ruk[4], Ana-Marija Domijan[5] & Vera Garaj-Vrhovac[1]

[1]Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2]ORESCANIN Ltd., Zagreb Croatia
[3]Advanced Energy Ltd., Zagreb, Croatia
[4]Municipal Company Komunalac, Koprivnica, Croatia
[5]Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

In modern days when population growth-related activities are in constant rise, the impact of man-made pollution towards aquatic environment follows the same trend. Heavy metals are usual components of polluted water released into the environment, however such water is not always treated before its release. Even in Europe, some areas have percentages of wastewater treatment below 25%, and for some areas such as Caribbean that values are lower than 10%. The development of new methods for treatment of polluted water enables more efficient and cost-effective removal of metals from the aquatic matrix. However, toxicological assessment of water purification process is necessary for the evaluation of removal efficacy and for monitoring of potential unwanted by-products. Join the grand tour where several identified pollution spots will be presented such as landfills, marinas, IT factories, and products of recycled materials. The comet assay was put to the front in conjunction with other available methods for detection of possible genotoxicants in polluted water, as well as in treated samples, providing a reliable, rapid, and sensitive tool for toxicological assessment. As a conclusion, more efficient methods for water treatment are expected to be installed throughout the world and the toxicological evaluation with the comet assay as one of its components will facilitate their implementation for the safe environment, for clean water, for us.

O27 - Recent advances in the development of the comet assay on the hemocytes of zebra mussels and its application in field studies for evaluating environmental genotoxicity

Marc Bonnard [1], Laurence Delahaut [1], Isabelle Bonnard [1], Maxime Louzon [1], Damien Rioult [1], Véronique Gaillet [1], Christelle Lopes [2], Benoît Xuereb [3] and Alain Geffard [1]

[1]Université de Reims Champagne-Ardenne (URCA), UMR-I 02 SEBIO (Stress Environnementaux et BIOsurveillance des milieux aquatiques), 51687 Reims cedex 2, France. [2]Université Claude Bernard Lyon 1, Laboratoire de Biométrie et de Biologie Evolutive (LBBE), UMR 5558, 69622 Villeurbannes cedex, France.[3]Université de Le Havre Normandie (ULHN), UMR-I 02 SEBIO (Stress Environnementaux et BIOsurveillance des milieux aquatiques), 76063 Le Havre cedex, France.

The contamination of surface waters by anthropogenic activities have conducted scientists to develop the comet assay in a variety of aquatic organisms used as sentinel species and its application in the evaluation of environmental genotoxicity (ERA). Due to a high filtration rate and bioaccumulation capacity of water contaminants, the zebra mussel *Dreissena polymorpha (Pallas, 1771*) demonstrated its relevance in ecogenotoxicity studies and consequently is considered as the freshwater counterpart of marine mussels [1]. Hemocytes of mussels are convenient cells for the comet assay as i) they are already individualized into the hemolymph, ii) may be punctured in adductor muscles through the year and iii) may reflect the general health status of individuals, as they are involved in many physiological functions. This communication will synthetize in four interconnected parts the recent advances regarding the application of the comet assay in the zebra mussel. The first part will focus on methodological improvements for the measure of DNA strand breaks and oxidative lesions (*Fpg-sensitive sites*) in hemocytes of zebra mussels. The second part will depict the strategy used for the definition of the reference DNA strand breaks level, with the transplantation of a control population of zebra mussels (n ≈ 300 mussels) in many rivers in the North-Eastern of France. The third part will replace and discuss the levels of DNA strand breaks (until 30% of tail intensity) measured in 3 week-encaged mussels in different sites of the *Seine* River through an upstream-downstream gradient of water contamination. In the end, the philosophy of research projects in progress, regarding the comparison and the inter-calibration of the response of genotoxicity biomarkers between sentinel species along the *continuum* between fresh- and marine-waters in the *Seine* watershed will be presented.

[1] Binelli A. et al. (2015). Does zebra mussel (*Dreissena polymorpha*) represent the freshwater counterpart of Mytilus in ecotoxicological studies? A critical review. Environmental Pollution, 196: 386-403.

O28 - Measuring DNA Strand Breaks in Plant Models

John Einset[1], Sergey Shaposhnokov[2] and Andrew R. Collins[1,2]

1. *Faculty of Medicine, University of Oslo, Oslo, Norway*
2. *Norgenotech As, Oslo Cancer Cluster, Radiumhospitalet, Oslo, Norway*

The comet assay has been used extensively for medical and public health research but relatively little for plant studies. On the other hand, plants can serve as useful models for fundamental questions related to radiation damage and repair of DNA as well as to mechanisms involved in aging. For example, it is known that DNA repair after acute X-ray exposures involves a rapid initial phase followed by a delayed phase of repair. Using ATM and BRCA1 mutants of Arabidopsis, we can show that the delayed phase involves repair of double-strand breaks and that it is associated with gene induction. These results raise the interesting possibility that low doses of exposure might cause damage that accumulates and is not repaired. Another example, using plants as models, involves the question of the relationship between genome size and sensitivity to X-ray damage. By exposing isolated nuclei from 6 different plant species, varying in genome size 2.6-19.2 Gbp, we show that larger genomes are more sensitive to damage by a relationship corresponding to the cube-root of the nuclear volume. Finally, during the aging of squash, cucumber and melon seeds, extensive DNA damage occurs over time. In the case of melon seeds which lose viability before squash and cucumber, DNA strand breaks occur faster in melon compared to squash and cucumber, suggesting that DNA damage is the cause of reduced viability.

O29 - Spheroids – a 3D cell based system for genotoxicity assessment

Bojana Žegura, Sonja Žabkar, Katarina Fras and Martina Štampar

Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia

Testing of chemicals and products for their potentially harmful side effects is important for the hazard identification and risk assessment in order to protect human health. During the early stages of the development of drugs, chemicals, cosmetic products, food and feed additives, pesticides, and others, it is essential to identify the ability of substance to induce DNA damage. Currently, hepatic two-dimensional (2D) cell cultures are used for the purpose of safety evaluation. However, they have several limitations, including the lack of many biological functions such as cell–cell and cell–matrix contacts, resulting in reduced cell differentiation, flattened cell morphology with altered cytoskeleton, reduced viability, altered cell signalling pathways and, most importantly, low expression of metabolic enzymes. Thus, there is a strong demand for the development of physiologically more relevant three-dimensional (3D) *in vitro* cell-based system, which hold promise for applications in drug discovery, cancer cell biology, stem cell research, safety studies and many other cell-based analyses by bridging the traditional 2D cell cultures and whole-animal systems. In this respect, hepatic 3D cell based systems (spheroids) can restore highly complex microenvironment and provide more predictive data for human exposure compared to 2D cell models, as they have improved cell-cell and cell-matrix interactions, have preserved complex *in vivo* cell phenotypes and exhibit higher levels of liver-specific functions, including metabolic enzymes. The presentation will discuss the development and the optimization of techniques and culture conditions for obtaining viable spheroids from human hepatocellular carcinoma (HepG2) cell line grown under static and dynamic conditions. Spheroids were first characterised and then used to assess the (geno)toxicity of various chemicals by studying classical genotoxicity endpoints (single-stranded and double-stranded DNA breaks) in combination with toxicogenomic analyses (targeted gene expression). In addition, the presentation will address the modification of the comet assay on 3D HepG2 spheroids.

Acknowledgement to projects: ARRS (P1-0245, J1-2465, Z1-3191) and CA16119 COST Action.

O30 - Comet assay from a new perspective introducing the Flash-comet - from hairpins to low conductive medium

Erik Bivehed[1], Ola Söderberg[2] and Björn Hellman[1]

[1] *Department of Pharmaceutical Biosciences/Drug Safety and Toxicology, Uppsala University, Uppsala, Sweden*
[b] *Department of Pharmaceutical Biosciences/Pharmaceutical Cell Biology & Biotechnology, Uppsala University, Uppsala, Sweden*

Since the comet assay was introduced over 30 years ago, a lot of work and much effort has been laid on evaluating if and how the different parameters in the comet assay protocol may affect the assay effectiveness as well as the obtained results. The main goal of these investigations has been to improve the robustness of the comet assay and reduce the inter- and intra assay variability through optimizing the protocol steps.
The basic electrochemical principal behind the comet assay is the same as for any other electrophoresis technique, namely that charged particles (DNA fragments or loops) are separated by their ability to move through a sieving matrix (an agarose gel) when an electrical gradient is applied. Consequently, the duration and voltage of the electrophoresis are key variables when it comes to the degree of DNA migration in a comet assay. Higher voltage, or increased speed, and/or longer duration, i.e. increased runtimes, will lead to an increase in DNA migration.
However, the composition of the electrophoresis medium has remained unchanged since the introduction of the method, being composed by a strong sodium hydroxide solution with a small addition of EDTA. Consequently, most of the work surrounding the electrophoresis has been focused on optimization of the duration of the electrophoresis.
Our work has focused on increasing the speed of the electrophoresis by changing the composition of the electrophoresis medium. We have shown that by replacing the sodium ions with lithium ion it is possible to lower the conductivity of the electrophoresis solution by ten times. This lowered conductivity allows for much stronger field strengths or higher voltages to be applied from 25 to 150 V. The increased voltage reduces the required runtime and sufficient DNA migration is achieved within one minute, a method we today call the Flash-comet.

O31 - HepaCometChip: A Cell-Based Platform to Replace Animal Studies of Liver Genotoxicity

Bevin P. Engelward

MIT Dept. of Biological Engineering. Cambridge, MA, USA

A major challenge to public health are the tens of thousands of chemicals used by industry for which we know little about their genotoxicity. While the *in vivo* comet assay is routinely performed to test drugs and environmental chemicals for their DNA damaging potential in the liver, it is imperative that we develop an *in vitro* substitute. In a collaboration between Integrated Laboratory Systems and MIT, the HepaCometChip is now being validated for this purpose. The most important aspect of the HepaCometChip is that it is based on HepaRG cells that have relatively robust P450 expression. This enables detection of carcinogens that require metabolic activation. In addition, the HepaCometChip can detect bulky lesions based on 'repair trapping.' In brief, bulky lesions are repaired by nucleotide excision repair (NER). During NER, there is a requisite repair synthesis step. By inhibiting DNA replication, NER cannot go to completion, leading to a persistent single strand break (SSB). As such, HU and AraC enable cells to convert undetectable bulky lesions into detectable SSB repair intermediates. Together, the use of replication inhibitors along with hepatocytes with robust metabolic capacity gives rise to a modified CometChip platform with broad sensitivity. The Engelward lab at MIT and Integrated Laboratory Systems, Inc. are now working together with many colleagues as part of the HepaCometChip Cooperative to perform a ring trial so as to validate the assay. This is the next step toward OECD approval, and it is being made possible by the contributions of comet experts (many of whom are part of the ICAWG) who have volunteered to participate in the ring trial. Ultimately, it is hoped that the HepaCometChip Cooperative will give rise to an effective *in vitro* assay that can be used to reduce the use of animals in research in regulatory science.

O32 - Electrophoresis with vertically-oriented comet slides: advantages and disadvantages

Aliy K. Zhanataev, Elena A. Anisina, Zlata V. Chayka, Andrei D. Durnev

Zakusov Research Institute of Pharmacology, Moscow, Russia

One of the drawbacks of conventional electrophoretic chambers used for comet assay is the low throughput due to the limited capacity of the slides. A new high-throughput approach has been developed where electrophoresis is performed with the slides vertically oriented. The goal of the study was to determine the specifics of electrophoresis with vertically oriented slides. In our study, we used a COMPAC-50 chamber (Cleaver Scientific), which has a unique proprietary design.

By using two polyoxymethylene racks, up to 50 slides can be manipulated simultaneously. Slides can remain in the racks throughout all stages of comet assay, which, on the one hand, minimizes the risk of damaging the gels adhered to the slides and, on the other hand, speeds up the process. The chamber requires less buffer volume, with the accompanying cost savings.

At the manufacturer's recommended voltage (21 V), the current and field strength were 420 mA and 0.6 V/cm, respectively. Analysis of comet slides revealed differences in DNA damage assessed for the same cell sample in a conventional chamber (actual voltage of 1 V/cm) and COMPAC-50: 12.8±2.2%DNA in the tail versus 4.6±0.6. A similar level of DNA damage was obtained when the electrophoresis time was increased from 20 to 30 min (12.9±2.1 %DNA in the tail). A field strength of 1 V/cm was achieved at 28 V, but by the end of electrophoresis, increasing the current to a limiting value of 700 mA resulted in a voltage drop at the power supply output and stabilized the voltage-to-current transition.

Thus, the advantages of electrophoresis with vertically oriented comet slides are an increase in the number of samples analyzed, a decrease in the number of slide manipulations and, accordingly, in the analysis time, a decrease in the risk of damaging the slides. The only disadvantages are the need to use a special rare power source with a current of more than 400 mA.

Posters

P1 **Transcriptional profile and fertility impairment in Drosophila melanogaster larvae and adults exposed to the potential biopesticide 2-dodecanone**
Mónica Aquilino, Rosario Planelló, Lola Llorente, David Siaussat, Óscar Herrero

P2 **Internal validation of the in vitro comet assay modified for detecting altered bases and DNA cross-links**
Amaya Azqueta, Damián Muruzabal, Adela López de Cerain, Ariane Vettorazzi

P3 **Analysis of in vitro genotoxicity of different nanoparticles in human blood lymphocytes by comet assay**
Milda Babonaitė, Juozas Rimantas Lazutka

P4 **Consumption of Aronia juice-based food supplement protects DNA integrity in healthy volunteers**
Tamara Bakuradze, Peter Meiser and Elke Richling

P5 **Biosafety determination of innovative nanocomposites with potential application in regenerative medicine**
Lucia Bálintová, Alessandro Paolini, Andrea Masotti, Monika Šramková

P6 **Comparison of biological activity of thymol and newly synthesized derivative on colorectal cancer cells**
Michaela Blažíčková and Katarína Kozics

P7 **Alkaline comet assay in whole blood samples: application in forensic genetics**
Lejla Caluk Klacar, Tamara Cetkovic, Ivona Oroz, Jasmina Cakar, Anja Haveric

P8 **The comet assay and the Fpg-modified comet assay: optimization and field-application on erythrocytes of the three-spined stickleback**
Amélie Cant, Anne Bado-Nilles, Jean-Marc Porcher, Jean Prygiel, Audrey Catteau, Laurence Delahaut, Cyril Turiès, Alain Geffard, Marc Bonnard

P9 **Graphene oxide: DNA damage induction in HEK 293T cells** Tamara Cetkovic, Sanjin Gutic, Sanin Haveric, Lejla Caluk Klacar, Maida Hadzic Omanovic, Anja Haveric

P10 **Does the duration of lysis affect in vivo comet assay results?**
Collia M., Vettorazzi A., López de Cerain A. and Azqueta A.

P11 **Are results with CometChip® and the two gels/slide comet assay comparable?**
Collia M., Vettorazzi A., López de Cerain A. and Azqueta A.

P12 **Could DNA damage in Ligustrum vulgare L. leaves also indicate soil pollution?**
Mujo Hasanović, Tamara Ćetković, Anesa Ahatović, Adaleta Durmić-Pašić

P13 **Double-stranded DNA breaks in the sperm of patients with normozoospermia and pathozoospermia**
Haurylchyk I.D., Ryabokon N.I.

P14 **The sensitivity of human peripheral blood lymphocytes to the DNA-damaging effect of pesticides and their combinations in vitro**
Ilyushina N.A., Averianova N.S., Kara L.A., Grabovskaia A.A. Ignatiev S.D., Egorova O.V.

P15 **Evaluation of genotoxic potential of the middle section of the Danube River and its major tributaries**
Jovana Jovanović Marić, Margareta Kračun-Kolarević, Stoimir Kolarević, Jelena Đorđević, Karolina Sunjog, Jovana Kostić-Vuković, Momir Paunović, Branka Vuković-Gačić

P16 **Effects of Di (2-ethyl hexyl) phthalate Exposure on Oxidative Stress and Epigenetic Profile in Early Adolescent and Adolescent Periods in Rat Brain**
S. Koc, E. Erdogmus, D. Ozkan-Vardar, U. Sur, P. Erkekoglu, B. Kocer- Gumusel

P17 **Genotoxicity of ambient PM10 at an urban site in Sarajevo, Bosnia and Herzegovina – assessment by comet assay in peripheral blood mononuclear cells.** Lejla Caluk Klacar, Tamara Cetkovic, Anja Haveric, Adna Softic, Lejla Haracic, Sabina Zero, Sanin Haveric

P18 **Heat killed paraprobiotic Lactiplantibacillus plantarum S1 reduces DNA damage in induced oxidative stress**
Deni Kostelac, Marko Gerić, Goran Gajski, Jadranka Frece

P19 **Sensitivity of invasive alien fish in Serbia black bullhead Ameiurus melas (Rafinesque, 1820) as a bioindicator of genotoxicity**
Jovana Kostić-Vuković, Karolina Sunjog, Stoimir Kolarević, Marija Smederevac-Lalić, Zoran Marković, Stefan Skorić1 and Milica Jaćimović

P20 **In vivo pharmacokinetics and biodistribution of gold nanoparticles**
Katarina Kozics, Monika Sramkova, Kristina Jakic, Tibor Dubaj, Peter Simon, Alena Gabelova

P21 **Bioactivity of the Joint Danube Survey 4 surface water samples collected by horizon large volume solid–phase extraction technique**
Margareta Kračun-Kolarević, Jovana Jovanović Marić, Peter Oswald, Martina Oswaldova, Jaroslav Slobodnik, Branka Vuković-Gačić, Momir Paunović, Katja Kološa, Bojana Žegura, Stoimir Kolarević

P22 **Effects of the 1,4-dihydropyridine salts assessed in vitro and in vivo**
Elina Leonova, Vitalijs Borisovs, Anatoly Mishnev, Egils Bisenieks, Gunars Duburs and Nikolajs Sjakste

P23 **The comet assay in Pieris brassicae (Lepidoptera, Pieridae): a useful tool to evaluate genotoxic effects induced by particulate matter (PM10)**
Manuela Macrì, Marta Gea, Irene Piccini, Luca Dessì, Alfredo Santovito, Simona Bonelli, Tiziana Schilirò, Sara Bonetta

P24 **DNA damage of PM2.5 samples during the COVID-19 pandemic: a comparison with the pre-lockdown period in Padana Plain.** Manuela Macrì, Marta Gea, Sara Bonetta

P25 **Genotoxicity assessment of some industrially processed meat products in the human Caco-2 cell line using the alkaline comet assay**
Chamlal Naima, Khallef Messaouda, Benouareth Djamel Eddine, Frenzilli Giad

P26 **Influence of antioxidants resveratrol and melatonin on telomere dynamics and DNA damage in liver and kidney of the Wistar rat model of aging**
Lucia Nanić, Marko Gerić, Goran Gajski, Jasna Jurasović, Dean Karaica, Marija Ljubojević, Vedran Micek, Ivana Novak Jovanović, Tatjana Orct, Maja Peraica, Dubravka Rašić, Ivana Vrhovac Madunić, Ivan Sabolić, Davorka Breljak, Ivica Rubelj

P28 **Mechanistic investigations of Mn-induced oxidative stress, DNA damage, DNA repair, and neurodegeneration in two different model systems**
Merle Marie Nicolai, Barbara Witt, Sharleen Friese, Nicola Winkelbeiner, Ann-Kathrin Weishaupt, Franziska Ebert, Andrea Hartwig, Tanja Schwerdtle, and Julia Bornhors

P29 **DNA damage in blood cells in relation to chemotherapy and nutritional status in colorectal cancer patients**
Anne Lene Nordengen, Congying Zheng, Andrew Collins, Truls Raastad, Rune Blomhoff, Sergey Shaposhnikov

P30 **DNA repair in ovarian cancer therapy response: The role of MRE11** Alena Opattova, Dominika Vallusova, Josef Horak, Pavel Vodicka

P31 **In vitro Evaluation of Cytotoxicity and Apoptotic Activities of Quercetin in BEAS-2B and A549**
D. Ozkan-Vardar, T. Duran, M. Aydin Terzioglu

P32 **Three weeks of 1500 vs. 567 kcal diet- differences in severely obese with BMI ≥ 35kg/m^2**
Ivan Ožvald, Martina Bituh, Hrvoje Radašević, Maja Nikolić, Martina Matovinović, Dragan Božičević, Lidija Duh, Ivana Vinković Vrček, Ana-Marija Domijan and Mirta Milić

P33 **Higher concentrations of zinc sulfate impair chromatin integrity, tight junction proteins, and lncRNA expression**
Stella Marie Reamon-Buettner, Lasse Liebisch, Ines Voepel, and Christina Ziemann

P34 **Comet Assay for human health purposes** NI Ryabokon

P35 **Genotoxicity of pyrrolizidine alkaloids in HepG2 cells in vitro** Naji Said Aboud Hadi, Ezgi Eyluel Bankoglu and Helga Stopper

P36 **Impact of cell type and exposure time on the quantitative outcome of in vitro genotoxicity tests**
J. Sanders, A. Thienpont, R. Anthonissen, T. Vanhaecke and B. Mertens

P37 **Novel formats for the comet assay**
Sergey Shaposhnikov, Congying Zheng, Adrew Collins, Kristine Bjerve Gutzkow, Gunnar Brunborg

P38 **Antioxidant, genotoxic and antigenotoxic properties of Ambrosia artemisiifolia L. (Asteraceae) deodorized water extract**
Biljana Spremo-Potparević, Lada Živković, Dijana Topalović, Jelena Kotur-Stevuljević, Stevan Samardžić, Zoran Maksimović

P39 **Genotoxicity of European chub (Squalius cephalus L. 1758) erythrocytes as an effective indicator in monitoring of water bodies under different pollution pressure**
Karolina Sunjog, Jovana Kostić-Vuković, Stoimir Kolarević, Jovana Jovanović Marić, Dušan Nikolić, Stefan Skorić

P40 **Mitochondrial DNA damage and DNA repair capacity in type 2 diabetic, obese rats**
Kristyna Tomasova, Sona Vodenkova, Pavel Vodicka, Roger Godschalk, Sabine A.S. Langie

P41 **Development of a high throughput HPLC-MS/MS method for the quantification of 16 DNA adducts in blood: Application to a French Agricultural cohort samples**
Quentin Vandoolaeghe, Valérie Bouchart, Isabelle Catro, Poppy Evenden, Pierre Lebailly, Matthieu Meryet-Figuiere, Raphaël Delépée

P42 **Optimized Fpg-modified comet assay for assessing differences in base excision repair activity**
Congying Zheng, Andrew Collins, Gunnar Brunborg, Frederik-Jan van Schooten, Anne Lene Nordengen, Roger Godschalk, Sergey Shaposhnikov

P43 **Dihydroquercetin (Taxifolin) and Biochaga attenutate oxidative DNA damage in lymphocytes of obese subjects in vitro**
Lada Živković, Dijana Topalović, Sunčica Borozan, Vladan Bajić, Vesna Dimitrijević Srećković, Andrea Pirković, Hristina Petrović, Biljana Spremo-Potparević

P1 **Transcriptional profile and fertility impairment in *Drosophila melanogaster* larvae and adults exposed to the potential biopesticide 2-dodecanone**

Mónica Aquilino[1], Rosario Planelló[1], Lola Llorente[1], David Siaussat[2], Óscar Herrero[1]

[1]Grupo de Biología y Toxicología Ambiental, Facultad de Ciencias, Universidad Nacional de Educación a Distancia (UNED), Madrid, Spain
[2]Institute of Ecology and Environmental Sciences of Paris, Department of Sensory Ecology, Sorbonne Université, Campus Pierre et Marie Curie, Paris, France

Synthetic pesticides have become a great public concern due to their associated health hazards. In this sense, natural products derived from plants are emerging as potent biorational alternatives for the integrated management of insect pests. Among the secondary metabolic compounds synthesised by plants, methyl ketones constitute a group of metabolites that have demonstrated their effectiveness in defending against phytophagous arthropods' attacks. However, information about their mode of action and molecular effects in target and non-target species is still scarce. This work aims to analyse the effect of the methyl ketone 2-dodecanone on different developmental stages of *Drosophila melanogaster*. Specifically, transcriptional alterations induced by sub-lethal concentrations (5 µg/L and 500 µg/L) of 2-dodecanone were evaluated in third instar larvae (acute 24-hour exposures), as well as in adult males and females (chronic full life-cycle exposures). Fertility (average number of eggs) in exposed adults was also analysed. Quantitative real-time PCR (qPCR) was used to measure the expression levels of selected biomarker genes related to the endocrine system (*EcR*, *ERR*, *HR3*, and *BR-C*), the cell-stress signalling pathway (*Tl*, *def*, *p38*, *hsf*, *hsp22*, *hsp40*, *hsp70*, and *hsc70*), and detoxification mechanisms (*cat*, *sod*, and *phgpx*). Our results showed that 2-dodecanone caused significant alterations in the transcriptional activity of most of the genes tested even in 24-hour exposures and that these toxic effects at the molecular level ultimately translated into a dose-dependent decrease in the fertility rate. This study provides for the first time in *D. melanogaster* novel and interesting results on the toxic effects of an isolated secondary metabolite naturally present in plants. It highlights the potential suitability of this organism to delve into the molecular effects of plant defences in insects.

P2 **Internal validation of the in vitro comet assay modified for detecting altered bases and DNA cross-links**

Amaya Azqueta[1,2], Damián Muruzabal[1], Adela López de Cerain[1,2], Ariane Vettorazzi[1,2]

[1]University of Navarra, Pamplona, Spain
[2]IdiSNA, Navarra Institute for Health Research, Pamplona, Spain

The comet assay is an important tool for elucidating the mechanism of action of genotoxic compounds. The conventional alkaline version of the assay only detects DNA strand breaks and alkali-labile sites; however, with certain modifications it is capable of detecting altered bases, cross-links and even bulky adducts.
In this work we internally validate the use of DNA-repair enzymes (i.e., formamidopyrimidine DNA glycosylase, endonuclease III, human 8-oxoguanine DNA glycosylase I and human alkyladenine DNA glycosylase) for detection of oxidized and alkylated bases as well as a modification for detecting cross-links.
Seven genotoxic compounds with different mechanisms of action (namely oxidizing and alkylating agents, and cross-links and bulky-adducts inducers), as well as a non-genotoxic and a cytotoxic compound were tested on TK-6 cells. The proliferation assay was used to assess the effect of the compounds on cell viability.
We were able to clearly differentiate oxidizing, alkylating and cross-linking agents, while, as expected, the bulky adduct inducer was not detected. These modifications of the comet assay significantly increase its sensitivity and its specificity towards DNA lesions, providing valuable mechanistic information.

Financial support: Spanish Ministry of Science and Innovation (BIOGENSA, AGL2015-70640-R and BIOGENSA2, PID2020-115348RB-I00).).

P3 **Analysis of *in vitro* genotoxicity of different nanoparticles in human blood lymphocytes by comet assay**

Milda Babonaitė[1], Juozas Rimantas Lazutka[1]

[1] *Institute of Biosciences, Vilnius University, Lithuania*

Nanoparticles (NPs) are widely used in different technologies, due to its unique properties. One of a main concern with nanoparticles exposure is their genotoxic potential [1]. The most frequently used technique to detect DNA damage induced by nanoparticles is comet assay. This is a relatively quick and sensitive technique to detect DNA strand breaks, alkali-labile sites, specific DNA lesions and DNA methylation [1,2]. We investigated the genotoxicity of differently sized cobalt oxide (Co_3O_4) NPs, silver (Ag) NPs and silica (SiO_2) NPs in human lymphocytes *in vitro*. Lymphocytes of 5 healthy donors were treated with different concentrations of NPs. In case of Co_3O_4 NPs, none of the tested 10-30 nm size NPs concentrations induced statistically significant increase in DNA damage, however all tested concentrations of 50 nm size NPs (20 - 80 μg/ml) significantly increased the amount of DNA in comet tail (TDNA%). Genotoxicity testing of 10-20 nm SiO_2 NPs suggested that after 1 hour incubation none of the tested concentrations (0 – 500 μg/ml) induced significant DNA damage, while after 24 hours incubation higher concentrations (100 – 300 μg/ml) induced statistically significant results. In case of Ag NPs only 60 μg/ml concentration caused statistically significant increase in TDNA%. However, in all cases, when evaluating the amount of TDNA% interindividual differences were observed. This has raised some concerns about appropriate interpretation of genotoxicity results. Therefore, more experiments must be done to analyze possible variability within a specific donor in order to ensure, that individual donor sensitivities to nanoparticles can occur.

[1] Azquetta A., Dusinska M. (2015). The use of the comet assay for the evaluation of the genotoxicity of nanomaterials. *Front. Genet*. 6:239
[2] Gosh I., Sadhu A., et al. (2017). Application of comet assay in the assessment of nanoparticle induced genotoxicity and DNA methylation**.** Mutagenesis, 12th International Comet Assay Workshop. Vol 32.

P4 Consumption of Aronia juice-based food supplement protects DNA integrity in healthy volunteers

Tamara Bakuradze[1], Peter Meiser[2] and Elke Richling[1]

[1]Technische Universität Kaiserslautern, Kaiserslautern, Germany;
[2] Ursapharm Arzneimittel GmbH, Saarbrücken, Germany

Several intervention studies reported that consumption of anthocyanin and polyphenol-rich fruit juice may improve DNA integrity in humans [1, 2]. However, many of these studies tested the intake of rather large volume of polyphenol-rich juices. We investigated the impact of small amounts of Aronia (Aronia melanocarpa) juice-based food supplement on DNA integrity in a randomized, controlled, human intervention study with 91 healthy volunteers [3]. Over a period of eight weeks, the volunteers consumed either the food supplement (2x25 mL ampules, n=45) or water (n=46) on a daily base. Before and after intervention times, the background and total DNA strand breaks in whole blood and H2O2-induced DNA damage in isolated peripheral blood lymphocytes were determined by comet assay. Compared to the baseline, the significant decrease of background DNA strand breaks ($p<0.05$) was observed after four and eight weeks of supplement intake, whereas the total DNA strand breaks were not significantly modulated. Additionally, consumption of the food supplement was associated with significant reductions of H_2O_2-induced DNA damage, compared to the baseline ($p<0.001$) as well as to the control ($p<0.05$). In conclusion, these findings suggest that the intake of small amounts of the investigated anthocyanin rich food supplement has the potential to reduce DNA damage in humans.

[1] Bakuradze, T.; Tausend, A.; Galan, J.; Groh, I.A.M.; Berry, D.; Tur, J.A.; Marko, D.; Richling, E. Antioxidative activity and health benefits of anthocyanin-rich fruit juice in healthy volunteers. Free Radic. Res. 2019, 53, 1045–1055.https://doi.org/10.1080 /10715762.2019.1618851
[2] Groh, IA.; Bakuradze, T; Pahlke, G.; Richling, E.; Marko, D. Consumption of anthocyanin-rich beverages affects Nrf2 and Nrf2-dependent gene transcription in peripheral lymphocytes and DNA integrity of healthy volunteers. BMC Chemistry. 2020, 14, 39. https://doi.org/10.1186/ s13065-020-00690-6.
[3] Bakuradze, T.; Meiser, P.; Galan, J.; Richling, E. DNA Protection by an Aronia Juice-Based Food Supplement. Antioxidants. 2021,10, 857. https:// doi.org/10.3390/antiox10060857.

P5 Biosafety determination of innovative nanocomposites with potential application in regenerative medicine

Lucia Bálintová[1], Alessandro Paolini[2], Andrea Masotti[2], Monika Šramková[1]

[1]Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia; [2]Bambino Gesù Children's Hospital-IRCCS, Research Laboratories, Rome, Italy

Wound healing is a complex regeneration process. Current advanced methods are trying to develop suitable materials that can restore and reproduce the favorable and natural environment needed for skin regeneration. The development of advanced multifunctional materials for wound treatment with the ability to provide multiple functions at once is crucial for clinical application. The utilization of nanohydrogels in regenerative medicine provides an innovative way to treat skin injuries.
As it is very important to evaluate the biosafety of nanohydrogel as a degradable biomaterial for use in the biomedical field, the aim of this study was to determine the biological effects of newly prepared nanocomposites. The model system represents different types of skin cells, HaCaT and HFF-1, and 3D *in vitro* MatTek's EpiDermFT skin model. The experiments are focused on determining the cytotoxic and genotoxic effect of nanocomposites and their individual components in the *in vitro* conditions. Three hydrogels (Alginate, Pluronic F127, and Gelatin metacrylate) with a different chemical composition and iron oxide nanoparticles were used for nanohydrogel build-up. Initial results of individual nanohydrogel components, measured by LDH assay showed increased cytotoxicity, observed in the case of all alginate hydrogel concentrations and at 20% concentration of gelatin metacrylate hydrogel after 24 h exposure. Additionally, genotoxicity measured by comet assay did not show any significant increase in DNA damage. We also did not observe any pathological changes in the skin model by histological staining with hematoxylin and eosin. Currently, we are determining the biological effect of nanohydrogel, consisting of both nanoparticles and hydrogel.

This work was supported by ENM III/2019/861/TENTACLES; DoktoGrant no. APP0316 and project VISION (Strategies to strengthen scientific excellence and innovation capacity for early diagnosis of gastrointestinal cancers) No. 857381.

P6 Comparison of biological activity of thymol and newly synthesized derivative on colorectal cancer cells

Michaela Blažíčková and Katarína Kozics

Cancer Research Institute, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

Thymol has a proven bioactive effect on colorectal cancer cells. However, its properties such as low solubility and cell penetration prevent its wider application. Therefore, a new hydrophilic derivative - acetylthymol - was synthesized. Our study focused on the comparison of the bioactive effect of thymol and acetylthymol on the colorectal cancer cell lines (HT-29, HCT-116) after 24 hours of exposure to the concentration scale. Cytotoxic effect was determined by MTT method, genotoxicity by comet assay, and reactive oxygen species (ROS) formation using the ROS-Glo™ H_2O_2 assay.
Cytotoxicity results showed that acetylthymol was more effective at much lower concentrations compared to thymol on both cell lines. IC_{50} values for thymol were 52 µg/ml (HT-29) and 65 µg/ml (HCT-116). For acetylthymol, the IC_{50} values were 0.08 µg/ml for both lines. Comet assays have also shown a significant increase in DNA damage for the newly synthesized derivative even at non-cytotoxic concentrations. The HCT-116 cell line showed higher DNA damage values than HT-29. There was also an increase in ROS production after acetylthymol treatment for both cell lines.
The results confirmed our assumption that the newly synthesized hydrophilic derivative can act more effectively than thymol. Further tests are followed to assess the effect of the substance in more detail, such as cell cycle analysis and apoptosis. Western blot will be performed to assess changes in the expression of selected proteins. Acetylthymol has the potential to act more effectively on colorectal cancer cells at much lower concentrations than thymol.

P7 **Alkaline comet assay in whole blood samples: application in forensic genetics**

Lejla Caluk Klacar[1], Tamara Cetkovic[1], Ivona Oroz[2], Jasmina Cakar[1], Anja Haveric[1]

[1]Institute for genetic engineering and biotechnology, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina
[2]Faculty of Science, University of Sarajevo, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina

Comet assay is a widely used and versatile method for the visualization and measurement of DNA fragmentation. DNA fragmentation is an important concern in forensic genetics, directly affecting DNA profiling. Therefore, this study aimed to estimate the potential of comet assay to correlate level of DNA fragmentation (DNA damage) determined by comet assay in blood samples with the ambial temperature, and duration of exposure. The whole blood sample was collected from a female healthy donor and placed in Petri dishes in two replicas for each duration time until processing. The comet assay sampling was carried out at different time intervals (0h, 2h, 24h, 48h, and 144h). As a positive control, 70μM H_2O_2 was used. Alkaline comet assay was performed and comets analyzed using Comet Assay IV software. Generally, room temperature exposure resulted in higher DNA damage, except for 2h interval. The significantly highest DNA damage measured as tail intensity (TI) was observed in the sample exposed to room temperature for 144h. Alkaline comet assay is a potent method for quality assessment of the whole blood samples prior to other forensic genetics analysis. Analysis of other biological samples or samples collected at crime scenes could be further assessed.

P8 **The comet assay and the Fpg-modified comet assay: optimization and field-application on erythrocytes of the three-spined stickleback**

Amélie Cant[1,2], Anne Bado-Nilles[1], Jean-Marc Porcher[1], Jean Prygiel[3], Audrey Catteau[2], Laurence Delahaut[2], Cyril Turiès[1], Alain Geffard[2], Marc Bonnard[2]

[1] Institut National de l'Environnement Industriel et des Risques (INERIS), UMR-I 02 SEBIO (ESMI unit), Parc Technologique Alata, BP 2, 60550 Verneuil-en-Halatte, France
[2] Université de Reims Champagne-Ardenne (URCA), UMR-I 02 SEBIO, UFR Sciences Exactes et Naturelles, Campus Moulin de la Housse, BP 1039, 51687 Reims, France
[3] Agence de l'Eau Artois-Picardie, 200 rue Marceline—Centre Tertiaire de l'Arsenal, BP 80818, 59508 Douai, France

In the context of aquatic biomonitoring, the measure of damages at different genomic scales may be appropriate and provide an integrated view of the genotoxic risk of exposed organisms. Genotoxic effect is considered as a major endpoint to assess aquatic pollution-related toxicity. In this aim, the finality of our research is to propose the use of biomarkers of genotoxicity as early-warning signals of potential alterations on individual. Notably, the alkaline comet assay (Single Cell Gel Electrophoresis assay, SCGE) which detects many DNA strand breaks like alkali-labile sites, DNA-double- and single- strand breaks coupled with flow cytometry (FCM) which measures the variations of the nuclear DNA content, have shown their relevance on erythrocytes of the three spined stickleback (*Gasterosteus aculeatus*, L.). Indeed, blood cells of fish offer many advantages as cellular models as their ease of collection, give a high density of erythrocytes already dissociated allowing the monitoring of several biomarkers on the same biological sample. Oxidation of DNA bases induced by the oxidative stress related to the production of reactive oxygen species (ROS) can be also measured by the comet assay. Specifically, the use of the modified comet assay coupled with the Fpg (formamidopyrimidine DNA glycosylase) seems to be relevant, as it has demonstrated its ability to improve the sensitivity and specificity of the SCGE on different cell lines including fish erythrocytes in field studies but remains less developed. The Fpg-modified comet assay optimization on stickleback erythrocytes has been carried out and allowed it field application. The response of this new biomarker in stickleback has been compared with responses of the other genotoxic biomarkers.

P9 **Graphene oxide: DNA damage induction in HEK 293T cells**

Tamara Cetkovic[1], Sanjin Gutic[2], Sanin Haveric[1], Lejla Caluk Klacar[1], Maida Hadzic Omanovic[1], Anja Haveric[1]

[1]University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina
[2]University of Sarajevo, Faculty of Science, Department of Chemistry, Sarajevo, Bosnia and Herzegovina

Graphene oxide (GO), a derivative of graphene, is one of the most potential candidates of graphene family nanomaterials members for biomedicine applications. Its increased use leads to concerns about the safety and potential toxicity effects on biological systems. Testing of nanomaterials toxicity *in vitro* by comet assay is considered a sensitive and simple method to evaluate DNA damage. The objective of this study was to evaluate the genotoxicity of one commercially available GO prepared by Hummers method, in human embryonic kidney (HEK 293T) cell line by alkaline comet assay. In order to exclude the potential DNA damage by trypsinization (0.25% trypsin/EDTA), prior the evaluation of GO genotoxicity and effects of different trypsin/EDTA treatment periods at 37°C were evaluated. To examine HEK 293T cells sensitivity to H_2O_2 and to optimize time of H_2O_2 (70 μM) exposure for positive control, different times of exposure at +4°C were applied. DNA damage was evaluated by tail intensity (TI) in a total of 200 scored cells for each treatment using the Comet Assay IV (Instem, UK). Compared to control cells (dH_2O), GO increased the DNA damage in a concentration-dependent manner. Minimal cell DNA damage was observed after exposure of HEK 293T cells to trypsin/EDTA for 1 min. For positive control, H_2O_2 treatment was optimal at 1 min exposure. Results suggest genotoxic potential of GO. Concerns regarding nanoparticles are related to their direct interaction with DNA, therefore further studies on biological effects of GO in various cell types and using broad spectrum of assays are necessary to confirm these findings.

This work has been supported by the Ministry of Education, Science and Youth of Sarajevo Canton (grant No: 27-02-11-41250-2/21)

P10 **Does the duration of lysis affect in vivo comet assay results?**

Collia M. [1], Vettorazzi A.[1,2], López de Cerain A.[1,2] and Azqueta A.[1,2]

[1]*Department of Pharmacology and Toxicology, School of Pharmacy and Nutrition, Universidad de Navarra, 31008 Pamplona, Spain.*
[2]*IdiSNA, Navarra Institute for Health Research, Pamplona, Spain.*

Lysis is a critical step in the comet assay protocol. Its duration affects the comet assay results when certain types of lesions are present and it is advisable to use the same time of lysis to make fair comparisons between experiments. The impact of the duration of lysis on comet assay results has been studied using the *in vitro* comet assay, but there is a lack of evidence concerning the *in vivo* version. In this work, we have tested the effect of using different lysis periods on the *in vivo* comet assay outcome.
A single oral dose of 200 mg/Kg methyl methanesulfonate (MMS) – to induce strand breaks – or 5 mg/Kg MMS – to induce formamidopyrimidine DNA glycosylase (Fpg)-sensitive sites – was administered to male Wistar rats (n=3 rats/group). Rats treated with dimethyl sulfoxide were used as controls. After 3 hours animals were sacrificed, and liver, kidney and duodenum were extracted. These tissues from animals administered with 200 mg/Kg MMS and from control animals were processed using the standard alkaline comet assay after no lysis, 5 min, 1 hour or overnight lysis. Liver and duodenum from animals administered with 5 mg/Kg MMS and control animals were processed using the Fpg-modified alkaline comet assay after periods of lysis as above.
Although an increase in the amount of lesions detected by the standard comet assay was observed with increasing lysis duration up to 1 hour, no statistical differences were found among different lysis lengths, including the absence of lysis. However, as expected, the lysis step is necessary to measure Fpg-sensitive sites. The levels of Fpg-sensitive sites detected increased when increasing the lysis duration; this could be due to the nature of the Fpg-sensitive sites detected in animals administered with MMS.

Financial support: Spanish Ministry of Science and Innovation (BIOGENSA2, PID2020-115348RB-I00).

P11 **Are results with CometChip® and the two gels/slide comet assay comparable?**

Collia M. [1], Vettorazzi A.[1,2], López de Cerain A.[1,2] and Azqueta A.[1,2]

[1]*Department of Pharmacology and Toxicology, School of Pharmacy and Nutrition, Universidad de Navarra, 31008 Pamplona, Spain.*
[2]*IdiSNA, Navarra Institute for Health Research, Pamplona, Spain.*

Several technical papers have been published aiming at increasing the throughput of the comet assay. There is increasing interest in a 96-well platform with an array of micropores patterned in agarose (CometChip®), which can substantially improve the number of samples processed per experiment.
A comparison between the two gels/slide version of the comet assay and CometChip® was carried out. For that purpose, methyl methanesulfonate (MMS) and hydrogen peroxide (H_2O_2), at different concentrations, were employed as DNA damage inducers in TK6 cells. After the treatment, cells were washed and resuspended. The same cellular suspensions were used to charge the CometChip® and to embed cells in agarose for the two gels/slide format. In both versions, cells were lysed for 1 hour and incubated with the alkaline solution for 40 minutes at 4ºC. CometChip® was electrophoresed at 1 V/cm for 50 minutes and two gel slides at 1.2 V/cm for 20 minutes. DAPI was employed as fluorescent dye in both versions; however, comets on the CometChip® were stained overnight whereas those on the two gel slides were stained for 1 hour.
Dose-response curves were obtained from 3 independent experiments using both formats in parallel. Some differences were found in values of % DNA in tail; these tended to be higher in the two gels/slide version in the case of H_2O_2-treated cells, while in MMS-treated cells the opposite was the case (except at the highest concentration tested). Inter-experiment variability was modestly higher for the two gels version. Considering CometChip® features, it promises to be a useful tool for increasing the throughput of the assay.

Financial support: Spanish Ministry of Science and Innovation (BIOGENSA2, PID2020-115348RB-I00).

P12 **Could DNA damage in *Ligustrum vulgare* L. leaves also indicate soil pollution?**

Mujo Hasanović, Tamara Ćetković, Anesa Ahatović, Adaleta Durmić-Pašić

University of Sarajevo-Institute for Genetic Engineering and Biotechnology

In our earlier study, we explored the potential of widely distributed and stress resilient ornamental plant *Ligustrum vulgare* L. to serve as air pollution indicator in urban settings. The results of comet assay applied to selected *L. vulgare* leaves indeed show an increase in DNA damage proportional to the level of exposure to the air pollution. The control leaves did not show observable DNA damage. However, the examined leaves originated from the environment whereas the control leaves originated from the potted plants, which raised a concern that soil content may have contributed to the results. Various soil pollutants may induce a variety of deleterious effects thus hindering plant growth. Therefore, in order to assess DNA damage in *L. vulgare* leaves caused by soil pollution we conducted a controlled experiment. *L. vulgare* cuttings were rooted in serpentine soils rich in heavy metals (Ni, Cr, Mn, Co, Fe) collected from two sites (Bljuva and Papratnica) in the serpentine rich ophiolitic region of central Bosnia and Herzegovina. Concentrations of mineral nutrients and heavy metals in the samples were measured using Flame Atomic Absorption Spectroscopy (FAAS). The pH values of soil samples were determined in ddH_2O and KCl (Bljuva =7.5; 6.7; Papratnica = 7.9; 7.2). The FAAS analysis showed low Ca/Mg ratio (<1) as well as K concentration (Bljuva = 0.033%; Papratnica = 0.037%). Concentration of Fe exceeded recommended values by threefold. Extremely high concentrations of Ni were recorded on both serpentine sites (Bljuva = 2052.53 ppm Papratnica = 1352.51 ppm). The analyzed soil samples also showed high concentrations of other potentially toxic heavy metals (Ni, Cr, Co, Mn). The control cuttings were potted in a mixture of hummus and vermiculite. The plants were maintained in the control environment. DNA damage in newly grown *L. vulgare* leaves was evaluated using plant comet assay.

P13 **Double-stranded DNA breaks in the sperm of patients with normozoospermia and pathozoospermia**

Haurylchyk I.D., Ryabokon N.I.

Institute of Genetics and Cytology, National Academy of Sciences of Belarus, Minsk, Belarus

The key objective of the present study was to assess double-stranded DNA breaks (DSDBs) in human sperm as a diagnostic tool for male infertility. The frozen sperm samples of patients with normozoospermia (the controls, n=34) and pathozoospermia (n=44) were provided by the Republican DNA Bank of a Human, Animals, Plants and Microorganisms (Minsk, Belarus). The spermatozoa were studied using the neutral version of the comet assay for DSDB detection and the alkaline comet assay was used to detect the total number of single-stranded DNA breaks, DSDBs and alkaline-sensitive sites. The levels of DNA damages were analysed by visual scoring in arbitrary units and the number of cells with damaged (fragmented) DNA was estimated as the DNA fragmentation index (DFI) presented in per cent.
The DSDB values measured by the neutral comet assay were about 40% of DNA damages estimated by the alkaline comet assay in the control subjects and patients with pathozoospermia. Significantly higher levels of DSDBs were observed in patients with pathozoospermia ($p=1.1\times10^{-5}$) and confirmed in the most numerous subgroup with astenozoospermia (n=23, $p=3.4\times10^{-5}$) compared with the control. This way, the conducted testing allowed discriminating men with reduced fertility from fertile ones. A primary analysis of DSDBs induced in spermatozoa *in vitro* by the well-known radiomimetic bleomycin sulphate showed that the levels of DSDBs recorded in pathozoospermia correspond to the mutagenic effect of bleomycin sulphate at a concentration of 20-30 μg/ml, and, thus, it confirms a high degree of sperm DNA disintegrity in reduced fertility.
In conclusion, our data demonstrate that not only the sperm DNA fragmentation of different origin measured by the alkaline comet assay, but also the sperm DSDBs analysed separately are strongly associated with male infertility and can be used for a diagnosis of this pathology after additional studies.

P14 **The sensitivity of human peripheral blood lymphocytes to the DNA-damaging effect of pesticides and their combinations *in vitro***

Ilyushina N.A., Averianova N.S., Kara L.A., Grabovskaia A.A. Ignatiev S.D., Egorova O.V.

The Federal Budgetary Establishment of Science «Federal Scientific Center of Hygiene named after F.F. Erisman» of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing, Mytishchi, Russian Federation.

Assessment of individual sensitivity to genotoxicants is of particular importance in genetic toxicology. Some pesticides are known to cause genetic damage. Our prior studies showed that captan induces mutagenic/ genotoxic effects in the Ames test and *in vivo* micronucleus analysis. Fludioxonil was cytotoxic in the Salmonella/microsome test and did not increase the micronucleus incidence in mouse bone marrow erythrocytes. The formulations based on these pesticides are used in agriculture separately and in tank mixtures.The aim of the study was to evaluate the sensitivity of human peripheral blood lymphocytes (PBL) to captan, fludioxonil, and their mixture using alkaline DNA comet assay.PBL were incubated with 5 concentrations of pesticides in DMSO for 3 hours in RPMI-1640 with antibiotics, FBS, and glutamine at 37ºC in 5% CO_2. DNA damage was evaluated with Comet Assay IV software. The individual sensitivity was compared based on the fold increase of $\%DNA_{tail}$ after exposure over the background level of 1% DMSO. Statistically significant genotoxic effects were seen after cultivation of PBL with captan (2.5-25 μg/ ml), fludioxonil (125 μg/ml) and their mixture (2.5+2.5–25+125μg/ml).
The most pronounced effect of captan was observed in the absence of S9. The level of DNA damage at the high concentration outreached 400 times the negative control. The difference between donors was about 110 times. The %DNAtail in PBL of the most sensitive donor exceeded 30 times over 1% DMSO level after exposure with fludioxonil only in the absence of S9, thus suggesting that fludioxonil is probably a direct mutagen. The difference in responses between PBL of the donors was 7.5 times. The mixture of captan and fludioxonil induced DNA damage only without S9.Thus, captan, fludioxonil, and their mixture can cause DNA damage in human cells. The level of DNA damages in PBL from different donors varied to a great extent.

P15 **Evaluation of genotoxic potential of the middle section of the Danube River and its major tributaries**

Jovana Jovanović Marić[1], Margareta Kračun-Kolarević[1], Stoimir Kolarević[1], Jelena Đorđević[2,3], Karolina Sunjog[2], Jovana Kostić-Vuković[2], Momir Paunović[1], Branka Vuković-Gačić[3]

[1] University of Belgrade, Institute for biological research "Siniša Stanković", National Institute of Republic of Serbia, Department of Hydroecology and Water Protection, Bulevar despota Stefana 142, 11000 Belgrade, Serbia
[2]University of Belgrade, Institute for Multidisciplinary Research, Department of Biology and Inland Waters Protection, Kneza Višeslava 1, 11000 Belgrade, Serbia
[3] University of Belgrade, Faculty of Biology, Chair of Microbiology, Center for Genotoxicology and Ecogenotoxicology, Studentski trg 16, 11000 Belgrade, Serbia

The middle section of the Danube River basin in the previous three Joint Danube Surveys (JDS) was found to be under intensive pollution, mainly affected by wastewaters. As a consequence of the lack of wastewater treatment plants, complex xenobiotics mixtures may get into surface water and decrease water quality and organisms' health. Some xenobiotics may induce different types of DNA damage. Hence, DNA damage could be a useful biomarker in the detection of "early warning signals" of organisms' exposure to genotoxic compounds, while the usage of bioassays batteries can provide better insight into a genotoxic potential. This study was conducted in 2019, during the JDS4 expedition, to assess the genotoxic potential of nine sites on the section of the Danube River which goes through Serbia and its major tributaries: the Sava, Tisza and Velika Morava. Blood and muscle of *Alburnus alburnus* (bleak) were used for genotoxicity assessment by comet, micronucleus and RAPD assays. In comet assay, the highest level of DNA damage was recorded at the Velika Morava mouth (JDS 39) and on the Danube River, downstream Radujevac (JDS 41). The highest frequency of micronucleus was recorded at the Sava mouth (JDS 36) and on the Danube River, downstream Pančevo (JDS 37). The lowest level of DNA damage in both tests was recorded on the Sava, site Jamena (JDS 35). The RAPD analysis distinct three major groups: Tisza mouth (JDS 33) and sites on the Sava (JDS 35, JDS 36), then two sites on the Danube River (JDS 37, JDS 41) and one site on the Danube River, Ram (JDS 40) with the Velika Morava mouth (JDS 39). Analyzed bioassays showed different sensitivity where the comet assay had the highest potential in discrimination of sites based on genotoxicity. Bleak was proved to be a reliable bioindicator in eco/genotoxicological studies.

P16 **Effects of Di (2-ethyl hexyl) phthalate Exposure on Oxidative Stress and Epigenetic Profile in Early Adolescent and Adolescent Periods in Rat Brain**

S. Koc[1], E. Erdogmus[1], D. Ozkan-Vardar[2], U. Sur[3], P. Erkekoglu[4], B. Kocer-Gumusel[1]

[1]Lokman Hekim University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkey;
[2]Lokman Hekim University, Vocational School of Health Services, Pharmacy Services, Ankara, Turkey;
[3]Katip Celebi University, Faculty of Pharmacy, Department of Toxicology, İzmir, Turkey;
[4]Hacettepe University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkey

Endocrine disrupting chemicals (EDCs) are known to mimic hormone signalling and to change normal functions of metabolism, energy balance, thyroid function, and reproduction. Considered the effects of EDCs on epigenetic mechanisms they may affect the health of subsequent generations particularly when exposed in early life stages. Di (2-ethyl hexyl) phthalate (DEHP) is an endocrine disrupting chemical which is widely used in many consumer products. In this study, preadolescent Sprague Dawley male rats were exposed to 30 mg/kg/day (D1) and 60 mg/kg/day (D2) DEHP until the end of adolescence period and the toxicity of DEHP on brain were evaluated in adulthood by measuring oxidative stress parameters (total glutathione and lipid peroxidation) and epigenetic changes (DNA methylation and histone acetylation). Total glutathione levels in brain tissues increased significantly in the D1 group (51.3%) and the D2 group (98.5%) possibly as an adaptive response to oxidative stress induced by DEHP. Lipid peroxidation levels did not change in both groups compared to control. Global DNA hypomethylation and increased histone acetylation levels which are the most important indicator of the changes in epigenetic profile, were observed in both groups. These results indicate that DEHP exposure in early stages of life may be critical for epigenetic modifications in brain tissues. More detailed and mechanistic studies are needed in order to better understand the phthalate exposure on epigenetic alterations.

P17 **Genotoxicity of ambient PM_{10} at an urban site in Sarajevo, Bosnia and Herzegovina – assessment by comet assay in peripheral blood mononuclear cells**

Lejla Caluk Klacar[1], Tamara Cetkovic[1], Anja Haveric[1], Adna Softic[2], Lejla Haracic[2], Sabina Zero[2], Sanin Haveric[1]

[1]University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina
[2]Univesity of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina

Sarajevo, the capital of Bosnia and Herzegovina (B&H), is among the most air-polluted cities in Europe during the winter months. Approximately 20 percent of deaths in B&H are related to air pollution. The aim of this study was to evaluate DNA damage in normal peripheral blood mononuclear cells (PBMCs) treated with water-soluble fraction of ambient PM_{10} samples using alkaline comet assay. Daily PM_{10} samples were collected in February 2020 at an urban zone in Sarajevo, B&H, using a medium-volume air sampler. PM samples were extracted with ultrapure water Type 1 using ultra-sonication at room temperature, followed by filtration of water suspensions, and stored at -20°C. Four days out of the seven days of the collection period were selected for the analysis: two with the highest determined PM_{10} concentrations (77.08 $\mu g/m^3$; 102.29 $\mu g/m^3$) and two with the lowest concentrations (14.07 $\mu g/m^3$; 18.61 $\mu g/m^3$). Six treatments were set, four for each selected day, positive (70μM H_2O_2) and negative control (dH_2O). PBMCs were treated for 3h. DNA damage was analyzed in 100 cells for each treatment using Comet Assay IV software and measuring tail intensity (%). The results showed a concentration-dependent manner of DNA damage compared to controls.

P18 **Heat killed paraprobiotic *Lactiplantibacillus plantarum* S1 reduces DNA damage in induced oxidative stress**

Deni Kostelac[1], Marko Gerić[2], Goran Gajski[2], Jadranka Frece[1]

[1]*Faculty of Food Technology and Biotechnology, Zagreb, Croatia*
[2]*Institute for Medical Research and Occupational Health, Zagreb, Croatia*
deni.kostelac@pbf.unizg.hr

Oxidative stress is characterized by the inability of the organism to detoxify reactive oxygen species (ROS) and can be defined as a condition in which the balance between prooxidants and antioxidants in the cell is disturbed, leading to DNA hydroxylation, protein denaturation, lipid peroxidation, and cell apoptosis. The antioxidant effects of probiotic cells and probiotic-enriched foods have been previously noticed, but the exact mechanisms of antioxidant action are not fully understood. Literature suggests that probiotic therapy improves host oxidative status, which may lead to prevention of some chronic diseases. The concept of paraprobiotics has been proposed to refer to the use of inactivated microbial cells or cell fractions that provide health benefits to the host. Hence, we aimed to exert H_2O_2-induced oxidative stress on isolated human lymphocytes and investigate the protective effect of heat killed probiotic bacteria *Lactiplantibacillus plantarum* S1 and extracted bacterial metabolites smaller than 2.000 Daltons on nuclear DNA of isolated lymphocytes, using the comet assay. Both, metabolites and heat killed cells significantly reduced the H_2O_2-induced DNA damage (from 7.59±1.51% in induced stress to 4.07±1.61% for metabolites and 3.86±1.58% for heat killed cells), displaying a protective role. In combined treatment of heat killed cells and metabolites, the DNA damage was significantly lower (2.62±1.02%) than oxidative stress sample and the levels of primary DNA strand breaks did not differ compared to control sample (1.33±0.14%). Probiotic metabolites and heat-killed cells could potentially be used in a modified model to reduce the adverse effects associated with oxidative stress in the lower parts of the gastrointestinal tract.

P19 **Sensitivity of invasive alien fish in Serbia black bullhead *Ameiurus melas* (Rafinesque, 1820) as a bioindicator of genotoxicity**

Jovana Kostić-Vuković[1], Karolina Sunjog[1], Stoimir Kolarević[2], Marija Smederevac-Lalić[1], Zoran Marković[3], Stefan Skorić[1] and Milica Jaćimović[1]

[1] University of Belgrade, Institute for Multidisciplinary research, Department of biology and inland waters protection, Belgrade, Serbia
[2] University of Belgrade, Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia, Department of Hydroecology and Water Protection, Belgrade, Serbia
[3] University of Belgrade, Faculty of Agriculture, Institute of Animal Science, Department of Growing and Reproduction of Domestic and Raised Animals, Belgrade, Serbia

Invasive alien fish species represent one of the greatest threats to the biodiversity and ecological status of freshwater ecosystems. Within their management, their application as bioindicators of pollution in monitoring programs should be considered. The aim of this study was to examine the use of black bullhead *Ameiurus melas* (Rafinesque, 1820), invasive alien fish in Serbia, as a bioindicator of water genotoxicity by alkaline comet assay. Sampling was performed from June to September 2021 on Markovačko Lake, which is exposed to diffuse pollution from the application of pesticides on the surrounding agricultural land. Monitoring also included the assessment of water quality by basic physical and chemical parameters, as well as microbiological indicators of faecal pollution. Alkaline comet assay was performed on fish erythrocytes, on ten specimens per month. The study also included ten control fish from the recirculating aquaculture system (RAS), which were transferred from Markovačko Lake for breeding, and treatment of their erythrocytes with H_2O_2 as a positive control. Scoring of comets was performed by CometScore 2.0 software. The lowest level of DNA damage was recorded in the control group and the highest in the positive control group. August was the month with the lowest level of DNA damage, and September with the highest. Statistically significant differences were found between all groups indicating a high sensitivity of the alkaline comet assay to discriminate fine differences in the genotoxic potential of water. Knowing that black bullhead is highly abundant in stagnant waters and relatively easy to sample, this study draws additional attention to its use as a bioindicator of genotoxicity.

P20 ***In vivo*** **pharmacokinetics and biodistribution of gold nanoparticles**

Katarina Kozics[1], Monika Sramkova[1], Kristina Jakic[1], Tibor Dubaj[2], Peter Simon[2], Alena Gabelova[1]

[1]Department of Nanobiology, Cancer Research Institute, Biomedical Research Center SAS, Dubravska cesta 9, 845 05 Bratislava, Slovak Republic
[2]Institute of Physical Chemistry and Chemical Physics, Slovak University of Technology in Bratislava, Radlinskeho 9, 812 37 Bratislava, Slovak Republic

The outstanding physicochemical properties, well-established synthetic procedures, and easy surface modifications make gold nanoparticles (AuNPs) an emerging platform for a wide range of pharmaceutical and biomedical applications. Despite the obvious advantages of gold nanoparticles for biomedical applications, controversial and incomplete toxicological data hamper their widespread use.
Here, we present the results from an *in vivo* toxicity study using gold nanoparticles coated with polyethylene glycol (PEG-AuNPs). The pharmacokinetics and biodistribution of PEG-AuNPs were examined in the rat's liver, lung, spleen, and kidney after a single i.v. injection at different time intervals. PEG-AuNPs had a relatively long blood circulation time and accumulated primarily in the liver and spleen, where they remained for up to 28 days after administration. We identified significant changes in lipid metabolism, altered levels of liver injury markers, and elevated monocyte count 24 h and 7 days after PEG-AuNPs exposure, suggesting the immunomodulatory effects of PEG-AuNPs. In blood cells, no DNA damage was present in any of the studied time intervals.
Our results indicate that the tissue accumulation of PEG-AuNPs might result in late toxic effects.

Funding: This study is based upon work from COST Action CA 17140 "Cancer Nanomedicine from the Bench to the Bedside" supported by COST, project HISENTS No. 685817, project VISION (Strategies to strengthen scientific excellence and innovation capacity for early diagnosis of gastrointestinal cancers) No. 857381, VEGA grants 2/0055/20, 2/0121/21.

P21 **Bioactivity of the Joint Danube Survey 4 surface water samples collected by horizon large volume solid–phase extraction technique**

Margareta Kračun-Kolarević[1], Jovana Jovanović Marić[1], Peter Oswald[2], Martina Oswaldova[2], Jaroslav Slobodnik[2], Branka Vuković-Gačić[3], Momir Paunović[1], Katja Kološa[4], Bojana Žegura[4], Stoimir Kolarević[1]

1 *University of Belgrade, Institute for Biological Research ¨Siniša Stanković¨, National Institute of Republic of Serbia, Department of Hydroecology and Water protection, Bulevar despota Stefana 142, 11000 Belgrade, Serbia*
2 *Environmental Institute, Okružna 784/2, 97241 Koš, Slovak Republic*
3 *University of Belgrade, Faculty of Biology, Chair of Microbiology, Center for Genotoxicology and Ecogenotoxicology, Studentski trg 16, 11000 Belgrade, Serbia*
4 *National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Večna pot 111, 1000 Ljubljana, Slovenia*

Within the Joint Danube Survey 4 (JDS4) (2019) surface water samples were collected at 24 localities along the Danube River and were extracted by horizon large volume solid-phase extraction (LVSPE) technique. LVSPE technique provided 25,000x relative enrichment factor (REF) of the river samples. The bioactivity of the LVSPE samples was investigated employing prokaryotic *SOS/umuC* assay (S9+ and S9-) and battery of bioassays analyzing different endpoints – cytotoxicity (MTS assay), genotoxicity (comet assay), cell cycle analyses of ZFL cells and embryo toxicity (zFET) in eukaryotic models (zebrafish liver cell line – ZFL and *Danio rerio* embryos – zebrafish). In the case of the *SOS/umuC* assay none of the tested samples (REF100) has induced genotoxic effect. The cytotoxic effect was detected for 11 samples at REF100 (MTS assay). The comet assay results indicated genotoxic potential for nine samples – one in the Upper Danube, six in the Middle Danube and two in the Lower Danube. The most potent were JDS4-37 and JDS4-41 samples collected at two sites in the Serbian part of the Danube River. These samples were analyzed at REF13. The analysis of the cell cycle was performed in the case of eight samples for which were previously recorded genotoxic effect. For one sample (JDS4-41) was found the G1 phase cell cycle arrest. The zFET assay was performed for all the samples at REF100 and there was no embryo toxicity or teratogenicity recorded. The results obtained within this study indicate a higher sensitivity of eukaryotic models compared to prokaryotic *SOS/umuC* assay. Likewise, it was found that most of the genotoxic samples originate from the middle part of the Danube.

P22 **Effects of the 1,4-dihydropyridine salts assessed in vitro and in vivo**

Elina Leonova[1], Vitalijs Borisovs[1], Egils Bisenieks[2], Gunars Duburs[2] and Nikolajs Sjakste[1]

[1]University of Latvia, Jelgavas Street 1, Riga, Latvia
[2] Latvian Institute of Organic Synthesis, Aizkaraukles Street 21, Riga, Latvia

Some data indicate ability of metal ions forming salts with organic compounds to modify biological effects of the latter. We have assessed several effects of different salts of the 1,4-dihydropyridine AV-153. Affinity of the AV-153 salts to DNA measured by a fluorescence assay was dependent on the metal ion forming a salt in position 4 of the 1,4-DHP, and it decreased as follows: Mg>Na>Ca>Li>Rb>K. All AV-153 salts under study quenched the fluorescence of ethidium bromide and DNA complex that points to intercalation binding mode. The binding *via* intercalation was confirmed by means of cyclic voltammetry and circular dichroism spectroscopy. It was shown that *in vitro* all salts of AV-153 could interact with four DNA bases. Data of FTIR spectroscopy indicated to the interaction of AV-153 salts both with bases and phosphate groups of DNA. Preference for base interaction was observed. AV-153 salts interacted mostly with G and C bases, however highest differences were detected in the spectral region assigned to phosphate groups X-ray diffraction experiments revealed unattended spatial position of the metal ions and carboxy group. AV-153-K and AV-153-Rb could not react chemically with peroxynitrite as opposed to AV-153-Mg and AV-153-Ca, the latter increased the decomposition rate of peroxynitrite. AV-153-Na and AV-153-Ca effectively reduced DNA damage induced by peroxynitrite in HeLa cells, while AV-153-K and AV-153-Rb were less effective, AV-153-Li did not protect the DNA, and AV-153-Mg even caused DNA damage itself. The Na, K, Ca and Mg AV-153 salts were also shown to reduce the level of DNA damage induced by the HIV Tat-protein in human B-cells. Thus metal ions modify both DNA-binding and DNA-protecting effects of the AV-153 salts. In animals the salts produced different effects on DNA repair-involved and proteasomal gene expression.

P23 **The comet assay in *Pieris brassicae* (Lepidoptera, Pieridae): a useful tool to evaluate genotoxic effects induced by particulate matter (PM_{10})**

Manuela Macrì[1], Marta Gea[2], Irene Piccini[1], Luca Dessì[1], Alfredo Santovito[1], Simona Bonelli[1], Tiziana Schilirò[2], Sara Bonetta[2].

[1]*Department of Life Science and Systems Biology, University of Torino, Torino, Italy*
[2]*Department of Public Health and Pediatrics, University of Torino, Torino, Italy*

Atmospheric pollution poses a serious threat to human health and particulate matter (PM) is one of the major contributors. Genotoxicity induced by PM has been investigated in different studies using prokaryotic/ mammalian cells and laboratory animals; however, the use of invertebrates for environmental risk assessment has been poorly explored.
The aim of this study was to evaluate the usefulness of the comet assay on a common butterfly species (*Pieris brassicae)* for investigating the genotoxic effects induced by PM_{10} samples.
PM_{10} was collected from April to September in a traffic site (T) and two rural sites (R1 and R2) with different pollution levels located in the North of Italy. *P. brassicae* caterpillars (n=117) were grown on cabbage plants (10 days) and they were exposed to PM_{10} organic extracts (40 m^3/mL) or dimethyl sulfoxide (controls) through vaporization. After exposure, caterpillars were dissected and cells were used for the comet assay. Different cell suspensions, method of collection and slide preparation were evaluated.
The comet assay protocol was optimised with the cell suspension obtained from the insect abdomens allowing to obtain an adequate number of cells and a good quality of the comets.
Results of the comet assay showed that all PM extracts induced significant DNA damage in exposed caterpillars compared to controls (Generalized Linear Model, $p<0.001$). Moreover, the highest genotoxic effect was induced by extract of T site (Tail Intensity %=15.48) while a lower effect was observed for the extracts of less polluted rural sites (R1 TI%=9.55; R2 TI%=9.67).
In conclusion, the comet assay was found to be a useful tool for detecting the genotoxic effect of PM samples with different concentration and composition in caterpillars of a butterfly species. Moreover, *P. brassicae* seems to be a sensitive and promising bioindicator to investigate air quality and PM genotoxicity.

P24 **DNA damage of $PM_{2.5}$ samples during the COVID-19 pandemic: a comparison with the pre-lockdown period in Padana Plain.**

Manuela Macrì[1], Marta Gea[2], Sara Bonetta[2].

[1]Department of Life Science and Systems Biology, University of Torino, Torino, Italy
[2]Department of Public Health and Pediatrics, University of Torino, Torino, Italy

In 2020, Sars-CoV-2 caused the COVID-19 pandemic. To limit virus spread, containment measures were applied in all countries (e.g. lockdown). Restrictions influenced air pollutant sources (e.g. traffic, domestic heating) and affected air quality, including particulate matter (PM), in many world area. PM is an important air pollutant and its finest fraction ($PM_{2.5}$) has been classified as carcinogenic to humans.
The aim of the study was to evaluate the role of lockdown and restriction measures on PM genotoxicity in four sites in Piedmont Region (Padana Plain): urban traffic (T), urban (U), suburban near incinerator (I) and rural (R).
Daily $PM_{2.5}$ samples collected in 2020 (lockdown) were weighted and pooled according to restriction measures: January/February (no restrictions), March (lockdown), April (lockdown), May/June (low restrictions), July/August/ September (low restrictions), October/November/December (medium restrictions). 2019 samples (pre-lockdown period) were pooled as 2020 samples for comparison. Organic extracts of $PM_{2.5}$ were tested on human bronchial cells (BEAS-2B) to evaluate genotoxicity using comet assay.
No significant difference was observed for $PM_{2.5}$ concentrations and organic pollutants (benzo(a)pyrene, benzo(a)anthracene, indeno(1,2,3-cd)pyrene, benzo(b,j,k)fluoranthene) between 2020 and 2019 in all investigated sites. The results of the comet assay showed that, during lockdown months (2020), $PM_{2.5}$ genotoxicity was lower in some sites (sites U and I) with respect to previous year (2019). However, in the other sites no differences were observed. During the medium restriction period (2020) some sites (sites I and R) showed lower level of DNA damage than in 2019, while in the site U a higher DNA damage was observed.
In conclusion, although the lockdown and the restriction measures may have contributed to reduce or modify pollutant emissions, the $PM_{2.5}$ genotoxicity seems to be decreased only in some sites. This trend is probably related to the complex origin and formation of PM and to the particular conformation of Padana Plain.

P25 **Genotoxicity assessment of some industrially processed meat products in the human Caco-2 cell line using the alkaline comet assay**

Chamlal naima[1], Khallef messaouda[2], Benouareth djamel eddine[2], Frenzilli giada[3]

[1] Laboratoire de biologie, eau et environnement, Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre et de l'Univers, Département de Biologie, Université 8 Mai 1945 Guelma BP401, Guelma 24000, Algeria
[2]Département de Biologie, faculté des sciences de la nature et de la vie et sciences de la terre et de l'univers, Université 8 Mai 1945 Guelma BP401, Guelma24000, Algeria
[3]Department of Clinical and Experimental Medicine-Section of Applied Biology and Genetics, University of Pisa, 56126 Pisa, Italy
khallef.messaouda@univ-guelma.dz

Industrially processed meat products are widely consumed by the Algerian population, particularly because of their availability in different flavors and their low cost compared to raw meats. Previous studies showed that these products have a positive correlation with the incidence of cancer. Nevertheless, the genotoxic risk associated with these products has not yet been assessed. Therefore, the aim of this study is to evaluate the potential genotoxic effect of five industrially processed meat products sold in Algeria (Salami, smoked roast chicken, smoked roast beef, chicken chawarma and beef jambon). Possible genotoxic compounds were extracted from the meat samples by maceration using a polar solvent (methanol) and a non-polar solvent (n-hexane). The alkaline version of the comet assay was used to evaluate DNA damage stemming from meat extracts exposure in human Caco-2 cells. To avoid false positive results, cytotoxic threshold was firstly established using the trypan blue exclusion assay. The results showed that all hexane extracts tested were genotoxic. Whereas, three out of the five methanol extracts investigated were also genotoxic. Our findings imply that in Algeria today, there are still products the production procedures of which should be refined to reduce the potential risk of genotoxicity to consumers. Consequently, more investigations should be performed with an accurate analytical approach in order to be in a position to suggest new recommendations on the permissible daily intake for those meat products.

P26 **Influence of antioxidants resveratrol and melatonin on telomere dynamics and DNA damage in liver and kidney of the Wistar rat model of aging**

Lucia Nanić[1], Marko Gerić[2], Goran Gajski[2], Jasna Jurasović[,2], Dean Karaica[,2], Marija Ljubojević[,2], Vedran Micek[,2], Ivana Novak Jovanović[,2], Tatjana Orct[,2], Maja Peraica[,2], Dubravka Rašić[,2], Ivana Vrhovac Madunić[,2], Ivan Sabolić[,2], Davorka Breljak[,2], Ivica Rubelj[1]

[1]Ruđer Bošković Institute, Zagreb, Croatia
[2]Institute for Medical Research and Occupational Health, Zagreb, Croatia

Aging is the major risk factor for the emergence of numerous pathological conditions leading to the onset of age-related diseases. Large body of evidence suggests that telomere shortening and DNA damage in cells may lead to the progression of senescence and aging. Oxidative stress also plays an important role in telomere dynamics. Various strategies for the neutralization of oxidants are used. Most widespread among them involves the use of antioxidant supplements, which are commonly considered healthy. Today, frequently used antioxidants are resveratrol and melatonin, both for experimental purposes and in various diets. To test whether oxidative stress could be ameliorated by antioxidants, we treated male and female Wistar rats for 9 or 21 months with melatonin and resveratrol (daily dose 300−400 μg/kg b.m.). We investigated their effect on telomere attrition rate in the liver and different zones of the kidney. Telomere length was assessed by Southern blotting of genomic DNA. We also investigated the baseline DNA damage using the comet assay in kidneys and liver of treated animals. Significant reduction in telomere loss in the liver of female Wistar rats after 21-month long resveratrol treatment has been observed, while other tissues did not show significant differences. Contrary to expected beneficial properties of tested antioxidants, in our experimental system following long-term treatment with melatonin and resveratrol in both Wistar male and female rats, these effects have not been observed. These findings call for more cautious use of these antioxidants as anti-aging supplements in the human population.

Supported by the Croatian Science Foundation projects IP-2018-01-8654 (REPROSKIN) and IP-11-2013-1481 (AGEMETAR).

P29 **DNA damage in blood cells in relation to chemotherapy and nutritional status in colorectal cancer patients**

Anne Lene Nordengen[1,2,3], Congying Zheng[1,4], Andrew Collins[1], Truls Raastad[3,5], Rune Blomhoff[3,5], Sergey Shaposhnikov[1]

[1]*Norgenotech AS, Oslo, Norway*
[2]*Department of Nutrition, University of Oslo, Oslo, Norway*
[3]*Department of Public Health, Sport and Nutrition, University of Agder, Kristiansand, Norway*
[4]*Maastricht University, Maastricht, The Netherlands*
[5]*Department of Physical Performance, Norwegian School of Sport Science, Oslo, Norway*
[6]*Department of Clinical Service, Division of Cancer Medicine, Oslo University Hospital, Oslo, Norway*

Background: Dietary factors are recognized to have an impact on the risk of cancers, with different dietary elements both increasing and reducing risk. In the specific case of colorectal cancer (CRC), World Cancer Research Fund (WCRF)/American Institute of Cancer (AICR) has reported that intake of whole grains, non-starchy-vegetables and fruits, and foods containing dietary fibre reduce the risk, while intake of red- and processed meat, alcoholic drinks and body fatness increase the risk of this disease[1]. CRC therefore represents a cancer type with great potential for dietary and lifestyle prevention. Much less is known about the role of the post diagnostic diet on CRC progression, relapse, and survival.
Method/design: The randomized controlled trial 'CRC-NORDIET' is the first study to test whether dietary intervention in CRC patients after surgery can improve disease-free survival. CRC patients are randomly assigned either to a group receiving intensive encouragement to adopt a diet in accordance with the WCRF recommendations, or to a control group receiving standard care. In addition to the WCRF recommendations, foods and drinks with the potential of dampening inflammation and oxidative stress are emphasized. CRC patients are clinically assessed at baseline, 6 months, 1 year and 3 years, and will be monitored for up to 15 years after diagnosis. For each patient, the comet assay will be used to measure basal DNA breakage, antioxidant resistance and endogenous oxidative damage. In addition, we are obtaining data on individual capacity for DNA repair.

References

1. World Cancer Research Fund/American Institute for Cancer Research. Diet, nutrition, physical activity, and cancer: a global perspective. Continuous update project expert report. 2018 Aug 1

P30 **DNA repair in ovarian cancer therapy response: The role of MRE11**

Alena Opattova[1,2,3], Dominika Vallusova[1,4,5], Josef Horak[1,6], Pavel Vodicka[1,2,3]

[1]Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic
[2]First Faculty of Medicine, Charles University, Prague, Czech Republic
[3]Biomedical Center in Pilsen, Charles University, Pilsen, Czech Republic
[4]Faculty of Science, Charles University, Prague, Czech Republic
[5]GHC Genetics, Prague, Czech Republic
[6]Third Faculty of Medicine, Charles University, Prague, Czech Republic

Cancer therapy failure, mainly represented by development of chemotherapy resistance or outgrowth of metastasis, is the major complication for cancer patients. Cancer chemotherapy resistance is the ability of cancer cells to blunt and counteract the effects of chemotherapeutics. The important mechanism in cancer therapy and chemoresistance is an alteration of DNA repair pathways. The overexpression of DNA repair genes in the tumor may confer more efficient repair of chemotherapy-induced damage, thus contribute to the chemoresistance. On the other hand, downregulation of the DNA repair genes may lead to a better therapy response but may also give a foundation for arising of new mutations and cancer progression. Therefore, there is a crucial clinical need for the better understanding of cellular processes associated with drug resistance.
The main aim of our research is to define a role of DNA repair pathways, mainly homologous recombination, in cancer therapy response in ovarian cancer. MRN complex, protein complex consisting of MRE11-RAD50-NBS1, plays an important role in initial processing of DSBs repair, mainly HR. Our previous findings showed association of MRE11 with progression free survival in colorectal cancer. Current results showed that inhibition of MRE11 by mirin leads to accumulation of DNA damage and increases the sensitivity of the ovarian cells to carboplatin ($p<0.05$). Moreover, our experiments on ovarian cells with acquired resistance to carboplatin showed that inhibition of MRE11 by mirin could overcome ovarian cancer resistance ($p<0.01$).
Our results suggest that downregulation of MRE11, could be associated with a better therapy response in carboplatin-treated ovarian patients.

Acknowledgement: GACR 19-10543S, AZV NU21-03-00145

P31 **In vitro Evaluation of Cytotoxicity and Apoptotic Activities of Quercetin in BEAS-2B and A549**

D. Ozkan-Vardar[1], T. Duran[2,] M. Aydin Terzioglu[3]

[1]*Lokman Hekim University, Vocational School of Health Services, Pharmacy Services, Ankara, Turkey*
[2]*KTO Karatay University, Medicinal Faculty, Konya*

People need to eat healthy in order to grow, develop and sustain their lives. Quercetin is a phenolic compound found in many plants. There are many in vitro and in vivo toxicology studies on the effects of quercetin in this area. In the literature review, there is no study examining the changes in genes related to cytotoxicity and apoptosis in lung cancer cell lines. Lung cancer has a special importance in terms of incidence and mortality rate in the world. In this study, it was aimed to investigate the cytotoxic and apoptotic effects of quercetin on BEAS-2B and A549 cell lines cultured from lung cancer tissues. The determination of their cytotoxic effects on cell culture in vitro will be determined by applying the MTT (3, (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) test. Apoptosis, also defined as genetically controlled programmed cell death, is an event that maintains the existing homeostasis in the organism. Particularly associated with cancer, apoptosis is a mostly energy-dependent process involving the activation of a group of cysteine proteases called caspases. In order to determine the apoptotic effect, Bax, Bad, Bak1, Bcl-2, Casp2, Casp3, Casp9, Casp12, Bcl-xl, Apaf1, P53 gene expressions will be examined. It is thought that quercetin will reveal the positive or negative aspects of the active ingredient and add a new perspective to cancer treatment. Different concentrations (6-145 µM) of quercetin have been applied in BEAS-2B and A549 cell lines. Quercetin decreased MTT cell viability at 24, 48 and 72 hours and caused cell death by apoptotic pathway. It has excellent potential to be converted into an antitumor precursor compound.

P32 **1500-kcal-nutritionally balanced reduction *vs.* 567-kcal-hospitally controlled diet differences on anthropometric, biochemical, oxidative, primary and permanent DNA damage parameters in severely obese patients with BMI ≥ 35kgm^{-2} after 3 weeks of diet–preliminary results**

Ivan Ožvald[1], Martina Bituh[2], Hrvoje Radašević[3], Maja Nikolić[4a], Martina Matovinović[5], Dragan Božičević[1], Lidija Duh[1], Ivana Vinković Vrček[4b], Ana-Marija Domijan[6] and Mirta Milić[4a]

[1]Special Hospital for Extended Treatment of Duga Resa, Duga Resa, Croatia [2]Laboratory for Food Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, Zagreb, Croatia [3]Andrija Stampar Teaching Institute of Public Health, 10000 Zagreb, Croatia [4]Institute for Medical Research and Occupational Health (IMROH), Zagreb, Croatia. [4a]Mutagenesis Unit, [4b]Analytical Toxicology and Mineral Metabolism Unit, [5]Department of Internal Medicine, University Hospital Centre Zagreb, Zagreb, Croatia [6]University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Pharmaceutical Botany, Zagreb, Croatia

We wanted to check the differences of anthropometric, biochemical, oxidative, primary and permanent DNA damage parameters in severely obese patients with BMI ≥ 35kgm^{-2} after 3 weeks between 567 kcal-hospitaly-controlled VLCD (26 patients) and nutritionally balanced reduction diet of 1500 kcal daily (10 patients in this preliminary study). Both diets did not cause significant differences in triglycerides, TSH, ft4 and ft3 levels, but both caused decrease in anthropometric and other biochemical parameters. Among anthropometric parameters only VLCD group had significant decrease in weight. Diets caused BMI reduction (VLCD-in average 3-4 BMI units' loss, 1500-kcal-1 unit), excessive weight loss (between 10-35%-VLCD and 13%-1500 kcal) and weight loss (average 9 kg, range 4.8-14.4kg for VLCD and only 4 kg average for 1500 kcal). Also, only VLCD caused significant decrease in glucosis, urea, total cholesterol-C, HDL-C, LDL-C, insulin and HOMA-IR levels, while 1500-kcal caused only slight decrease. Changes were also observed in DNA damage in the following manner: VLCD caused significant decrease in TI comet assay values, while both diet caused significant decrease in Net FPG TI values. In cytochalasin-B-blocked micronucleus assay, VLCD caused significant decrease of MN (micronuclei), NB (nuclear buds), NPB (nucleoplasmic bridges) and apoptosis frequencies, while 1500 kcal caused also decrease, but only significant one was in the frequency of NB and apoptosis. According to this preliminary results it seems that more strict diet has better results on human health and DNA stability improvement in severely obese patients with comorbidities, but both were able to decrease oxidative DNA damage, that is considered as the first factor leading to increased genomic instability, that again can lead to higher cancer risk and mortality.

P33 **Higher concentrations of zinc sulfate impair chromatin integrity, tight junction proteins, and lncRNA expression**

Stella Marie Reamon-Buettner, Lasse Liebisch, Ines Voepel, and Christina Ziemann

Fraunhofer Institute for Toxicology and Experimental Medicine, Nikolai-Fuchs-Strasse 1, 30625 Hannover, Germany

Zinc is an essential trace element of biological processes such as in growth, development, and immunity. Thus, zinc deficiency has been implicated in several diseases. But increased zinc levels can also lead to toxicity through inhalation, oral, and dermal exposures. To gain further insights into molecular cues of zinc (geno)toxicity, we analyzed different concentrations (50 – 350 µM) of zinc sulfate ($ZnSO_4$), an inorganic compound and an important source of dietary zinc. We determined various endpoints in mouse fibroblasts (L929) as well as in human mesothelial (LP9) and lung epithelial (NHBE) cells treated with $ZnSO_4$ for 19 – 24 h. Cells were grown under submerse or air-liquid interface (ALI) conditions during treatment. In L929 cells, we measured cytotoxicity by cell count, WST-1 assay, and lactate dehydrogenase release. At 50 – 150 µM $ZnSO_4$, we obtained concentration-dependent cytotoxicity and increased frequency of micronuclei. Fluorescence in situ hybridization (FISH) with centromere-specific probes showed that about 90% of micronuclei had FISH signals, suggesting aneugenic events and genomic instability. In LP9 cells, at >200 µM $ZnSO_4$, FISH with centromeric probes showed micronuclei with FISH signals, and large cell nuclei with distended centromeric satellite DNA. The distension of satellite repeats at centromere is referred to as senescence-associated distension of satellites (SADSs), considered as an early marker of cellular senescence. In ALI-cultured NHBE cells at >200 µM $ZnSO_4$, immunofluorescence also showed impairment of tight junction proteins (ZO1 and OCLN) and the microtubule protein, beta-tubulin IV. Furthermore, by RNA FISH with two lncRNAs implicated in lung cancer, we observed alterations of *NEAT1* and *MALAT1* expression associated with abnormal chromatin morphology. Overall, we demonstrate that higher concentrations of $ZnSO_4$ can induce various cellular damage that may help in better understanding of zinc (geno)toxicity.

P34 **Comet Assay for human health purposes**

Ryabokon N.I.

Institute of Genetics and Cytology, National Academy of Sciences of Belarus, Minsk, Belarus

The purpose of this work was to assess human genome stability in the course of various diseases using the comet assay.
Previously, we demonstrated that the alkaline version of the comet assay is a suitable tool for the study of DNA base excision repair, in particular during the first 5 min after *in vitro* genotoxic stress in human cells (culture cell lines or peripheral blood mononuclear cells). We found that this time interval is crucial for the DNA repair rate and efficiency and depends on the level of poly(ADP-ribose) formation, which can be modulated upwards and downwards by various groups of compounds.
The further study showed that the alkaline comet assay allows detecting increased DNA damage levels and genome sensitivity to oxidative stress *in vitro*, as well as a decreased DNA repair rate and efficiency in peripheral blood mononuclear cells of children with autoimmune diseases (juvenile idiopathic arthritis or systemic lupus erythematosus). These data may indicate genomic instability in humans with autoimmune diseases and its importance for a disease prognosis should be studied in more detail.
In a more recent study, we found that both alkaline and neutral versions of the comet assay detect increased DNA fragmentation levels in human sperm in association with decreased male fertility. Besides, our study evaluates that some somatic diseases elevate DNA damage in sperms of patients and controls. Thus, our results show that alkaline and neutral versions of the comet assay may be used for the identification of a possible genetic cause of decreased male fertility and for monitoring the efficiency of subsequent therapeutic treatment and/or lifestyle change.
Altogether, our data demonstrate the importance of genome stability analysis for human health purposes, as well as the high sensitivity and broad usefulness of the comet assay for experimental research work and human health risk assessment.

P35 **Genotoxicity of pyrrolizidine alkaloids in HepG2 cells *in vitro***

Naji Said Aboud Hadi [1, 2], Ezgi Eyluel Bankoglu [1] and Helga Stopper [1]

[1] *Institute of Pharmacology and Toxicology, University of Wuerzburg, Wuerzburg, Germany.*
[2] *School of Health and Human Sciences, Pwani University, Kilifi, Kenya.*

Hundreds of different pyrrolizidine alkaloids (PAs) have been discovered in thousands of plants as natural constituents. Certain PAs are hepatotoxic and genotoxic and are potentially harmful to humans via food, food supplements and herbs/spices. PAs can cause hepatic sinusoidal obstruction syndrome and are carcinogenic in animals.
Here we investigated the genotoxicity of PAs of different esterification types, such as europine, retrorsine and lasiocarpine, in HepG2 cells using the cytokinesis-block micronucleus (CBMN) assay and the comet assay. DNA-crosslinking activity was investigated using a modified comet assay. Chemical inhibitors were used to investigate the role of metabolic activation and of in- and outward trans-membrane transport.
An increase in micronucleus formation was found with all tested PAs of different chemical structures in the micromolar range. In the comet assay, PAs did not cause increased DNA damage after 24 hours treatment, but in the modified crosslink comet assay, the diester type PAs reduced tail formation after hydrogen peroxide treatment, while an equimolar concentration of the monoester europine did not significantly reduce DNA migration. In addition, micronucleus induction by lasiocarpine was abolished after pre-treatment with the cytochrome P450 inhibitor ketoconazole and reduced by the addition of inward transporter inhibitors, while addition of outward transporter inhibitors slightly increased the genotoxic damage.
In conclusion, PAs are genotoxic and the extent and quality of DNA-damage is influenced by their ester-type. Metabolic activation plays a crucial role in PAs genotoxicity, while trans-membrane transporters only affect it to a limited extend.

P36 **Impact of cell type and exposure time on the quantitative outcome of *in vitro* genotoxicity tests**

J. Sanders[1,2], A. Thienpont[1,2], R. Anthonissen[1], T. Vanhaecke[2]* and B. Mertens[1]*

[1]Risk and health impact assessment, Sciensano, Brussels, Belgium
[2]Department of In-vitro Toxicology and Dermato-Cosmetology, Vrije Universiteit Brussel, Brussels, Belgium
**Equally contributing senior scientists*

Although genotoxicity data have long only been used in a qualitative way, there is currently a paradigm shift ongoing towards the quantification of genotoxic effects. Such a quantitative use of genotoxicity data requires a better understanding of the factors that may affect the sensitivity i.e. the lowest concentration at which a genotoxic effect is detected, in particular for *in vitro* tests. In this context, we analysed the impact of two parameters, i.e. cell type and exposure time, on the sensitivity of the *in vitro* micronucleus and the *in vitro* comet assay. Hereto, two known genotoxic compounds, i.e. aflatoxin B1 and ethyl methanesulfonate, were tested in both assays using two different cell types (i.e. CHO-K1 and TK6 cells) and two exposure times (i.e. 3h and 24h). Analysis of the results was done with the benchmark dose covariate approach. Our data showed that the cell type may indeed have an impact on the sensitivity of the *in vitro* micronucleus test to detect a genotoxic compound, but only in the 24h scenario. For the *in vitro* comet assay, the sensitivity was not affected by the cell type for the investigated compounds. The exposure time had a significant impact on the sensitivity of both assays when using CHO-K1 and TK6 cells, where the 24h scenario resulted in a higher sensitivity. Overall, these results suggest that factors such as cell type and exposure time should be considered when using *in vitro* genotoxicity data in a quantitative way.

P37 **Novel formats for the comet assay**

Sergey Shaposhnikov[1], Congying Zheng[1], Adrew Collins[1,2], Kristine Bjerve Gutzkow[3], Gunnar Brunborg[1,3]

[1]Norgenotech AS, Oslo, Norway
[2] University of Oslo, Oslo, Norway
[3]Norwegian Institute of Public Health, Oslo, Norway

The comet assay (single cell gel electrophoresis) is used for detecting DNA damage in single cells. It is widely used in human bio-monitoring and for industrial testing of chemicals and drugs, and has a potential as a diagnostic tool in personalized medicine. The traditional comet assay format has 2 gels on a microscope slide, and there is a limit of 40 gels per experiment given the size of a typical electrophoresis tank. To increase throughput, we have developed two alternative formats, high- and medium- throughput. Medium throughput comet assay involves 12 minigels set on one microscope slide, and a total of 240 minigels can be run in one experiment. High-throughput assay involves 96 minigels supported by hydrophilic polyester film, giving a total of 384 gels in one experiment. The novel comet assay formats are suitable for large screening studies in which a high-capacity robust and standardised assay are needed. They save time at all stages and are suitable for further automation.

P38 **Antioxidant, genotoxic and antigenotoxic properties of Ambrosia artemisiifolia L. (Asteraceae) deodorized water extract**

Biljana Spremo-Potparević[1], Lada Živković[1], Dijana Topalović[1], Jelena Kotur-Stevuljević[2], Stevan Samardžić[3], Zoran Maksimović[3]

[1]Department of Pathobiology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia
[2]Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia
[3]Department of Pharmacognosy, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

Ambrosia artemisiifolia L. (*Asteraceae*) is a well-known allergenic plant that causes allergic rhinitis across its whole range of distribution, especially in Central and Eastern Europe, in late summer and autumn when it pollinates. Numerous campaigns have been undertaken so far to eradicate or control this plant species across its European areal, but with arguable success. However, if this weed becomes attractive as a raw material for the pharmaceutical and chemical industry, the situation can be changed. The aim of this study was to assess antioxidant, genotoxic and antigenotoxic properties of LC-MS characterized aqueous extract remaining after the hydrodistillation of dried herb of *A. artemisiifolia* – (deodorized water extract - DWE). Antioxidant activity was assessed *in vitro* in the human serum pool after the induction of oxidative stress using exogenous oxidant tert-butyl hydroperoxide (TBH). Genotoxic and antigenotoxic properties of DWE were examined in human peripheral blood cells by the comet assay. Biochemical assays revealed remarkable antioxidant capacity of DWE, comparable to the same feature of Trolox as a proven antioxidant. None of the 3 concentrations tested (100, 200 and 400 μg/mL) were genotoxic, while antigenotoxicity in post-treatment *vs.* hydrogen peroxide was significant at all concentrations ($p \leq 0.05$). The results of this study demonstrate notable *A. artemisiifolia* medicinal potential, which calls for further investigations.

P40 **Mitochondrial DNA damage and DNA repair capacity in type 2 diabetic, obese rats**

Kristyna Tomasova[1,2], Sona Vodenkova[1,2], Pavel Vodicka[1,2], Roger Godschalk[3] and Sabine A.S. Langie[3]

[1]Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[2]Department of Molecular Biology of Cancer, Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic
[3]Department of Pharmacology & Toxicology, School for Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University, The Netherlands

Metabolic syndrome represents a cluster of conditions, including insulin resistance, hypertension, obesity, and hyperglycemia. A characteristic of all these conditions is excess generating of reactive oxygen species, creating oxidative stress, and contributing to higher susceptibility to oxidative DNA damage, especially in mitochondria. As mitochondria play an important role in metabolism, efficient DNA repair to prevent the accumulation of DNA lesions is crucial for their proper functioning.
Our study aims to look closely at mitochondrial 8-oxoguanine DNA damage and repair in ZSF1 (Zucker fatty and spontaneously hypertensive) rats, an experimental model suitable for studying type 2 diabetes. ZSF1 obese and -lean (control) rats have two different mutations in leptin receptors. Obese rats develop insulin resistance, hyperglycemia, and mild hypertension; lean ones are only hypertensive. We intend to demonstrate if increased oxidative stress leads to mutagenic DNA lesions and compromised base excision repair capacity, specifically in mitochondria.
In our experiment, 10 lean and 10 obese rats were sacrificed at 8-9 weeks of age. Mitochondria were isolated from the liver using density gradient ultracentrifugation, and their protein purity was verified on Western blot. The protein extracts will be tested by alkaline Comet assay to analyze base excision repair activity in mitochondria. Mitochondrial DNA oxidative lesions will be detected by using FPG (formamidopyrimidine [fapy]-DNA glycosylase) treatment and subsequent quantitative PCR. Currently, we are optimizing both methodologies. The abstract will be updated accordingly.

This study is financially supported by the Charles University (project GA UK No. 120), Grant Agency of the Czech Republic (21-04607X), Grant Agency of Ministry of Health of the Czech Republic (NU21-07-00247), and MEYS (CZ.02.2.69/0.0/0.0/16_018/0002455,CZ.02.2.69/0.0/0.0/18_053/0017000).

P41 **Development of a high throughput HPLC-MS/MS method for the quantification of 16 DNA adducts in blood: Application to a French Agricultural cohort samples**

Quentin Vandoolaeghe[1,2,3], Valérie Bouchart[1,4,5], Isabelle Catro[4], Poppy Evenden[1,2,3], Pierre Lebailly[1,2,3], Matthieu Meryet-Figuiere[1,2,3], Raphaël Delépée[1,2,3,4]

[1]Institut National de la Santé et de la Recherche Médicale (INSERM), Unité Mixte de Recherche (UMR) 1086 ANTICIPE, Caen, France ;
[2]Centre de Lutte Contre le Cancer François Baclesse, Caen, France;
[3]Université de Caen Normandie, Caen, France ;
[4]PRISMM platform, UNICAEN, CLCC François Baclesse, Caen, France;
[5]LABÉO, Pôle recherche développement et innovation, Caen, France;

Knowledge of health risk factors related to professional agricultural activity remains spotty and constitutes a major public health issue. It is now recognized that agriculture and occupational exposures to pesticides are associated with an increased risk of some cancers. Molecular epidemiology conducted on professional agricultural biobanks could highlight the health consequences of agricultural uses.
Various DNA adducts have been linked to various diseases including cancer and may serve as genotoxic and epigenetic biomarkers of both exposure and early carcinogenic process. Starting from a previous method developed in our laboratory [1], we have developed a new LC-MS/MS method able to quantify the original 12 DNA adducts combined with 4 epigenetic biomarkers. Most of these added adducts are linked with the DNA repair cycle.The blood samples were obtained from 795 adults residing on 410 randomly selected farms in Calvados (Normandy, France). The farms targeted by this biobank were mainly engaged in field crop and livestock activities. Thus, the method was optimized for the analysis of an important number of samples. Sample preparation contain the following steps: reduction, precipitation, washing, internal standards addition and Hydrolysis. These steps have been optimized and adapted to the use of a pipetting robot, allowing a high throughput analysis of the available samples. Stable isotopes labelled DNA adducts have been selected as internal standards for most of the compounds. Non-commercial compounds were synthetized in the laboratory. Two transitions have been selected for each compound, the first one used for the quantification and the second one used for the confirmation.
This method was applied for the analysis samples from the biobank to better understand the link between farmers' task and the effects in terms of DNA adducts.

[1] Alamil H, Lechevrel M, Lagadu S, Galanti L, Dagher Z, Delépée R. J Pharm Biomedical Anal. 2020;179:113007.

P42 **Optimized Fpg-modified comet assay for assessing differences in base excision repair activity**

Congying Zheng[1,2], Andrew Collins[1], Gunnar Brunborg[1], Frederik-Jan van Schooten[2], Anne Lene Nordengen[1], Roger Godschalk[2], Sergey Shaposhnikov[1]

[1]Norgenotech AS, Oslo, Norway
[2]Maastricht University, Maastricht, The Netherlands

We optimized Formamidopyrimidine DNA glycosylase (Fpg)-modified comet assay as a robust and sensitive method to assess individual DNA repair kinetics. The idea was to identify some factors affecting the rate of repair of oxidized bases, and to propose a reliable approach to the comparative study of DNA repair rates in different cell lines that could also be of use in studying inter-individual variations in DNA repair in humans. We analyzed the removal of DNA oxidation over different time-points in peripheral blood mononuclear cells (PBMCs) and 8 cell lines, after exposure to either 1 μM Ro19-8022 plus visible light or 10 mM potassium bromate ($KBrO_3$). After transformation of the data, DNA repair kinetics showed an early phase of 60 min with fast removal of Fpg-sensitive sites, followed by slower removal over the following the remaining 420 min. According to our findings, adjusting the initial damage to an equal level in the cellular repair assay based on the Fpg-modified comet assay could improve a more accurate analysis of cellular DNA repair kinetics.

P43 **Dihydroquercetin (Taxifolin) and Biochaga attenuate oxidative DNA damage in lymphocytes of obese subjects *in vitro***

Lada Živković[1], Dijana Topalović[1], Sunčica Borozan[2], Vladan Bajić[3], Vesna Dimitrijević Srećković[4], Andrea Pirković[5], Hristina Petrović[6], Biljana Spremo-Potparević[1]

[1] University of Belgrade - Faculty of Pharmacy, Department of Pathobiology, Belgrade, Serbia
[2] University of Belgrade - Faculty of Veterinary Medicine, Department of Chemistry, Belgrade, Serbia
[3] University of Belgrade - Institute for Nuclear Research "Vinča", Laboratory for Radiobiology and Molecular Genetics, Belgrade, Serbia
[4] University of Belgrade - Faculty of Medicine, Clinic for Endocrinology, Diabetes and Metabolic Diseases, Belgrade, Serbia
[5] University of Belgrade - Institute for Application of Nuclear Energy, Department for Biology of Reproduction, Belgrade, Serbia
[6] University Children's Hospital, Belgrade, Serbia

Systemic oxidative stress from increased free radicals and reduced antioxidant capacity are common characteristics of obese individuals. Data from human studies suggest that use of antioxidant nutrients mixtures rather than a single dietary component is beneficial in reducing obesity and its associated pathologies. Using hydrogen peroxide to induce oxidative stress *in vitro* in peripheral lymphocytes, we estimated the inhibition of DNA damage exposed in pre- and post-treatment to dihidroquercetin (DHQ) (Taxifolin) and Biochaga (B) alone, or in combination, as a DNA protective tool. Lymphocytes were obtained from 14 obese subjects (BMI 30-40) and 10 controls (BMI 18.4-24.9). DNA damage was evaluated by using the simple and reproducible technique, the Comet assay. The protective effect of individual and joined incubation with DHQ and B in pre and post-treatment showed a statistically significant decrease regarding DNA damage created by hydrogen peroxide in both groups. In a group of obese subjects, the more prominent attenuation was noted in the pre-treatment with DHQ (250 μg/mL) ($p<0.0001$), while in the control group it was pre-treatment with B (250 μg/mL) ($p<0.0001$). Conclusively, DHQ and B reduce oxidative stress in lymphocytes from obesity subjects and controls when challenged *in vitro*. A broader investigation of its profile in biological systems is needed.

Social Programme

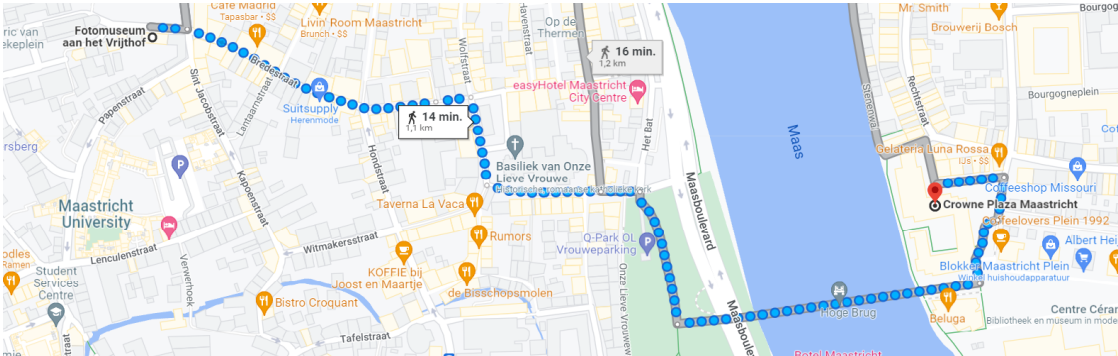
Social Programme

Welcome reception & Poster session
Enjoy some local spirits and snacks, while strolling along the posters.

Congress dinner & party - Tuesday, May 24th
A night out in the city!
Only a 15 minutes' walk away from your venue, in the heart of Maastricht, at Grand Café Soiron Maastricht, you will enjoy a fabulous congress diner & party. This amazing event location is located at Museum at the Vrijthof. The museum is situated in the former Spanish Government, a building dating back to the 16th century served as the outer palace of Emperor Charles V. We welcome you with a festive welcome drink, followed by a walking dinner. Our DJ will set the right mood and you will "dance" the night away!

Discover Maastricht - Wednesday, May 25th
You will get to know the beautiful city of Maastricht and the Burgundian lifestyle of its inhabitants!
In groups of 10 people, with the help of a GPS you will discover this amazing city.
You will meet "famous" Maastricht residents, taste delicious Maastricht delicacies, and learn all the ins and outs of the culture and sights. The inhabitants of Maastricht are known for some Chauvinism; they want to win! A competitive element between the teams should therefore not be missing. Will you be crowned as honorary visitor of Maastricht?

Directions to the dinner location



Notes

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

Meeting Sponsors

GOLD SPONSORS

sanofi

Instem
Information Solutions For Life

SILVER SPONSORS

MetaSystems

NOVARTIS

HESI Genetic Toxicology Technical Committee

BRONZE SPONSORS

BioSPX
a new focus on life science

toxys

phenion
COMPETENCE IN SKIN PHYSIOLOGY
www.phenion.com
A Henkel brand

ELSEVIER
MUTATION RESEARCH
Genetic Toxicology and Environmental Mutagenesis

mutagenesis

Hands-on training course sponsors

phenion
COMPETENCE IN SKIN PHYSIOLOGY
www.phenion.com
A Henkel brand

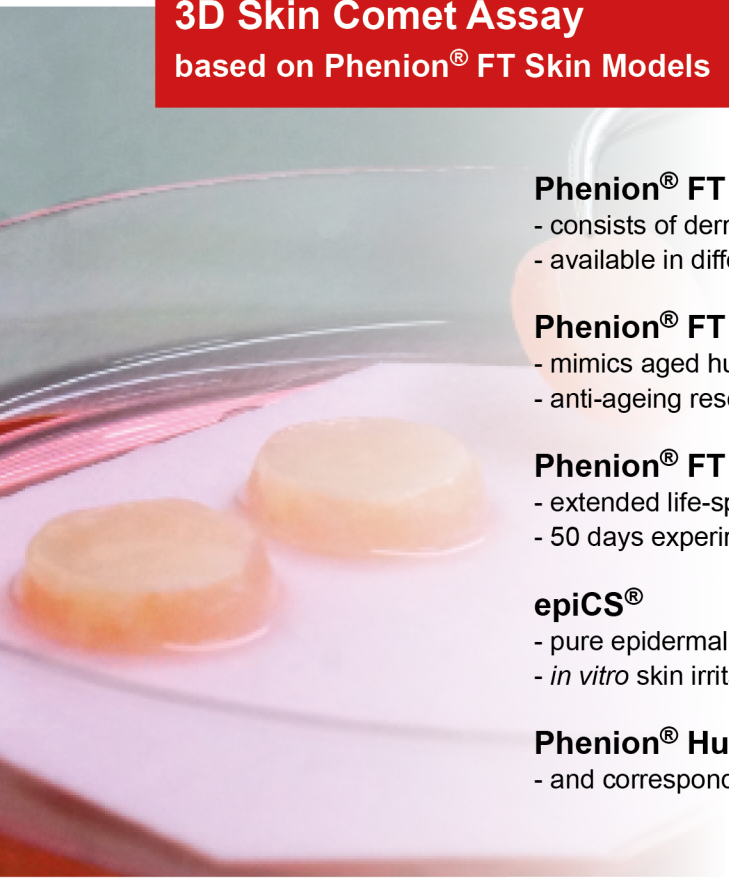
uhems

biotechne®

Enter a new dimension!!!

Phenion® 3D tissue models of the human skin
for *in vitro* R&D and risk assessment

3D Skin Comet Assay
based on Phenion® FT Skin Models



Phenion® FT Standard
- consists of dermis and epidermis
- available in different sizes and formats

Phenion® FT AGED
- mimics aged human skin
- anti-ageing research

Phenion® FT Long-Life
- extended life-span
- 50 days experimental phase

epiCS®
- pure epidermal model
- *in vitro* skin irritation / corrosion testing

Phenion® Human Skin Cells
- and corresponding cell culture media

Meet us and browse more detailed information at our website

www.phenion.com

phenion
COMPETENCE IN SKIN PHYSIOLOGY
www.phenion.com
A Henkel brand