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 popular technique. The Workshop has always been rather informal. It began as a satellite to the 2nd International Conference on Environmental Mutagens in 1995. At that time, the Comet Assay community was small, and they mostly knew each other. Even now, following exponential growth in popularity of this assay, the workshop retains the feeling of an extended family, reunited every two years, with a healthy intake of new family members. We try to keep registration costs as low as possible to make it easier for less established researchers to attend, and as a matter of policy no-one has expenses refunded; there are no invited speakers. All are equal, and the newest recruits can readily share ideas with the old-timers.

The first five meetings in this series were simply called 'Comet Assay Workshops'. 'International' was added in 2005. So far, all have been held in Europe, which perhaps reflects the fact that, while the Comet Assay has mixed North American/European parentage, more users of the assay are in Europe than anywhere else, and many innovations and new applications have come from Europe.

The programme will include oral and poster presentations depicting the highlights of the past, the present and especially the future of the Comet Assay; discussing the latest advances in the development and application of the Comet Assay. Different applications will be discussed, including: genotoxicity testing, human biomonitoring, DNA repair studies, environmental biomonitoring and clinical studies. The programme will also include an open discussion session to exchange ideas about practical issues and the latest innovations. This will encourage the exchange of information between specialists from both the academic and industrial sectors.

We very much look forward to joining us in Pamplona at the 12th International Comet Assay Workshop hosted by the University of Navarra.
KEYNOTE SPEAKER

PROF. DAVID KIRKLAND
GENETIC TOXICOLOGY CONSULTANT

Professor Kirkland has a BSc (microbiology) from the University of London and a PhD (cellular cancer studies) from Brunel University. Following 2 post-doctoral fellowships he became Research Director at Toxicol Laboratories. He then joined Microtest Research Limited in 1984, which became part of Covance where, over 25 years, he was Head of Genetic Toxicology, Vice-President of Toxicology and of Scientific and Regulatory Consulting. In 2009 he became an independent consultant. He has extensive experience with regulatory issues relating to genotoxicity data, has published >100 peer-reviewed papers and is a regular podium speaker/chairperson.

He was awarded Fellowship of the UKEMS in 2002, and made Honorary Professor of the University of Wales, Swansea in 2006. In 2010 he received the first Industrial Genotoxicity Group (UKEMS) Distinguished Toxicologist Award, and also the US Environmental Mutagen Society Alexander Hollaender Award for global leadership in the regulation of genetic toxicology testing. In 2014 he was awarded The Kitashi Mochizuki Award by the Japanese Environmental Mutagen Society for promotion of international harmonization of genotoxicity tests through the International Workshops on Genotoxicity Testing (IWGT) of which he was chair of the steering committee for 20 years. For many years he was Special Issues Editor for Mutation Research and editorial board member of the Journal of Applied Toxicology. He is currently a member of the UK Government Advisory Committee on Mutagenicity, UK expert to OECD for genotoxicity guidelines, and Past President of the European EMS.
TUESDAY 29 AUGUST 2017

8:30  REGISTRATION

SESSION 1: WELCOME AND KEY NOTES (9:00 - 10:30)
Chair: Amaya Azqueta (University of Navarra, Spain)

9:00  Welcome
Adela López de Cerain, Dean of the Faculty of Pharmacy and Nutrition of the University of Navarra (Spain).

9:15  Key note: Recent perspectives on the regulatory use of the comet assay
David Kirkland (Kirkland Consulting, UK)

10:10  hCOMET: a COST Action dedicated to the comet assay in human biomonitoring
Andrew Collins (University of Oslo, Norway)

10:30  COFFEE BREAK

SESSION 2: REGULATORY ISSUES, GENOTOXICITY TESTING & NANOTOX (11:00 - 13:00)
Chairs: David Kirkland (Kirkland Consulting, UK) and Gudrun Koppen (Flemish Institute for Technological Research, VITO, Belgium)

11:00 The comet assay in the evaluation of the genotoxicity of inhaled compounds
Desmond Cave (BioReliance, USA)

11:20 3D Skin Comet assay: Genotoxicity assessment addressing the dermal route of exposure
Kerstin Reisinger (Henkel AG & Co, Germany)

11:40 Application of comet assay in the assessment of nanoparticle induced genotoxicity and DNA methylation
Anita Mukherjee (University of Calcutta, India)

12:00 Silver nanoparticle-mediated cellular responses in isolated Sertoli cells in vitro
Diana Anderson (University of Bradford, UK)

12:15 Comparative toxicity of silver and titanium dioxide nanoparticles, their binary mixture and silver-doped titanium dioxide nanoparticles to mammalian cells
Marcin Kruszewski (Institute of Rural Health, Poland)

12:30 In vitro and in vivo comparison of nano aluminum, aluminum oxide and aluminum chloride genotoxicity
Pegah Jalili* (French Agency for Food, Environmental and Occupational Health Safety, ANSES, France)

12:40 Human renal proximal tubule epithelial cell line TH1 as a promising in vitro kidney model to predict the genotoxicity of xenobiotics and nanoparticles
Monika Sramkova (Cancer Research Institute, Slovakia)

12:50 Differentiated and exponentially growing HL-60 cells exhibit different sensitivity for genotoxic agents in the comet-assay
Helga Stopper (University of Würzburg, Germany)

13:00 LUNCH

POSTER SESSION 1 (14:30 - 17:00)

14:30 Pitch poster presentation
Chairs: Ariane Vettorazzi (University of Navarra, Spain) and Bertrand Pourrut (ISA-Lille, France)

- P1: DNA damage in enzymatic decolorization of Congo red
  Barbara Janovic** (Institute of Chemistry, Technology and Metallurgy, Serbia)

- P8: Toxicity of binary mixtures of metal(oxide) nanoparticles and quantum dots in two human cell lines HepG2 And A549
  Lucyna Kapka-Skrzyczczak** (Institute of Rural Health and University of Information Technology and Management, Poland)

- P12: Oxidatively damaged DNA in SHSY-5Y neuronal cells exposed to nicotine and cotinine
  Ana LH García** (Lutheran University of Brazil, Brazil)

- P13: Combinations of genotoxic tests for the evaluation of group 1 IARC
carcinogens  
*Jacky Bhagat** (Goa University, India)

- P14: Cellular uptake and toxicity of positively and negatively charged silica nanoparticles  
*Elisabeth Elje** (Norwegian institute for air research, NILU, Norway)

- P16: Tolerance of Carpobrotus acinaciformis L. halophyte plants exposed to high level of NaCl stress  
*Sema Karakas** (Harran University, Turkey)

- P22: Genotoxic effects produced by Vinclozolin on the aquatic insect Chironomus riparius (Diptera)  
*Monica Aquilino** (National University of Distance Education, Spain)

- P26: DNA damage response and biomarkers correlation following occupational exposure to pesticides mixtures.  
*Fernanda Rabaioli da Silva** (La Salle University, Brazil) on behalf of Vivian Kahl (Lutheran University of Brazil, Brazil)

- P35: Freezing procedure optimization of two human cell lines for comet assay analysis  
*Sónia Fraga** (National Institute of Health Dr. Ricardo Jorge, Portugal)

- P38: Moss Physcomitrella patens as a model system for study of DNA damage repair by comet assay  
*Radka Vágnerová** (Institute of Experimental Botany, Czech Republic)

- P43: Aerobic exercise reduces DNA damage possibly by increase neurotrophic signaling in hippocampus of old rats  
*Thais Vilela** (University of Southern Santa Catarina, Brazil)

15:30 Poster session while coffee is served – Authors of the odd number posters should be present in order to present their posters

17:30 Social programme:  
Visit to the Otazu winery (Coaches will be provided)

(*) In the running for the best young oral presentation  
(**) In the running for best pitch poster presentation

WEDNESDAY 30 AUGUST 2017

SESSION 3: ENVIRONMENTAL AND HUMAN BIOMONITORING (8:30 - 10:30)  
Chairs: Anita Mukherjee (University of Calcutta, India) and Roger Godschalk (Maastricht University, The Netherlands)

8:30 Live and born in a polluted area; mother-newborn relationship  
*Emilio Rojas (National Autonomous University of Mexico, Mexico)

8:50 Comet assay in salivary leukocytes for the evaluation of early biological effects of air pollution exposure in children  
*Massimo Moretti (University of Perugia, Italy)

9:10 Application of the comet assay to detect metal-induced DNA strand breaks in cultures of the marine sponge Hymeniacidon perleve and Amorphinopsis sp  
*Rachael Ununuma Akpiri (University of Birmingham, UK)

9:30 Acute exposures to the plasticizers di (2-ethylhexyl) phthalate (DEHP) and butyl benzyl phthalate (BBP) induce genotoxic damage in the aquatic larvae of the model species Chironomus riparius (Diptera)  
*Mónica Aquilino* (National University of Distance Education, Spain)

9:45 Use of sperm samples in the detection of DNA damage in boron-exposed workers  
*Nurşen Başaran (Hacettepe University, Turkey)

10:00 Genotoxic effects of 3T static magnetic field on in vitro human peripheral blood lymphocytes  
*Carina Ladeira (Lisbon School of Health Technology, Portugal)

10:15 Cytogenetic markers and DNA damage in workers exposed to a known carcinogen  
*Solange Costa*(National Institute of Health and Instituto de Saúde Pública da Universidade do Porto, Portugal)

10:30 Coffee break
Open discussion session: case study - Storing and processing of blood samples in human biomonitoring (11:00 - 12:30)

Chairs: Emilio Rojas (National Autonomous University of Mexico, Mexico) and Lisa Giovannelli (University of Florence, Italy)

11:00 5 min pitch presentations to introduce the session:

- P19: Use of whole blood to measure DNA damage in a group of wildland firefighters
  Solange Costa on behalf of Armanda Teixeira** (National Institute of Health and Instituto de Saúde Pública da Universidade do Porto, Portugal)

- P29: The comet assay in human biomonitoring: cryopreservation of whole blood and comparison with isolated mononuclear cells
  Gudrun Koppen (VITO, Belgium)

- P31: Comparison of DNA damage level with alkaline comet assay on peripheral blood mononuclear cells and peripheral whole blood that can be used in human biomonitoring studies
  Zsuzsanna Nemeth (National Public Health Institute, Hungary)

- P32: Comparison of the alkaline comet assay results on fresh and one year frozen whole blood
  Mirta Milic (Institute for Medical Research and Occupational Health, Croatia)

- P33: The impact of cryoprotectant use and washing steps on DNA damage levels assessed by comet assay
  Ana Inês Silva** (University of Trás-os-Montes and Alto Douro, Portugal)

11:25 Open discussion

12:30 Lunch

Poster session 2 (14:00 - 15:00)

14:00 Poster session – Authors of the even number posters should be present in order to present their posters

Session 4: Assay design and data processing (15:00 - 18:00)

Chairs: Stefano Bonassi (Scientific Institute for Research, Hospitalization and Health Care San Raffaele Pisana, IRCCS San Raffaele Pisana, Italy) and Kristine Gutzkow (Norwegian Institute of Public Health, Norway)

15:00 Searching for assay controls for the Fpg- and hOGG1-modified comet assay
  Peter Moller (University of Copenhagen, Denmark)

15:20 Calibration of the comet assay using x-rays
  Gunnar Brunborg (Norwegian Institute of Public Health, Norway)

15:40 Genome size and sensitivity to X-rays – plant comets tell the story
  John Einset (University of Oslo, Norway)

16:00 Coffee break

16:30 Validation of high throughput alkaline and FPG modified comet assay using a linear mixed model
  Eisa Boutet-Robinet (University of Toulouse/INRA, France)

16:50 4DLifetest: A highly accurate high throughput comet assay platform
  Juan Carlos Cassano (Empa- Swiss Laboratories for Material Science and Technology, Switzerland)

17:05 A comparison of different statistical strategies to analyze comet data
  Tug Timur* (Technical University of Dortmund, Germany)

17:20 Report from the Short-Term Scientific Mission (STSM) in Rome on joint work for adjusting database and making the Masterfile and performing first statistical analysis, discussion of the results and preparation for writing the scientific paper
  Mirta Milic (Institute for Medical Research and Occupational Health, Croatia)

17:30 A pre-validation study of comet assay on plants
  Priscilla Boccia (Italian Workers Compensation Authority, INAIL, Italy)

17:40 Medium throughput plant Comet Assay optimisation and development
of an automated scoring system
Julien Dubus* (ISA-Lille, France)

17:50 Performing the comet assay in combination with different human and bacterial repair enzymes in order to increase its sensitivity
José Manuel Enciso (University of Navarra, Spain)

19:00 Social programme
Guided tour in the old part of Pamplona

20:00 Conference dinner: ‘El Colegio’ restaurant

(*) In the running for the best young oral presentation
(**) In the running for best pitch poster presentation

THURSDAY 31 AUGUST 2017

SESSION 5: DNA REPAIR (9:00 - 10:30)
Chairs: John Einset (University of Oslo, Norway) and Sabine Langie (Flemish Institute for Technological Research, VITO, Belgium)

9:00 Identification of knowledge gaps and opportunities in the validation of comet assay based DNA repair phenotyping in human biomonitoring studies
Roger Godschalk (Maastricht University, The Netherlands)

9:20 Mobile phone specific electromagnetic fields induce DNA damage and nucleotide excision repair in human glioblastoma cells
Siegfried Knasmueller (Medical University of Vienna, Austria)

9:40 Kinetics of DNA double-strand breaks repair in moss Physcomitrella patens
Marcela Holá* (Institute of Experimental Botany, Czech Republic)

10:00 DNA damage and repair capacity in mother and newborns form the Spanish cohort (Newgeneris study)
Naouale El Yamani (Norwegian Institute for Aire Research, Norway)

10:15 The effect of DNA repair in target tissue on response to treatment, disease progression and long-term survival in colon cancer patients
Sona Vodenkova* (Institute of Experimental Medicine, The Czech Academy of Sciences, Czech Republic)

10:30 Coffee break

SESSION 6: NUTRITIONAL AND CLINICAL ASPECTS (11:00 - 12:30)
Chairs: Andrew Collins (University of Oslo, Norway) and Solange Costa (National Institute of Health and Instituto de Saúde Pública da Universidade do Porto, Portugal)

11:00 DNA damage among Inflammatory Bowel Disease patients
Cristiana Costa-Pereira (National Institute of Health, Portugal)

11:20 An attempt to modify DNA single-strand breaks in diabetes mellitus patients and in rat models of the disease by 1,4-dihydropyridien
derivatives

Nikolajs Sjakste (University of Latvia, Latvia)

11:35  Modulation of genomic instability in patients with type 2 diabetes mellitus treated with vitamin D

Vanessa Moraes de Andrade (University of Southern Santa Catarina, Brazil)

11:50  Comet Assay in evaluation of individual DNA-damage of blood leukocytes in breast cancer patients undergone chemotherapy with doxorubicin-containing regimens

Nikolay Sirota (Institute of Theoretical & Experimental Biophysics, Russian Academy of Sciences, Russia)

12:05  The comet assay as a means to monitoring nutritional effects on DNA integrity in humans

Tamara Bakuradze (University of Kaiserslautern, Germany)

12:20  Antigenotoxic potential of dry olive leaf extract against DNA damage induced by estradiol and diethylstilbestrol on human peripheral blood leucocytes in comet assay

Lada Zivkovic (Belgrade University, Serbia)

**CLOSING CEREMONY 12:30 - 13:00**

12:30  Closing ICAW 2017 by the co-chairs:

Amaya Azqueta (University of Navarra, Spain)
Sabine Langie (VITO, Belgium)
Bertrand Pournut (ISA-Lille, France)

Awards ceremony: best poster, pitch poster presentation and oral communication

12:45  ICAW 2019 announcement

Nikolay Sirota (Institute of Theoretical & Experimental Biophysics, Russian Academy of Sciences, Russia)

**13:30 Lunch**

(*) In the running for the best young oral presentation
O1 - Recent perspectives on the regulatory use of the comet assay

D J Kirkland
Kirkland Consulting, Tadcaster, UK

Although an OECD guideline now exists for the *in vivo* comet assay, its use in a regulatory setting is somewhat inconsistent. Some agencies have expressed a clear preference for use of the transgenic rodent mutation (TGR) assay as a follow-up to a substance that induces gene mutations *in vitro*. In order to investigate this thinking a working group of the ILSI/HESI Genetic Toxicology Technical Committee has compiled a database of 90 chemicals for which both *in vivo* TGR and comet assay data are publicly available. Published genotoxicity data for all other regulatory endpoints *in vitro* and *in vivo*, plus carcinogenicity data, have been added to the database. Preliminary analysis of the database indicates the comet assay does not “miss” any substances that are positive in TGR, and performs equally well as the TGR for detecting Ames-positive carcinogens. Some chemicals are positive in comet but negative in TGR, however there are reasons to question whether the TGR protocol in these cases was robust. Another regulatory issue with the comet assay involves choice of tissues. Some agencies have asked for data in both glandular stomach and duodenum for site-of-contact effects, as well as the liver for substances that might require metabolism. A preliminary analysis of the database indicates that liver + duodenum is an appropriate combination of tissues to detect genotoxic chemicals, and that there is no justification for also requiring glandular stomach. This database could be useful for addressing other questions regarding the regulatory use of the comet assay.
O2 - hCOMET: a COST Action dedicated to the comet assay in human biomonitoring

Andrew Collins¹, on behalf of COST Action 15132
¹ University of Oslo, Department of Nutrition, PB 1046 Blindern, 0316 Oslo, Norway

The COST Action hCOMET has members from 23 countries, with a common interest in using the comet assay to measure DNA damage and repair in humans. The purpose of this Action is two-fold: first, to collect as much human comet assay data as possible into a single database so as to allow a pooled analysis; and second, to improve the inter-laboratory reproducibility of the assay. In the first year we have succeeded in creating the database, with DNA damage estimates for around 20,000 human samples; analysis is now proceeding, to determine which factors (smoking, age, nutrition, sex, occupational exposure etc.) affect DNA damage and repair, and to what extent.

To achieve the second aim, we need first to understand better the technical factors that affect assay performance, in measurement of both DNA damage and DNA repair, and working groups are actively engaged in these topics. Standardised methods will be tested in a ring study; and the findings of this will be incorporated into standard operating procedures that, we hope, will be adopted as best practice in future biomonitoring studies. Another working group is studying the applicability of the comet assay to different cell types, for example cells from normal and tumour tissue, isolated peripheral blood mononuclear cells (the most commonly used cells) compared with whole blood or leukocytes isolated from frozen blood samples, and cells from the surface of the eye or from the buccal epithelium, representing target cells for environmental/occupational exposure to genotoxins.

An important aspect of hCOMET is the training of young researchers, through training schools and short-term scientific missions - for which anyone from member countries can apply.

O3 - The comet assay in the evaluation of the genotoxicity of inhaled compounds

Desmond Cave
BioReliance Ltd., 9630 Medical Centre Drive, Rockville, Maryland, 20850, USA

Inhalation is a common route of exposure for pharmaceuticals, agrochemicals and chemicals. The OECD Guideline for the comet assay states that the route of exposure should mimic the route of exposure in humans and we have been asked by many companies if we could perform the comet assay and evaluate nasal tissue in rats or mice. We had experience of this when performing the Transgenic Rodent Mutation Assay OECD 488 as we had been asked to perform this assay using inhalation as the route of exposure. The nasal epithelium is a difficult tissue to extract and we found that consistency of removal is important, whereas lung and lung lavage less so.

Five cohorts of 10 animals in each (A, B, C, D and E) were tested in five independent experiments. In each cohort, five animals were dosed with the vehicle, 0.9% saline and five were dosed with the positive control EMS at 200 mg/kg b.w. Animals were dosed 3-4 hours prior to euthanasia. All animals were euthanized by CO₂ inhalation, nasal tissues (turbinate and septum lining) were collected. Tissues were minced with scissors and single cells were prepared and processed for the comet assay. EMS was dosed at 200 mg/kg b. w. The vehicle control was 0.9% saline (10 mL/kg). The positive control, EMS, was dissolved in 0.9% saline and was prepared fresh just prior to dosing.

Vehicle control dosed male rats had % tail DNA values in all 5 cohorts ranging from 0.07 to 0.89 with a mean value of 0.30 ± 0.22 in nasal tissue.

Positive control EMS dosed male rats had % tail DNA values in all 5 cohorts ranging from 7.24 to 29.02 with a mean value of 15.73 ± 5.18 in nasal tissue. These values were statistically significant compared to the concurrent vehicle control values.
O4 - 3D Skin comet assay: Genotoxicity assessment addressing the dermal route of exposure

Kerstin Reisinger, Joep Brinkmann, Tom Down, Anja Fischer, Andrea Haase, Frank Henkler, Sebastian Hoffmann, Manfred Liebsch, Andreas Luch, Claudia Petrick, Ralph Pirow, Astrid Reus, Andrea Said, Monika Schäfer-Korting, Markus Schulz, Stefan Pfuhler

1 Henkel AG & Co. KGaA, Düsseldorf, Germany
2 Seh consulting + services, Paderborn, Germany
3 German Federal Institute for Risk Assessment, Department of Chemicals and Product Safety, Berlin, Germany
4 Procter & Gamble, Mason, OH
5 Triskelion B.V., Zeist, The Netherlands
6 Institute for Pharmacy, Freie Universität Berlin, Berlin, Germany.
7 Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany

The 3D Skin Comet assay was developed as follow-up tool for positive results from standard in vitro test batteries. It addresses two aspects underrepresented in current in vitro genotoxicity testing: in vivo-like route of exposure and metabolism. To mimic the dermal exposure route full-thickness skin tissues were combined with a classical readout-parameter, the migration of DNA fragments in the electric field. The fragments represent DNA damage that may lead to clastogenic as well as to mutagenic lesions.

We here report on a validation study in which 30 compounds were tested blinded in five laboratories. Data analysis revealed a specificity of 87% and sensitivity of 73% which is comparable to the predictively obtained in vivo. According to our findings, the 3D Skin Comet assay can be used as a direct replacement of animal studies when following-up on positive results from the standard test battery.

The work was funded by Cosmetics Europe and the German Ministry for Research and Education.

O5 - Application of comet assay in the assessment of nanoparticle induced genotoxicity and DNA methylation

Ilika Ghosh, Abhishek Sadhu, Yuji Moriyasu, Maumita Bandyopadhyay, Anita Mukherjee

1 Cell Biology and Genetic Toxicology Laboratory, Centre of Advanced Study, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata – 700019, India
2 Plant Molecular Cytogenetics Laboratory, Centre of Advanced Study, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata – 700019, India
3 Graduate School of Science and Engineering, Saitama University, Shimo-Okubo 255, Sakura-ku Saitama-shi, 338-8570, Japan

We utilized the versatility of comet assay as a tool to define the role of genotoxicity and DNA methylation as vital regulators of nanotoxicology of Mn$_3$O$_4$ nanoparticles (MnNps) (~50 nm) and fullerene soot nanoparticles (FNPs) (~50 nm) in model plant and animal systems. Tobacco BY-2 cells, Physcomitrella patens and human peripheral blood lymphocytes were exposed to MnNPs (5, 10 and 20 µg/mL) and FNPs (25, 50, 100 µg/mL) at various time intervals. Modified nuclei isolation procedures were adapted for each test system for optimal yield; unwinding and electrophoresis times were adjusted for least DNA migration. Global DNA methylation pattern at the level of single cells was detected by comet assay in combination with the methylation sensitive isochizomeric restriction endonucleases HpaII (digests unmethylated and hemimethylated DNA) and MspI (digests 5’-CmCGG-3’). Our results suggest dose independent genotoxicity response in each test system. Significant DNA hypomethylation was noted at the genotoxic concentrations of MnNPs. This was confirmed by colorimetric estimation and/or HPLC. Thus, modified comet assay techniques can be implemented for epi-genotoxicity analyses of environmentally relevant nanomaterials in diverse biological systems ranging from plants to mammalian cells.
**O6 - Silver nanoparticle-mediated cellular responses in isolated Sertoli cells in vitro**

Khaled Habas, Martin H Brinkworth, Diana Anderson  
*University of Bradford, Bradford, UK*

The growing use of silver nanoparticles (AgNPs) in various applications, including consumer, agriculture and medical products, has raised many concerns about the potential risks of nanoparticles (NPs) to human health and the environment. Silver nanoparticle exposure has been reported recently to be related to male reproductive toxicity in mammalian models. The interplay between Sertoli and spermatogenic cells is crucially significant for successful development of germ cells into spermatozoa, because Sertoli cells form sites of attachment to spermatogenic cells. Unattached spermatogenic cells in the seminiferous epithelium are frequently associated with Sertoli cell dysfunction. The aim of the present study is to explore the mechanism of cytotoxic and genotoxic effects of AgNPs on primary cultures of mouse Sertoli cells, using changes in various cellular parameters of Sertoli cells involving viability, DNA damage, oxidative stress and apoptosis. DNA damage was evaluated by the Comet assay, apoptotic cells were detected using the TUNEL assay and apoptosis markers such as p53 and bcl-2 were analysed by real-time PCR (qPCR), Superoxide anions were detected using the nitroblue tetrazolium (NBT) reduction assay and activity of antioxidant enzymes such as catalase (CAT), glutathione peroxidase 1 (GPX-1) and superoxide dismutase 1 (SOD-1) were quantified using qPCR. Our results indicate that AgNPs caused severe Sertoli cell oxidative damage and apoptosis, accompanied by excessive production of superoxide anions and changes in gene expression and DNA damage. Our study suggests that AgNP exposure increased oxidative stress levels, changed gene expression and oxidative DNA damage which are involved in the mechanistic pathways of AgNP-induced apoptosis in the Sertoli cells *in vitro*. This may lead to reduced numbers of Sertoli cells in early male germ cell differentiation, thus affecting satisfactory germ cell production.

**O7 - Comparative toxicity of silver and titanium dioxide nanoparticles, their binary mixture and silver-doped titanium dioxide nanoparticles to mammalian cells**

Sylwia Męczyńska-Wielgosz¹, Anna Kędziora², Maria Wojewódzka³, Lucyna Kapka-Skrzypczak³,⁴, Marcin Kruszewski¹,³,⁴  
¹Institute of Nuclear Chemistry and Technology, Centre for Radiobiology and Biological Dosimetry, Dorodna 16, 03-195 Warszawa, Poland  
²Institute of Genetics and Microbiology, Wrocław University, Poland  
³Department of Molecular Biology and Translational Research, Institute of Rural Health, Jacezkiewicza 2, 20-090 Lublin, Poland  
⁴Department of Medical Biology and Translational Research, Faculty of Medicine, University of Information Technology and Management, Sucharskiego 2, 35-225 Rzeszów, Poland

Increasing body of evidence indicates that combined action of different nanomaterials may have different effect as compared with sum of the effects of nanomaterials alone. In this work, we compared cytotoxicity and genotoxicity of AgNPs (20 nm) and TiO₂NPs (21 nm) alone, in binary mixture and silver doped TiO₂NPs (100 nm TiO₂ with 20 nm Ag speckles) in two human cell lines – A549 and HepG2. Cytotoxicity was estimated by MTT or NR assay 24 h and 48 h after exposure. Genotoxicity was studied by comet assay after 2 h or 24 h exposure. In HepG2 cells, TiO₂NPs did not induce cytotoxicity, neither DNA breakage nor base damage that differed substantially from the control. In the contrary, AgNPs, and the respective silver-doped NPs, expressed concentration-related toxicity and induction of both forms of DNA damage. In A549 cells, previously characterised as relatively resistant to AgNPs 20 nm, toxicity and DNA damage induction were similar to those in the controls notwithstanding the type or concentration of NPs. In both cell lines, the toxicity and induction of DNA damage by the AgNPs and TiO₂NPs mixture followed a complicated pattern, depending on cell line, endpoint studied the ratio of NPs in the mixture, only partly reflecting their previously studied sensitivity to AgNPs and TiO₂NPs.

This work was supported by the statutory grant to INCT (SMW, MW), statutory grant to University of Wroclaw (AK), National Science Centre grant DEC-2013/09/B/NZ7/03934 (LKS, MK) and COST Action CA15132 (LKS, MK).
O8 - **In vitro and in vivo comparison of nano aluminium, aluminium oxide and aluminium chloride genotoxicity**

ANSES, Laboratoire de Fougères, Unité de Toxicologie des Contaminants, 10 B rue Claude Bourgelat, 35306 Fougères, France

Human oral exposure to aluminium (Al) and aluminium oxide (Al$_2$O$_3$) is reported through numerous goods: food additives, medication, beverage, water treatment as well as cooking utensils. According to EFSA opinion, the tolerable weekly intake (TWI) of 1 mg/kg bw is likely to be exceeded especially for children [1].

Due to the increasing use of nanoparticles (NP), different forms of Al NPs can be involved in human exposure. It has been outlined that aluminium can cross the brain barrier, reach the placenta, persist in some organs before being excreted in urine [1, 2] and can induce several side effects including neurotoxicity and embryotoxicity. However, the effects on the intestinal tract as the first barrier for oral exposure and on liver as the main target organ for Al accumulation have been poorly studied.

In this study, we investigated the **in vitro** genotoxicity of Al, Al$_2$O$_3$ NP and the ionic form AlCl$_3$ via comet assay, on the human intestinal Caco2 and hepatic HepaRG cells after 24h treatment from 0.6 to 256 µg/cm$^2$. We also explored their **in vivo** genotoxicity on liver and duodenum of male Sprague dawley rats after 3 feedings as well as after 28 days treatment with 6, 12 and 25 mg/kg bw/day.

Results showed that the **in vitro** genotoxicity of Al and Al$_2$O$_3$ on intestinal and hepatic cells was not confirmed on liver and duodenum in **in vivo** with or without using the Formamidopyrimidine DNA glycosylase enzyme to detect oxidative DNA damage.

This project was funded by l'Agence Nationale de la Recherche (ANR-13-IS10-0005-01).


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O9 - **Human renal proximal tubule epithelial cell line TH1 as a promising in vitro kidney model to predict the genotoxicity of xenobiotics and nanoparticles**

Monika Sramkova, Katarina Kozics, Annamaria Srancikova, Alena Gabelova
Cancer Research Institute, Biomedical Research Center SAS, Dubravska cesta 9, 845 05 Bratislava, Slovakia

The kidneys are vital organs that perform important physiological functions such as blood filtration, reabsorption of small molecules, and production of some hormones. In addition, kidneys play a central role in excretion of toxic metabolites and drugs, and renal excretion is also involved in elimination of nanoparticles after systemic application. In general, kidney toxicity is one of the most frequent adverse events notified during drug development. Therefore, more accurate predictive cell culture models are required for a comprehensive evaluation of potential nephrotoxicity. Human renal proximal tubule epithelial cells TH1 cultivated on Transwell membrane filters resulted in enhanced epithelial cell polarization. Moreover, cultivation of these cells under a shear stress conditions mimics the in vivo-like physiology found in the renal system. Here we compared the capacity of TH1 cells and human embryonic kidney HEK293 cells to detect the genotoxicity of selected chemicals with different mechanisms of action such as chlorpromazine (CPZ), colchicine (COL) and methyl methanesulfonate (MMS), and inorganic nanoparticles (Au, TiO$_2$, SiO$_2$, Fe$_3$O$_4$). Medium-throughput version of the alkaline SCGE assay, with 12 minigels was utilized to speed up the hazard assessment of selected chemicals and inorganic NMs. Neither CPZ nor COL induced any significant increase in DNA migration in TH1 and HEK293 cells, as both chemicals are non-genotoxic agents. In contrast, higher level of DNA damage was detected in TH1 cells compared to HEK293 cells after exposure to MMS, a strong alkylating agent. In line with the cytotoxicity results, none of the inorganic nanoparticles caused any increased DNA damage comparing to the control cells.

Based on our results, TH1 cells might serve as a useful tool for evaluating human-relevant renal toxicity in biomedical safety studies.
The research leading these results has received funding from European Union’s Horizon 2020 (H2020/2014-2020) under grant agreement no. 685817 – HIS-ENTS. The authors would also like to acknowledge the contribution of the COST Action CA15132

O10- DIFFERENTIATED AND EXPONENTIALLY GROWING HL-60 CELLS EXHIBIT DIFFERENT SENSITIVITY FOR GENOTOXIC AGENTS IN THE COMET-ASSAY

Helga Stopper and Gracia Montag
Institute of Pharmacology and Toxicology, University of Würzburg, Würzburg, Germany

To investigate the impact of the differentiation state on sensitivity for genotoxic damage induction by chemicals, we used the human promyelocytic leukaemia cell line HL-60. For differentiation, they were treated with 1.25 % DMSO for 3 days. At that time, differentiation was assessed by microscopic quantification of granulocyte-like cells with lobed nuclei. Differentiation efficiency was around 90% after DMSO-treatment and around 10% as basal level in normal cultures. Cell density during treatment was kept constant between differentiated and undifferentiated cells, and fresh medium was always added the day before the treatment. Cell vitality was assessed by enzymatic activation/dye exclusion (fluorescein diacetate/safe red) and evaluation in the comet-assay was limited to treatments with less than 20% non-vital cells at the highest substance concentration. Treatment was performed with methyl methane sulfonate (MMS), doxorubicin and hydrogen peroxide (H₂O₂). All three chemicals caused a dose-dependent increase of DNA-damage. While MMS induced similar damage in the differentiated and the undifferentiated cells, there was a remarkable difference for doxorubicin and H₂O₂, with undifferentiated cells being more sensitive. Further chemicals and other models for cellular differentiation will be added to this investigation.
O11 - Live and born in a polluted area; mother-new-born relationship

Emilio Rojas\textsuperscript{1,2}

\textsuperscript{1} Present address: Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Pisana, Rome, Italy
\textsuperscript{2} Permanent address: Instituto de Investigaciones Biomedicas. Universidad Nacional Autónoma de México. México

Actual consequences of changes in global environment play a major role in the etiology of Non-Communicable Diseases (NCDs), including hypertension, obesity, cardiovascular diseases, diabetes, and cancer. These diseases could be generated by a cluster of compounds. It is well documented the complexity of the characterization of the exposure to mixtures present in the environment by its dynamics. The achieving of the Millennium Development goals focus a great deal of attention and effort on improving health outcomes for the most vulnerable groups in our population, namely foetuses and women of childbearing age. In this way, it is important the improvement of biological biomarkers such as single cell gel electrophoresis assay to address the genotoxic consequences of pollution. Using this versatile test, we have studied for 20 years the relationship between environmental exposure and genotoxicity in various human biomaterials. Our present concern is a better understanding of the potential effects of environmental exposures on foetal and childhood growth as a marker of public health. Air pollutants has been detected in both maternal and cord blood, which means that some pollutants cross the placental barrier to reach the foetus directly and consequentially weaken relevant transplacental functions. We assessed basal DNA damage in cord blood cells collected at delivery of new-born children from different zones in the Metropolitan Area of Mexico City, exposed \textit{in utero} to air pollution, principally ozone and 10 \(\mu\text{m}\) air particles (PM\textsubscript{10}) in a cross sectional study. Stablishing correlation with the DNA damage of their mothers, and different parameters of the environmental exposure and complications during pregnancy. Our data indicate that some environmental pollutants and gestational conditions have relation to DNA damage.

O12 - Comet assay in salivary leukocytes for the evaluation of early biological effects of air pollution exposure in children

Massimo Moretti\textsuperscript{1}, Marco Verani\textsuperscript{2}, Antonella De Donno\textsuperscript{3}, Sara Bonetta\textsuperscript{4}, Alessio Perotti\textsuperscript{5}, Elisabetta Ceretti\textsuperscript{6}

\textsuperscript{1}Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy
\textsuperscript{2}Department of Biology, University of Pisa, Pisa, Italy
\textsuperscript{3}Department of Biological and Environmental Science and Technology, University of Salento, Lecce, Italy
\textsuperscript{4}Department of Public Health and Pediatrics, University of Torino, Torino, Italy.
\textsuperscript{5}Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy
\textsuperscript{6}Department of Medical and Surgical Specialties, Radiological Science and Public Health, University of Brescia, Brescia, Italy.

By detecting biological effects before the development of clinical diseases, the use of biomarkers of early effects in human biomonitoring allows a prompt detection of the biological consequences of a specific situation, such as the exposure to air pollutants. Furthermore, biomarkers of early effects are measurable in a wide number of individuals, representing in more appropriately the global burden of effects that environmental exposures may determine in a population. Primary DNA damage detected by the comet assay showed to be sensitive and effective in the evaluation of the impact of human exposure to environmental mutagens and carcinogens. Several studies found a positive association between high level of air pollutants (PM and ozone, in particular) and DNA damage detected by comet assay. Most of these studies used blood cells.

In the MAPEC\_LIFE project, we assessed primary DNA damage performing comet assay on salivary leukocytes of children exposed to different levels of air pollution. This type of cells, directly exposed to air passage through the mouth, represents the target cells of this exposure and so is more appropriate than blood cells for the evaluation of its effects. Moreover, they are easy to retrieve, especially in children, using a simple and non-invasive method, which allows to obtain a great sample size. Despite these considerations, we encountered some difficulties working with buccal cells, especially regarding the number of cells retrieved per sample, the interference played by the buccal epithelial cells on the comet images, and the consequent extremely time-consuming analysis of the slides.
Nevertheless, final results showed significantly different levels of DNA damage in child cells by the sampling season and town of residence. Moreover, statistical significant associations were found between primary DNA damage in salivary leukocytes and levels of some air pollutants, ozone in particular.

O13 - Application of the comet assay to detect metal-induced DNA strand breaks in cultures of the marine sponge Hymeniacidon perleve and Amorphinopsis sp

Rachel U Akpiri1, Nikolas J Hodges1, Roseline S Konya2
1Scholl of Biosciences University of Birmingham, UK B15 2TT, UK
2Department of Animal and Environmental Biology, University of Port Harcourt, Port Harcourt, Nigeria

In this study a novel in vivo exposure sponge culture model (sponge cell aggregates) from field collected and cryopreserved sample of Hymeniacidon perleve was developed to investigate the genotoxic effects of selected metals in laboratory exposures. Sponge cells were treated with non-cytotoxic concentrations of CdCl2, NiCl2, Na2Cr2O7, and AlCl3 (0, 0.1, 0.3, and 0.4 mg/L) for 12 hours. Following treatment there was a statistically significant (P < 0.05) concentration-dependent increase in the level of DNA strand breaks and reactive oxygen formation as assessed by the comet assay and dichlorofluoroscein oxidation respectively. The method was further developed to study potential genotoxicity in field-collected sponges (Amorphinopsis sp) from polluted sites in Niger Delta, Nigeria. Sponge samples from Niger Delta were analysed for heavy metals by inductively coupled plasma mass spectrometry and DNA damage quantified using the comet assay. There was a statistically significant positive correlation (r²=0.7114) between levels of DNA strand breaks and Al concentrations in sponge tissue. In conclusion, we have developed for the first time a novel in vivo sponge culture-model compatible with the alkaline comet assay and ROS quantification and described for the first time a new sponge species from the Niger Delta area of Nigeria. We have also shown using laboratory exposures, that non-cytotoxic concentration of the genotoxic metals Cd, Cr, Ni and Al all result in a concentration-dependent increase in DNA damage. Genotoxicity in marine sponges measured by the comet assay technique may be a useful tool for bio-monitoring research and risk assessment in aquatic ecosystems. [1]

O14 - ACUTE EXPOSURES TO THE PLASTICIZERS DI (2-ETHYLHEXYL) PHTHALATE (DEHP) AND BUTYL BENZYL PHTHALATE (BBP) INDUCE GENOTOXIC DAMAGE IN THE AQUATIC LARVAE OF THE MODEL SPECIES CHIRONOMUS RIPARIUS (DIPTERA).

Mónica Aquilino, Rosario Planelló, Óscar Herrero
Grupo de Biología y Toxicología Ambiental. Facultad de Ciencias. Universidad Nacional de Educación a Distancia, UNED. Paseo de la Senda del Rey 9, 28040 Madrid, Spain

Plasticizers are additives that increase the plasticity or viscosity of a plastic material, especially polyvinyl chloride (PVC), making it soft and flexible. Other uses outside the PVC industry include their presence in fragrances and personal care products, adhesives, paints, household cleaning products, printing inks, textiles, and many more. Among the many substances used for these purposes, phthalates are a widely-used group of chemicals with a very high production worldwide, being considered nowadays as ubiquitous environmental pollutants. Two of the world’s most used phthalates are di (2-ethylhexyl) phthalate (DEHP) and butyl benzyl phthalate (BBP). Both have been identified as contaminants in numerous environmental compartments, detected in tissues of exposed animals and humans, and classified as toxic in numerous studies, especially regarding their activity as endocrine disrupting compounds (EDCs). In addition, both pollutants can bioaccumulate in the trophic chains of contaminated ecosystems. While DEHP has been classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC) and the U.S. National Toxicology Program (NTP), no evidences of carcinogenicity have been found for BBP. Both xenobiotics are also considered non-genotoxic. In the present study, we used the comet assay to assess their ability to induce DNA damage in insects. Chironomus riparius larvae—a model species in ecotoxicology studies—were exposed for 24 hours to environmentally relevant concentrations (1 and 100 µg/L) of DEHP or BBP. Several parameters (mainly %DNA in tail) demonstrated that 100 µg/L DEHP or BBP altered significantly the DNA integrity (2.4-fold and 1.7-fold, respectively), while 1 µg/L showed a genotoxic response only in the case of DEHP (1.8-fold). These findings provide new insights into the genotoxic potential of DEHP and BBP, particularly in invertebrates, and bring serious concerns about environmental and health risk assessments.

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O15 - USE OF SPERM SAMPLES IN THE DETECTION OF DNA DAMAGE IN BORON-EXPOSED WORKERS

Nurşen Başaran¹, Yalçın Duydu², Sevtap Aydın¹, Aylin Üstündağ²
¹ Hacettepe University Faculty of Pharmacy Department of Pharmaceutical Toxicology, Ankara, Turkey
² Ankara University Faculty of Pharmacy Department of Pharmaceutical Toxicology, Ankara, Turkey

Industrial production and use of boron compounds have increased during the last decades, especially for the manufacture of borosilicate glass, fiberglass, detergents/bleaches, metal alloys and flame retardants. Turkey holds the world’s largest boron deposits and is one of the largest manufacturers. On the other hand, the reproductive toxicity of boric acid and borates is the matter of current regulatory concern. Boron treatment of rats, mice and dogs was dose-dependently associated with testicular toxicity, characterized by inhibited spermatogenesis at lower dose levels, and reduction of epididymal sperm counts at the highest dose levels. Based on the experimental studies in rats, NOAELs were found to be 17.5 boron (B)/kg b.w. for male fertility and 9.6 mg B/kg b.w. for developmental toxicity. According to the current EU safety assessments, boric acid and sodium borates have been classified as toxic to reproduction. However, such effects have not been proven in humans so far. The aim of the study was to investigate the reproductive toxicity indicators in highly exposed workers employed in a boric acid production plant in Turkey. The DNA integrity of cryopreserved sperm cells (n=204) was determined by using neutral COMET assay. DNA strand breaks (% tail intensity) in the neutral COMET assay do not increase with increases in blood boron concentration but rather, that a negative correlation apparently exists between this pair of variables. Our findings suggest that exposure to environmental boron within expected ranges is not detrimental to sperm status and may even have “beneficial” properties.

This project was funded by BOREN and Eti Mine Works General Management.
O16 - Genotoxic effects of 3T static magnetic field on in vitro human peripheral blood lymphocytes

Carina Ladeira1,2,3,4, Carina Ramos2, Margarida Pereira1, Luís Freire1
1Escola Superior de Tecnologia da Saúde de Lisboa – IPL, Portugal
2Environment and Health Research Group, Escola Superior de Tecnologia da Saúde de Lisboa – IPL, Lisboa, Portugal
3Research Group in Genetics and Metabolism, Escola Superior de Tecnologia da Saúde de Lisboa – IPL, Lisboa, Portugal
4Centro de Investigação em Saúde Pública - Escola Nacional de Saúde Pública, (CISP-ENSP), Universidade Nova de Lisboa, Portugal

Over the last two decades, Magnetic Resonance Imaging (MRI) became a widespread medical imaging technique, which is partially explained due to the use of non-ionizing radiation. However, biological effects of static magnetic fields have been documented and positive findings have been reported. Yet, only few reports on the genotoxic effects of static magnetic fields were produced, and controversy about their impact on cellular DNA integrity remains.

An in vitro study was designed to analyse if the static magnetic field used in MRI can induce genomic instability, measured by comet assay. Cultures of peripheral lymphocytes from 3 individuals (2 females and 1 male) were exposed to the static magnetic field of a 3 T-MRI equipment for 0h (control), 1h, 6h and 12h.

The samples were placed inside the MRI scanner, close to its isocentre, where the static magnetic field can be considered homogeneous. The control was placed in another room of the same building, at room temperature. At the end of the experiment, all the samples were cryopreserved and comet assay was performed at equal conditions.

The results showed an increase of DNA damage from 1h (8.89±7.98) to 6h (10.85±12.22), both higher than the control (4.62±2.08). However, regarding to the 12h, the damage was the lowest (3.26±1.88), which may suggest that the DNA damage induced by the static magnetic field starts being repaired between 6h and 12h after exposure.

In terms of oxidative DNA damage, no consistent trend was found between 1h (3.00±6.28), 6H (1.36±11.25) and 12h (6.08±1.52) relatively to controls (2.45±0.90), although the increase observed at 12h may indicate that cells are in stressful conditions.

The overall data strongly suggests the need for further studies, namely the increase of the number of samples and human biomonitoring studies assessing the occupational exposure of workers that handle with MRI technology.
O17 - CYTOGENETIC MARKERS AND DNA DAMAGE IN WORKERS EXPOSED TO FORMALDEHYDE, A RECENT KNOWN HUMAN CARCINOGEN

Solange Costa¹,², Carla Costa¹,², Vanessa Valdiglesias¹,³, Patrícia Coelho², Susana Silva³, Luís Silva Santos⁴, Beatriz Porto⁵, Blanca Laffon⁶, João Paulo Teixeira¹,²

¹ ISPUP-EPIUnit, Universidade do Porto, Porto, Portugal
² Environmental Health Department, National Institute of Health, Porto, Portugal
³ Toxicology Unit, Department of Psychobiology, University of A Coruña, A Coruña, Spain
⁴ Health Sciences Department, Portuguese Catholic University, Viseu, Portugal
⁵ Laboratory of Cytogenetics, Instituto de Ciências Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal

Cytogenetic endpoints have long been applied in surveillance of human genotoxic exposure and early effects of genotoxic carcinogens. Comet assay has been applied in human biomonitoring for the detection of different levels of DNA damage at the individual cell level. The simultaneous use of cytogenetic tests and comet assay in occupational studies allows a comparison of the presence of DNA strand breaks due to both acute and chronic exposure and of chromosome damage due to clastogenic and aneugenic events. Formaldehyde is a high-volume production chemical produced worldwide to which many people are exposed both environmentally and occupationally. Formaldehyde was recently acknowledged as a human carcinogen. Although formaldehyde’s genotoxicity is confirmed in a variety of experimental systems, data from human studies is conflicting particularly the biological evidence for genotoxicity in other tissues besides sites of the first contact. The aim of the present study was to evaluate the levels of cytogenetic markers and DNA damage in formaldehyde-exposed workers. Air monitoring was performed to estimate the level of exposure during shift work, the mean level was 0.38±0.03 ppm. The study population was tested for chromosomal aberrations, micronucleus, and sister-chromatid exchange, DNA damage was assessed by comet assay (% tail DNA). Cytogenetic alterations and DNA damage were significantly increased in formaldehyde-exposed workers compared to controls. Correlation between biomarkers was found. The present study addresses the need for a policy-oriented research focused on the development of integrated approaches regarding the health risk assessment of populations occupationally exposed to hazard compounds. Our data indicates a potential health risk situation thus the implementation of security and hygiene measures may be crucial to decrease the risk. This may be achieved by engineering control measures, regular monitoring and surveillance activities, medical surveillance and implementation of written policies and procedures.

O18 - SEARCHING FOR ASSAY CONTROLS FOR THE FPG- AND hOGG1-MODIFIED COMET ASSAY

Peter Møller, Kim Jantzen, Mille Løhr, Maria Helena Andersen, Ditte Marie Jensen, Martin Roursgaard, Pernille Hogh Danielsen, Annie Jensen, Steffen Loft

Department of Public Health, Section of Environmental Health, University of Copenhagen, Øster Farimagsgade 5A, DK-1014 Copenhagen K, Denmark

The formamidopyrimidine DNA glycosylase (Fpg) and human 8-oxoguanine DNA glycosylase (hOGG1)-modified comet assays have been widely used in human biomonitoring studies. An assessment of the literature shows a large variation in the reported levels of Fpg-sensitive sites (range 0.05 – 1.31 lesions/10⁶ bp). The levels of Fpg-sensitive sites are lower in studies where Fpg has been obtained from commercial suppliers or unknown sources as compared to Fpg from one particular non-commercial source. The levels of hOGG1-sensitive sites are lower (range: 0.04 – 0.18 lesions/10⁶ bp in leukocytes) compared to the Fpg-sensitive sites. Surprisingly, many biomonitoring studies have not reported data from assay controls for the enzyme-modified comet assay. This may be due to a lack of consensus about suitable controls for the Fpg- and hOGG1-modified comet assay. A major challenge is to find an agent that only oxidizes nucleobases and does not generate DNA strand breaks because this reduces the dynamic range of Fpg- and hOGG1-sensitive sites in the comet assay. The photosensitizer Ro19-8022 plus light, KBrO₃, 4-nitroquinoline-1-oxide, Na₂Cr₂O₇ and ferric nitrilotriacetate are commonly used assay controls. Ro19-8022 plus light and KBrO₃ have also been used as exposures for the generation of oxidatively damaged DNA in the in vitro base excision repair activity. It is recommended to include positive assay controls in the comet assay and report the results in publications.
O19 - CALIBRATION OF THE COMET ASSAY USING X-RAYS

Gunnar Brunborg, on behalf of the Inter-laboratory Dose-response Calibration Trial Consortium (IDCTC)
Department of Molecular Biology, Norwegian Institute of Public Health, Oslo, Norway

Inter-laboratory trials have attempted to identify sources of variability in the comet assay, hoping to achieve more similar responses between experiments and in different laboratories. A calibration trial was initiated several years ago, involving laboratories in Russia, Denmark, UK, Belgium, Czech Republic, Poland, and two laboratories in Norway. The idea was to compare ionizing radiation dose-response curves (Tail %DNA vs dose in Gy) obtained with peripheral blood cells in each participating laboratory, using defined radiation doses. Radiation dose calibrations were hence in focus. X-ray or gamma sources were calibrated by all partners using alanine pellet dosimeters then sent to a specialised laboratory for analysis, and in addition radiation doses were measured using thermoluminescence detectors and two aqueous chemical dosimeter solutions. We observed some deviations between the alanine pellet calibrated and the nominal dose rates. Chemical dosimetry performed locally was satisfactory. Fresh mononuclear cells were analysed after irradiation with 6-7 doses (0 – 15 Gy), using a standardised comet assay protocol and also the favourite protocol of each laboratory. The dose response curve regression slopes showed substantial variations based on nominal doses and less variation with dose corrections. The response of cells irradiated in suspension vs embedded in gels were similar indicating that post-exposure repair of DNA strand breaks before comet analysis was unimportant. The favourite protocols of each laboratory implied different electrophoresis conditions (V/cm, time and agarose concentrations); when correcting for these differences, some of the dose-response curve slope differences were explained. The study shows that comet assay data obtained in different laboratories are quantitatively more similar when results are compared based on reference curves obtained using fresh mononuclear blood cells and calibrated radiation doses. This is promising news for analyses of large databases of comet results obtained in studies of human samples in different laboratories using different electrophoresis conditions.

O20 - GENOME SIZE AND SENSITIVITY TO X-RAYS – PLANT COMETS TELL THE STORY

John Einset
University of Oslo, Oslo, Norway

Among several factors affecting radiation sensitivity, genome size has received limited attention during the last 50 years since research at Brookhaven National Laboratory (USA) and other locations demonstrated substantial differences in radiation sensitivities between tree species with large (e.g. conifers such as pines) versus small (e.g. dicots such as oaks) genome sizes. Taking advantage of the wide range of genome sizes among species, we conducted studies using whole plants or isolated nuclei exposed to X-rays and analyzed DNA damage with the alkaline comet assay. As a starting point, we considered two possible explanations for the high radiation sensitivity of conifers such as pines with large genome sizes: 1) impaired DNA repair and/or 2) inherently higher sensitivity of larger genomes. With regard to DNA repair, two conifer species, Sawara cypress (*Chamaecyparis pisifera*, 8.9 Gbp genome size) and Scots pine (*Pinus sylvestris*, 20 Gbp genome size), both effectively repaired DNA damage within 50 and 70 minutes, respectively, after acute X-ray exposures. In terms of genome size effects, experiments exposing isolated nuclei from 6 different plant species to X-rays, varying in genome sizes 2.6-19.2 Gbp, showed that larger genomes are more sensitive to DNA damage by a relationship corresponding to the cube-root of the nuclear volume; e.g. a 10-fold increase in genome size increases sensitivity by 2.15-fold.
O21 - Validation of high throughput alkaline and FPG modified comet assay using a linear mixed model.

Hervé Perdry1, Kristine B. Gutzkow2, Marianne Chevalier3, Gunnar Brunborg2, Elisa Boutet-Robinet3

1 Univ Paris-Saclay & Inserm CESP, Villejuif, France
2 Department of Molecular Biology, Norwegian Institute of Public Health, Oslo, Norway
3 Toxalim (Research Center in Food Toxicology), Université de Toulouse, INRA, ENVT, INP-Purpan, UPS, Toulouse, France

Even if comet assay has been widely used for decades, there are still two challenging issues: low throughput and variability of the assay. To improve these two points we further validate a high throughput comet assay using Gelbond® films. The aim of this study is to analyze both intra and inter-experiment variability and to decompose the source of these variabilities. Several experiments were performed using human lymphocytes either untreated or treated with different concentration of MMS (Methyl Methanesulfonate) to induce different levels of DNA damage. A positive control for Fpg (Formamidopyrimidine DNA glycosylase) modified comet assay (Ro-198022 with light) was also used. Each experiment (one electrophoresis) included 3 Gelbond® films with 40 deposits on each (15 µL of sample per deposit).

A total of 50 cells from the same condition and the same volunteer were analyzed on each deposit. Instead of summarizing the DNA damage measured on a deposit by the mean or median of the percentage tail DNAs, all the data points were analyzed using a Linear Mixed Model after appropriate data transformation. This method allows identification of the sources of variability of the assay, including intra-deposit variability.

The main source of variation in our experimental data were between cells within the same deposit, suggesting biological rather than technical causes. The second source of variation is the inter-experimental variation (day-to-day experiment). Variation between deposits in the same experiment is negligible. Moreover, there is no systematic bias due to positioning of deposits on the Gelbond® film nor to position of the Gelbond® film in the electrophoresis tank. These conclusions were the same for all conditions (either negative control, MMS or Ro-198022 with light treated cells).

This high throughput alkaline and Fpg modified comet assay is thus suitable for various applications of the comet assay and especially biomonitoring.

O22 - 4DLifetest: A highly accurate high throughput comet assay platform

Juan Carlos Cassano1, Cordula Hirsch1, Oliver Schicht2, Peter Wick1

1 Empa, Swiss Laboratories for Material Science and Technology, Particles-Biology Interactions Lab, CH-9014, St Gallen, Switzerland
2 4D Lifetec AG, CH-6330 Cham, Switzerland

Variability within the Comet Assay (CA) is a major issue, due to differences in parameters within CA protocols, such as agarose concentration, unwinding time, electrophoresis duration, voltage gradient, temperature of electrophoretic run, the type of electrophoretic tank used and the lack of adequate biological reference standards. These inevitably lead to inter-laboratory differences in results.

The aim of this study was to narrow the variation within the CA by influencing: 1) the physical parameters, namely the electrophoresis tank; 2) the sample preparation prior to electrophoresis, namely the protocol; and 3) the biological sample (reference standard).

For this study, we have used the 4DLifetank™, a new innovative electrophoresis tank developed by 4DLifetec AG based on SCGE technology. The 4DLifetank™ provides accurate voltage and temperature control, and a patented highly homogenous and controlled electric field. The 4DLifetank™ allows high throughput comet assay analysis through the development of the gel spotting plate (GSP), offering a flexible format in terms of sample number and allocation of spotting on the plate.

We have analyzed and quantified the variability between spots on the GSP, and the variation between different 4DLifetank™ units as well as CAs performed on different days, and shown the 4DLifetank™ to be highly reproducible. Using A549 cells exposed to increasing levels of X-ray irradiation (0, 1, 2, 4, 8 Gy), the 4DLifetank was able to distinguish between the amounts of increasing DNA damage in each treated population. Finally we demonstrate the improved precision of the 4DLifetest versus other electrophoresis units.
O23 - A COMPARISON OF DIFFERENT STATISTICAL STRATEGIES TO ANALYZE COMET DATA

Timur Tug1, Katja Ickstadt1, Bernd-Wolfgang Igl2
1 Faculty of Statistics, TU Dortmund University, Dortmund, Germany
2 Research and Clinical Sciences Statistics, Bayer AG, Berlin, Germany

In the pharmaceutical industry, the single cell gel electrophoresis assay (Comet assay) is one of the standard tests to evaluate the genotoxic potential of a test substance. The Comet assay is considered to be a sensitive follow-up in-vivo assay within a standard genotoxicity test battery that is able to detect chemically induced DNA damages in various tissues. A recent update of the OECD Guideline for the Testing of Chemicals No. 489 directly affects the statistical analysis of Comet data. We will demonstrate that the selection of different parameters to describe an average value per slide, like the geometric mean or a median, leads to fundamentally different test results. Under certain circumstances, these changes are dramatic, such that, for example, a positive outcome becomes a negative one. In this talk, we will compare different statistical strategies by means of original as well as simulated data, and we will suggest a generalized linear model to compare treatment and vehicle measurements.

O24 - REPORT FROM THE SHORT-TERM SCIENTIFIC MISSION (STSM) IN ROME ON JOINT WORK FOR ADJUSTING DATABASE AND MAKING THE MASTERFILE AND PERFORMING FIRST STATISTICAL ANALYSIS, DISCUSSION OF THE RESULTS AND PREPARATION FOR WRITING THE SCIENTIFIC PAPER

Mirta Milić1, Stefano Bonassi2,3, Emilio Rojas Del Castillo2,3, Irene Paximadas2, Marcello Ceppi4, COST hCOMET Consortium
1 Mutagenesis Unit, Institute for Medical Research and Occupational Health, Ksavarska cesta 2, 10 000 Zagreb, Croatia
2 Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Pisana, Rome, Italy
3 Department of Human Sciences and Quality of Life Promotion, San Raffaele University, Rome, Italy
4 Unit of Clinical Epidemiology, IRCCS AUO San Martino-IST, Genoa, Italy

The crucial task in deliverables and milestones of the Working Group 1 in the newly formed Cost Action hCOMET- The comet assay as a human biomonitoring tool, was to make files for data collection from the laboratories using alkaline comet assay in human biomonitoring, to contact and send them spreadsheets and other documents where methods and studies would be described in details. Collected spreadsheets from the participating laboratories were used to create a unified database-Masterfile. After the Masterfile, next task would be to discover firstly the technical factors influencing comet assay results and to check their comparability among the laboratories and give guidelines and protocols in order to minimize the technical influence on the comet assay results. For this reason, the Short-Term Scientific Mission (STSMs) has been done after collection of the data. We have collected data on more than 19 000 persons, from 41 laboratories all over the world with 90 methods described and with the results from 108 studies. In one month during STSM, the data were unified in one database and the first statistical analysis were performed with description of the similarities and differences in methods and performance and type of the studies. The results of the first statistical analysis will be represented and we will represent the changed masterfile after adjustment according to the type of the data received. Masterfile includes not only results on DNA damage parameters, but also DNA damage and repair measured with FPG, hOGG1, ENDO III, BER and NER repair, cellular repair assay, information about clinical parameters, exposure, life-style habits: diet, exercise, BMI; disease, drugs and, if measured, information about chromosomal aberrations, micronucleus or polymorphisms.
O25 - A pre-validation study of comet assay on plants

Boccia Priscilla¹, Pourrut Bertrand², Miriam Zanellato¹, Julien Dubus², Vinita Vijayaraj¹, Sturchio Elena¹

¹ Italian Workers Compensation Authority (INAIL), Department of Technological Innovation and Safety of Plants, Product and Anthropic Settlements (DIT), Via R. Ferruzzi 38, Roma, Italy
² Yncrea Hauts de France – ISA Lille – LGCGe, 48 boulevard Vauban, Lille, France

In the 1990s the comet assay began to be used in plant models, with several limitations in the protocol as a result of the differences in the structure of the plant cell as compared to the animal cell. Because of the conserved structure of plant genetic material, numerous varieties of species can be used in plant genotoxicity testing. In the case of plant cells, several discrepancies and dissimilarities in the protocol exist across labs, despite the fact that an optimized protocol was recently released [1]. In addition, several labs continue to adopt conventional methods into their protocol, which are both time-consuming and redundant, reducing the reliability of the assay.

The aim of this work was to initiate a collaboration between two research institutes in order to study the inter-laboratory variation in DNA strand breaks in plants and identify key factors affecting comet assay performance through a pre-validation study. Two model plants were selected; Vicia faba (broad bean) and Trifolium repens (white clover). Several optimizations in Pourrut’s protocol were evaluated at different steps such as chopping, unwinding, and electrophoresis time [1]. Preliminary significant results have been obtained, identifying the extraction as the main critical step. The chopping method resulted in good efficiency of nuclei isolation in terms of integrity and yield obtained for Trifolium repens while not for Vicia faba variety used because of its cellular structure, as its larger nuclei, thus a different method of isolating nuclei from Vicia faba has been required.

Further research is required in order to optimize the same protocols using different sensitive plant species to ensure reliability as well as an extrapolation of results to provide a guideline for plant comet assay.


O26 - Medium throughput plant comet assay optimisation and development of an automated scoring system

Julien Dubus¹, Charles Homsy², Michel Soussaline³, Françoise Soussaline³, Bertrand Pourrut²

¹ Équipe Sols et Environnement, Laboratoire Génie Civil et géo-Environnement (LGCGe-ISA Lille), Lille, France
² IMSTAR S.A., Paris, France

The comet assay applied to plant has been established as one of the most interesting techniques to detect the genotoxic effect of a pollutant in plant. Despite the growing uses of the plant comet assay in the past 10 years, this technique should be improved. Because, unlike the comet assay applied to animal cells, in the plant comet assay (i) it is difficult to isolate intact nuclei and (ii) the nuclei extraction rate is low. Then (iii) no standardized protocol is established and (iv) no high throughput comet assay scoring method are available. In these circumstances, the French-Norwegian project ComPack (2014-2017) tried to deal with these issues. Identification of key steps in plant comet assay has been identified and an optimized protocol to increase the reliability was established [1]. The major issue related to the throughput improvement of the plant comet assay is to conciliate a high number of nuclei extracted and a low background level. To realize that, firstly, an optimisation of the nucleus extraction was done. Secondly, an automated scoring system was adapted to the plant comet assay and the background was controlled by this system. As result, a new extraction protocol allows the isolation of several nucleus and its compatibility with the medium-throughput comet assay (12-gel system). In addition, the background is now clear and compatible with an automated scoring system. So, the automated scoring system Pathfinder™, developed by IMSTAR and initially designed for the human/animal cells is now compatible with the plant comet assay. In conclusion, these results are a technological breakthrough for the plant comet assay.

O27 - Performing the comet assay in combination with different human and bacterial repair enzymes in order to increase its sensitivity

José Manuel Enciso1, Kristine B. Gutzkow2,3, Ann-Karin Olsen2,4, Magnar Bjøras5, Rune J. Forstrøm2, Gunnar Brunborg, Adela López de Cerain, Amaya Azqueta1
1 Department of Pharmacology and Toxicology, University of Navarra, Pamplona, Spain
2Department of Molecular Biology, Norwegian Institute of Public Health, Oslo, Norway
3 Centre for Environmental Radioactivity (CERAD CoE), Norway
4 Department of Microbiology, Oslo University hospital, Oslo

Single-cell gel electrophoresis (the comet assay) has been used for more than 30 years in genotoxicity testing, human biomonitoring and ecogenotoxicology. Whereas the standard alkaline version of the comet assay is able to detect single and double strand breaks and alkali-labile sites (mostly apurinic or apyrimidinic sites), this assay can also be used in combination with lesion-specific enzymes in order to detect certain other classes of DNA damage. Most widely used is the *Escherichia coli* Formamidopyrimidine-DNA glycosylase (Fpg), which detects a variety of DNA lesions including oxidized bases (mainly 8-oxoguanine), ring-opened purines and formamidopyrimidine (FaPy)-products of adenine and guanine. Our aim was to establish the comet assay in combination with novel DNA repair enzymes to broaden the lesion specificity of the assay, and, in addition, to compare the sensitivity and specificity of the different repair enzymes. For this purpose, human lymphoblastoid cells (TK6) were treated with either methyl methanesulfonate (MMS), a known monofunctional alkylating agent producing 3-methyladenine (3 mA) and 7-methylguanine (7mG), or a photosensitizing agent (Chiron 8503, similar to Ro 19-8022) together with visible light producing oxidative DNA damage. The comet assay was performed in combination with the DNA repair enzymes Fpg, its human analogue OGG1 (8-oxoguanine DNA glycosylase) and human 3-methyladenine DNA Glycosylase (AAG). All the different DNA repair enzymes tested were able to detect DNA damage produced by both agents to different extents and increase the sensitivity of the assay.

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O28 - Identification of knowledge gaps and opportunities in the validation of comet assay based DNA repair phenotyping in human biomonitoring studies

Roger Godschalk2 and Sabine Langie2,3, on behalf of Workgroup 5 of the hCOMET project (CA15132)
1 Dept. Pharmacology & Toxicology, School for Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University, Maastricht, The Netherlands
2 Unit Environmental Risk and Health, Flemish Institute of Technological Research (VITO), Mol, Belgium
3 Faculty of Sciences, Hasselt University, Diepenbeek, Belgium

DNA damage levels represent the net effect of damage formation and removal. Therefore, to better interpret human biomonitoring studies, an individual’s ability to remove DNA damage should be characterized. Various comet assay based methodologies are available to measure DNA repair capacity, but their application in field studies depends on: i. DNA repair in accessible surrogate tissues should reflect repair capacity in target-tissues, ii. DNA repair kinetics should be defined to optimize sampling timing, iii. Sources of inter- and intra-individual variability must be controllable, and iv. Analyses of DNA repair capacity by the comet assay must have advantages compared to other techniques. Although DNA repair capacity is different between various tissues, a correlation was reported between the repair capacity in easily accessible peripheral blood mononuclear cells and internal organs for both nucleotide (NER) and base excision repair (BER). However, no correlation was found with tumor tissue. It is not yet sufficiently clear whether total white blood cells or other accessible cells (e.g. exfoliated cells) can be used. There are mixed reports concerning the regulation of DNA repair by environmental and dietary factors. Exposure to genotoxic agents has in general not been found to change BER activity, whereas *in vitro* and *in vivo* studies indicate that NER can be altered by various conditions. Thus, crucial questions concerning the factors regulating repair and inter-individual variation remain unanswered, and therefore, timing of sampling is a key issue that needs further investigation. Still, intra-individual variation over a period of days to weeks seems limited, which is favorable for DNA repair phenotyping in biomonitoring studies. Interestingly, DNA repair phenotyping cannot easily be replaced by genomic or transcriptomic approaches. In conclusion, although comet assay based approaches to measure BER/NER phenotypes are promising, more work is needed to optimize their application in human biomonitoring and intervention studies.
**O29 - Mobile phone specific electromagnetic fields induce DNA damage and nucleotide excision repair in human glioblastoma cells**

Siegfried Knasmüller¹, Franziska Ferk¹, Michael Kundi², Andrea Bileck³, Christopher Gerner⁴, Miroslav Mišik⁵, Armen Nersesyan⁶, Monika Waldherr⁷, Manuel Murbach⁴, Tamara T. Lah⁶, Christel Herold-Mende⁶, Andrew R. Collins⁷, Halh Al-Serori¹

¹Institute of Cancer Research, Department of Internal Medicine 1, Medical University of Vienna, Vienna, Austria
²Center for Public Health, Institute of Environmental Health, Medical University of Vienna, Vienna, Austria
³Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria
⁴IT IS Foundation, Zurich, Switzerland
⁵Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, Slovenia
⁶Experimental Neurosurgery, Department of Neurosurgery, University of Heidelberg, Heidelberg, Germany
⁷Department of Nutrition, University of Oslo, Oslo, Norway

It was found in epidemiological studies that the use of mobile phones causes brain tumors (glioblastomas). It is known that damage of the DNA leads to malignant transformation. Therefore, we investigated the impact of the UMTS signal on DNA in human glioblastoma and other cell lines under conditions which are relevant for users (SAR 0.25 to 1.00 W/kg). We found induction of comet formation in single cell gel electrophoresis assays in a p53 proficient glioblastoma line U87 when the cells were grown without serum, while no effects were seen in the p53 deficient line (U251). Subsequent experiments showed that the damage in U87 cells disappears after 1 hr; it is repaired by nucleotide excision repair (NER) and does not cause double strand breaks (DSBs). The observation of NER induction is supported by findings of a proteomic analysis indicating that several repair proteins are up-regulated after exposure to UMTS; additionally, we detected higher concentrations of proteins which are involved in the γ-interferon pathway. Our findings show that the signal causes transient genetic instability in glioma derived cells and activates defense systems.

**O30 - Kinetics of DNA double-strand breaks repair in moss Physcomitrella patens**

Marcela Holá, Radka Vágnerová, Karel J. Angelis

Institute of experimental botany AS CR, Na Karlovce 1, 160 00 Praha 6, Czech Republic

Plants are permanently exposed to genotoxic stress and therefore they evolved a number of effective DNA repair mechanisms. As a most deleterious lesion of DNA are considered to be double stranded breaks (DSB’s) which are usually repaired via homologous recombination (HR) or non-homologous end joining (NHEJ). Measurement by comet assay showed that kinetics of DSB-DNA repair in plants is biphasic with very rapid first phase in which most of DSB’s is removed.

A moss *Physcomitrella patens* is an attractive model organism for DNA repair study in particular for the preference of homologous recombination during DSB repair. Kinetics of DSB repair in *Physcomitrella* mutants deficient in HR revealed that fragmentation of DNA after bleomycin (BLM) treatment was removed at equal rates ($t_{1/2} = 3$ min) in each mutant as in wt. Similarly, in *Physcomitrella* mutants *pplig4* and *ku70* with defective NHEJ repair pathway kinetics of DSB’s was intact. This suggests a presence of alternative on HR and NHEJ independent repair pathway in plants. First characterization of the rapid repair pathway revealed its dependence on structural maintenance of chromosome (SMC) complexes and structurally related SMCHD proteins. Measurement of kinetics of the quick repair pathway is enabled by easy and fast sample processing during comet assay protocol allowing study of the fastest DSB’s repair phase.
O31 - DNA damage and repair capacity in mother and newborns from the Spanish cohort (Newgeneris study)

Naouale El Yamani, Amaya Azqueta, Andrew Richard Collins, Ricard Marcos
1 Health Effects Group, MILK, Norwegian Institute for Air Research, Kjeller, Norway
2 Department of Pharmacology and Toxicology, University of Navarra, Pamplona, Spain
3 Department of Nutrition, University of Oslo, Oslo, Norway
4 Department of Genetics and Microbiology, Autonomous University of Barcelona, Cerdanyola del Vallès, Spain

Exposure during pregnancy to genotoxic or preventive agents could increase or decrease the risk of adverse health effects later in a child’s life. Oxidative stress might contribute to birth defects and in many studies, 8-oxoguanine (8-oxoG) is considered to be a sensitive and reliable biomarker of oxidative damage to DNA. This biomonitoring study was carried out to measure the level of 8-oxoG in lymphocytes from healthy mothers and in their neonates and to establish whether the level differs between the two groups. We also used cellular extracts from lymphocytes of both mothers and newborns, containing repair enzymes, to determine their base excision repair (BER) capacity.

A total of 200 pairs of blood samples from mothers and new-borns were analyzed. The comet assay incorporating formamidopyrimidine DNA glycosylase (Fpg) and endonuclease III (EndoIII) was used. Significantly higher frequency of DNA breaks and oxidized bases were obtained in mothers compared to umbilical cords (p<0.001). Our results demonstrated also a positive correlation between mothers and newborns in DNA strand breaks (SBs) (p<0.001), Fpg-sensitive sites (p<0.001), EndoIII-sensitive sites (p<0.001). The frequency of SBs in mothers was not associated with age. However, when analysing the DNA oxidation damage in newborns, this was inversely associated with the mother’s age (p<0.05). Linear regression analysis showed that the level of DNA oxidation damage is lower in children born from mothers with pre-BMI above 24.12 kg/m² (p<0.001). Moreover, preterm newborns (<37weeks) had a significantly lower level of oxidised purines than that found in full-term newborns (p<0.001). We did not observe any correlation between DNA damage in mothers (both types; SBs and oxidised bases) and DNA repair in newborns. The DNA repair capacity in newborns was also inversely related to the pre-BMI of their mothers.

O32 - The effect of DNA repair in target tissue on response to treatment, disease progression and long-term survival in colon cancer patients

Sona Vodenkova, Michal Kroupa, Katerina Jiraskova, Marketa Urbanova, Veronika Vymetalkova, Ludmila Vodickova, Alena Opatlova, Pavel Vodicka
1 Department of the Molecular Biology of Cancer, Institute of Experimental Medicine, The Czech Academy of Sciences, Prague, Czech Republic
2 Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University, Prague, Czech Republic
3 Department of General Biology and Genetics, Third Faculty of Medicine, Charles University, Prague, Czech Republic
4 Faculty of Medicine and Biomedical Center in Pilsen, Charles University, Pilsen, Czech Republic

Colon cancer (CC) is one of the most common cancers with the highest mortality rate in Central Europe mainly due to late diagnosis and low treatment efficiency. A conventionally used chemotherapeutic agent in CC at locally advanced stages is 5-fluorouracil (5-FU) which, at molecular level, either incorporates into DNA/RNA or imbalances the synthesis of thymidine from uracil resulting in false uracil DNA incorporation. These DNA lesions are repaired by base excision (BER) and mismatch repair pathways.

Our hypothesis assumed that cells with high DNA repair capacity may show better survival and therefore patients with these molecular characteristics may suffer from poor response to treatment, recurrence and decreased survival. Our study aimed to correlate BER, microsatellite instability (MSI) and repair protein levels in target tissues as a predictive and/or prognostic marker in newly diagnosed CC patients.

Our set of patients consisted of 94 individuals. DNA from tumor tissue and adjacent mucosa was isolated for MSI status analysis. Protein extracts from tissues were isolated both for protein expression and BER capacity measurements. Protein expression levels were tested by Western Blot. Functional assessments of BER were performed by comet assay-based in vitro DNA repair assay.

We did not find differences in BER between tumor tissue and mucosa (p=0.103).
In our group of patients, BER did not affect survival or recurrence. Levels of OGG1 showed positive correlation with BER (p<0.0001). Expression of XPD was higher in tumor (p=0.04) and increased with further stages of the disease. MSI-high tumors showed higher BER.

Our results suggest that levels of BER alone do not have any predictive/prognostic significance. We propose that it is important to study multiple correction pathways simultaneously for deeper understanding of the DNA repair mechanism in the context of cancer therapy.

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O33 - DNA damage among Inflammatory Bowel Disease patients

Cristiana Costa-Pereira, Rosa Coelho, Daniela Grácio, Cláudia Dias, Marco Silva, Armando Peixoto, Pedro Lopes, Carla Costa, João Paulo Teixeira, Guilherme Macedo, Fernando Magro

1 National Institute of Health – Environmental Health Department, Oporto, Portugal
2 MedInUP - Centre for Drug Discovery and Innovative Medicines, University of Oporto, Oporto, Portugal
3 Gastroenterology Department, Faculty of Medicine, Centro Hospitalar São João, Oporto, Portugal
4 Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto, Oporto, Portugal
5 Health Information and Decision Sciences Department, Faculty of Medicine, University of Oporto, Oporto, Portugal
6 CINTESIS – Centre for Health Technology and Services Research, Oporto, Portugal
7 Department of Internal Medicine, Faculty of Medicine, University of Porto and Centro Hospitalar São João, Porto, Portugal
8 EPIUnit - Institute of Public Health, University of Oporto, Oporto, Portugal

Chronic inflammation is the hallmark in Inflammatory Bowel Disease (IBD), a group of conditions mainly referring to Crohn’s disease (CD) and ulcerative colitis (UC). Although its etiology is still unknown, research has identified genetic predisposition and environmental factors as key elements in the development of IBD. Inflammation is a major contributor to cellular oxidative damage and assumed to be involved in the promotion of carcinogenesis.

This work aimed to investigate the oxidative damage in IBD patients through a case-control and prospective study with 344 IBD patients and 294 healthy controls. DNA damage and oxidative DNA damage were measured by comet assay techniques and oxidative stress by plasmatic lipid peroxidation, protein carbonyls, and total antioxidant capacity.

Higher DNA damage (P<.001) was found both in CD (9.66 % Tail DNA (%TD); IQR: 6.21-14.01) and UC patients (7.13 %TD; IQR: 4.35-11.68) when compared to controls (5.41 %TD; IQR: 3.76-6.76) as well as oxidative DNA damage (CD: 3.56 %TD; IQR: 1.76-6.78; UC: 4.57 %TD; IQR: 2.35-8.07; and Controls: 2.31 %TD; IQR: 1.16-4.18). Stratifying patients by therapeutic groups (5-ASA, azathioprine, anti-TNF, and combined therapy with azathioprine and anti-TNF), significant differences were found for DNA damage, both in CD and UC, with
the combined therapy exhibiting the highest DNA damage levels (11.56 %TD; IQR: 9.54-14.32, and 12.37 %TD; IQR: 10.60-14.99, respectively). Among CD patients, disease behaviour (B1 and B2), and age at diagnosis over 40 years (A3) stood out as risk factors for DNA damage. For UC patients, the found risk factors for DNA damage were disease activity, treatment, age at diagnosis under 40 years (A1+A2) and disease locations (E2 and E3).

In conclusion, in IBD there is an increase in the DNA damage levels, and treatment, age at diagnosis and inflammatory burden seem to be risk factors.

O34 - An attempt to modify DNA single-strand breaks in diabetes mellitus patients and in rat models of the disease by 1,4-dihydropyridien derivatives

Elina Leonova¹,² Evita Rostoka¹,², Alise Dekante¹, Jelizaveta Sokolovska¹,², Nikolajs Sjakste¹,²
¹ University of Latvia, Faculty of Medicine
² Latvian Institute of Organic Synthesis

Abnormal production of free radicals and subsequent DNA damage are important in development of diabetes mellitus (DM) complications. Search for remedies which could alleviate DNA lesions in DM is a topical problem, and 1,4-dihydropyridines (DHP) appear to be promising compounds from this point of view. In the present study we have tested some compounds of this class on the level of DNA breaks in nucleated blood cells of DM patients and in animal models of DM.

Fifty-four type 1 DM patients and forty nine healthy subjects were enrolled in the study. In rats Type 1 DM was modelled by streptozotocin (STZ) or alloxan injections. Comet assay was performed as described (Azqueta and Collins, 2013).

The DNA of nucleated blood cells in the Type 1 DM patients contained more single-strand DNA breaks (83.98±9.45 AU) compared to the control group (58.03±6.15 AU; p=0.0264). When blood samples were incubated ex vivo with AV-153-Na, the DNA breaks were reduced to 49.59±16.18 AU by 1 nM and to 37.95±8.02 AU (p=0.0016) by 5 nM of the compound.

Induction of the STZ DM, but not alloxan DM, caused a three-fold increase of the level of DNA breakage (from 28.82 ± 2.11 AU to 88.43 ± 693 AU; p< 0.0001). Strikingly, administration of AV-153 to intact rats caused DNA breakage, the level of single-strand DNA breaks was raised up to 142.00 ± 13.70 AU, n= 4, the result was statistically significant (p<0.001). Some other 1,4-DHPs (metcarbatone, etcarbatone and stirylcarbatone also promoted the level of DNA breakage in intact and diabetic animals. However metcarbatone at dose 0.5 mg/kg and stirylcarbatone at dose 0.05 mg/kg alleviated DNA damage level. Thus 1,4-DHP appear to be prospective compounds for alleviating level of DNA breaks in DM. Additional studies on dosages and administration protocols should be performed.
O35 - Modulation of genomic and biochemical instability in patients with Type 2 Diabetes mellitus treated with vitamin D

Vanessa Moraes de Andrade1, Gabriela Elbio Fagundes1, Adriani Paganini Damiani1, Tamires Pavei Macan1, Paula Rohr1, Luciane Bisognini Ceretta1, João Paulo Fernandes Teixeira2

1 University of Southern Santa Catarina, UNESC, Criciúma, SC, Brazil
2 Portuguese National Institute of Health, Department of Environmental Health, Porto, Portugal

Vitamin D has been recognized as a vitamin with pro-hormone activity. Since its receptor is present in various tissues other than bones, like gut and kidneys, some studies suggest an association of vitamin D deficiency with the pathogenesis of various health conditions, such as cardiovascular disease and type 2 diabetes mellitus (T2DM), as well as an important role in essential stages of genomic instability development. The objective of this study was to investigate the influence of vitamin D3 supplementation on the modulation of genomic instability in patients diagnosed with T2DM. We evaluated 79 patients with T2DM, registered in the Integrated Clinic of University of Southern Santa Catarina. Participants received 4000 IU vitamin D3 supplementation daily for eight weeks. Blood samples were collected at the beginning of supplementation, at the end of supplementation, and after 4 weeks of the end of supplementation, and were evaluated: 25(OH)D, glucose homeostasis, lipid profile, hepatic enzymes, renal function, oxidative stress and DNA damage. Supplementation of 4000 IU of vitamin D3 over the eight weeks was sufficient to increase blood levels of 25(OH)D (P <0.0001) and to positively influence glycemic control, liver enzymes, oxidative stress and DNA damage. Vitamin D3 supplementation was shown to be effective on the reduction of free radicals generated by the poor glycemic control and consequently for the reduction of DNA damage, which becomes this vitamin a potential adjuvant in the treatment of T2DM.

O36 - Comet Assay in evaluation of individual DNA-damage of blood leukocytes in breast cancer patients undergone chemotherapy with doxorubicin-containing regimens

Ekaterina Igorevna Surikova1, Irina Alexandrovna Goroshinskaya1, Elena Mikhail'evna Frantsiysiants2, Ol'ga Vladimirovna Tarnopolskaya1, Nikolai Petrovich Sirota2, Ljubov' Jur'evna Vladimirova1, Natal'ja Mikhail'evna Tikhanovskaya1, Elena Ananevna Kuznetsova1

1 Rostov Research Institute of Oncology, Rostov-on-Don, Russian Federation
2 Institute of Theoretical and Experimental Biophysics, Pushchino, Russian Federation

Prediction of a chemotherapy effectiveness and development of hematological complications is important task for oncologist. Comet assay allow evaluating DNA damage in individuals undergoing chemotherapy. We assessed DNA damage in leukocytes (nontarget cells) of breast cancer patients undergone doxorubicin-containing neoadjuvant chemotherapy. We studied leukocytes of fresh (not frozen) capillary whole blood from 17 breast cancer patients (T1–2N1–2M0, infiltrating ductal carcinoma, all subtypes), during neoadjuvant chemotherapy with doxorubicin-containing regimens (FAC). The level of DNA damage (% tail DNA) was assessed by alkaline Comet Assay on the days 0 and 2-5 on the 1st and 2nd courses of chemotherapy. We used 1% agarose both types, electrophoresis at 20 min, 20V, 300 mA. The slides were stained by ethidium bromide. As a positive control we used the exposure of slides in H2O2 150 µM 10min. 400 comets per patient were evaluated. Before treatment % tail DNA was 2.6±0.3% (1.5-5.7%). Only in 1 patient significant increase of % tail DNA in 2 times was observed on day 2. Before the second course, % tail DNA in 11 patients was similar to the primary level and in 6 patients was increased by 3-5 times (with triple negative tumor, multicentric tumor or bilateral synchronous breast carcinoma). On the 2-4 days of the 2nd course % tail DNA was decreased by 3-4 times in these 6 patients. In positive control all patients had % tail DNA significantly increased up to 20 times before 1st and 2nd courses but during the 2-4 days its increase was only up to 4 times, showing reduction of sensitivity to H2O2.

The data obtained may be due to the mechanism of action of the used drugs (especially doxorubicin) and also could be specified by individual features of the patient (IHC subtype of tumor or clinical characteristics of the disease).
O37 - THE COMET ASSAY AS A MEANS TO MONITORING NUTRITIONAL EFFECTS ON DNA INTEGRITY IN HUMANS

Tamara Bakuradze¹, Isabel Groh², Doris Marko³, Jens Galan², Gerhard Eisenbrand¹, Elke Richling¹
¹ Department of Chemistry, Division of Food Chemistry and Toxicology, University of Kaiserslautern, Kaiserslautern, Germany
² Department of Food Chemistry and Toxicology, University of Vienna, Vienna, Austria
³ Gruenstadt, Hochgewannen 19, Gruenstadt, Germany

DNA damage is associated with the pathogenesis of cancer and other diseases. Consumption of foods rich in polyphenols (e.g. coffee and/or red fruit juice) was reported to improve DNA integrity in humans. The comet assay is a widely accepted technique for measuring DNA damage at the level of an individual cell and is widely used in human intervention studies. However, as effects of nutritional/lifestyle usually are rather small, it is challenging to measure potential effects on DNA integrity by the standard comet assay method. We therefore have adapted the method in distinct aspects: investigations were performed in whole blood and the monitoring of tail intensity is achieved via higher (63 x/1.25 oil) than standard magnification.

We have performed human intervention studies to investigate the consumption of coffee or anthocyanin rich fruit juice on DNA integrity in whole blood cells. In the first study reported here healthy volunteers consumed three cups of coffee (à 250 ml) per day for four weeks. The results showed that regular coffee consumption was associated with markedly reduced background DNA strand breaks as compared to the control. In the second intervention study, volunteers consumed coffee brew repeatedly during eight hours (four times 200 ml). Already two hours after the first coffee intake, background DNA strand breaks were significantly reduced in comparison to baseline. In another study, volunteers consumed an anthocyanin-rich fruit juice. The regular intake of this red fruit juice significantly decreased total DNA strand breaks already eight hours after consumption and prologs for many weeks.

In conclusion, our studies showed that consumption of coffee and/or anthocyanin-rich juice has the potential to reduce DNA damages during short as well as long time periods. Using this comet method variation, we were able to see significant effects on background DNA strand breaks.

O38 - ANTIGENOTOXIC POTENTIAL OF DRY OLIVE LEAF EXTRACT AGAINST DNA DAMAGE INDUCED BY ESTRADIOL AND DIETHYLSILBESTROL ON HUMAN PERIPHERAL BLOOD LEUCOCYTES IN COMET ASSAY

Dijana Topalovic¹, Lada Zivkovic¹, Andrea Cabarkapa-Pirkovic¹, Dragana Dekanski², Vladan Bajic³, Suncica Borozan⁴, Biljana Spremo-Potparevic¹
¹ Department of Biology and Human Genetics, Institute of Physiology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia
² Biomedical Research, R&D Institute, Galenika a.d., Pasterova 2, 11000 Belgrade, Serbia
³ The Laboratory for Radiobiology and Molecular Genetics, Institute for Nuclear Research “Vinca”, University of Belgrade, Mike Petrovica Alasa 12-14, 11000 Belgrade, Serbia
⁴ Department of Chemistry, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia

Long-term exposure to higher levels of estrogens and estrogen-like compounds has been associated with the range of adverse effects including development of breast, ovarian and endometrial cancer. It is well known that estrogen-like compounds mediate DNA damage by generation of reactive oxygen species (ROS), implying that their effects can be modulated by antioxidants. Dry olive leaf extract (DOLE), from the Mediterranean olive tree (Olea europaea L.) is known to contain large amounts of bioactive and antioxidative components. Its main constituent is oleanol, but it also contains triterpenes, flavonoids, caffeic acid and tannins. The protective effect of olive leaf extract on H₂O₂-induced oxidative DNA damage is described previously and strong DOLE capacity to attenuate adrenaline- and thyroxine-induced DNA damage in human peripheral blood leukocytes (PBL) is recently demonstrated. The main goal of this study was to investigate antigenotoxic potential of the standardized DOLE against DNA damage induced by 17β-estradiol and diethylstilbestrol (DES) in human PBL using the comet assay (single-cell gel electrophoresis). Our results show that both investigated estrogens (100 μM) expressed genotoxic effect. Dry olive leaf extract was efficient in reducing number of cells with estrogen-induced DNA strand breaks at all tested concentrations (0.125, 0.5 and 1 mg/ml) and under two different experimental protocols, pretreatment and post-treatment, exhibiting significant (p < 0.05) genoprotective and antigenotoxic properties. Based on our findings, DOLE can prevent estrogen induced DNA damage and we can presume that its antigenotoxic potential arises predominantly from the synergism of the cell’s antioxidant capacity stimulation and ROS scavenging.
P1 - DNA damage in enzymatic decolorization of Congo red

Barbara S. Janović, Zoran M. Vujčić and Miroslava T. Vujčić
1 Department of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia
2 Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia

Horseradish peroxidase (HRP) is an oxidoreductase which can catalyze degradation of numerous aromatic substrates, such as dye molecules, in the presence of hydrogen peroxide [1]. However, using the enzymatic decolorization needs to take into account the toxicity of degradation products as well as the percentage of color removed. Enzymatic degradation is known to break the azo bond in dye molecule leading to accumulation of aromatic compound with higher reactivity than the parent dye molecule [2]. We have applied medium throughput comet assay to measure DNA damage in MRC-5 cells treated with double azo dye, Congo red (CR), before and after decolorization by HRP. Obtained results showed that 30 min of in-gel exposure to CR induced DNA damage (in terms of % of DNA in comet tails) in MRC-5 cells. Before HRP treatment highest concentration of CR (200 µg ml⁻¹) induced 72% DNA damage, while after the HRP treatment DNA damage was 45%. Lowest concentration of CR (50 µg ml⁻¹) induced 31% DNA damage, while the same concentration of degradation products showed no DNA damage. We concluded that the enzymatic removal of CR decrease the DNA damage in MRC-5, indicating the potential of HRP for dye decolorization in terms of reduction of genotoxicity potential of dye tested.

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P3 - GENOTOXIC EFFECT OF SECONDARY MINERAL CRYSTALLIZED ON ROCKS FROM COAL MINE DRAINAGE USING IN VITRO ASSAYS

Adriane P. Nordin¹,², Juliana da Silva³, Claudia T. de Souza³, Johnny F. Dias³, Kátia da Boit³, Marcos L.S. Oliveira⁴, Ivana Grivicich⁵, Ana L.H. Garcia⁶, Luis F.S. Oliveira⁴, Fernanda R. da Silva³
¹ Centro Universitário La Salle (UNILASALLE), Canoas, RS, Brazil
² Laboratory of Genetic Toxicology, Lutheran University of Brazil, Canoas, RS, Brazil
³ Ion Implantation Laboratory, Institute of Physics, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

Coal extraction activities, such as coal beneficiation, generates a large volume of waste that are, in many cases, handled or disposed of incorrectly way, causing damage not only to human health but also to the environmental. Often these wastes produce acidic drainage where there several minerals crystallized (evaporites), still little studied by toxicological science. This study aimed identify secondary minerals (evaporites), as well as the genotoxic potential of coal mine drainage which flow into the coal areas. The samples were collected in two places along the Rocinha River in the state of Santa Catarina Brazil: (1) directly from the source of the acid drainage (Evaporites 1), and (2) in the river bank (Evaporites 2). The crystalline samples characterization was realized by X-ray diffraction and by emission technique of X-ray induced by particles. To identify genotoxic and mutagenic potential in vitro of mineral crystallized on rocks in coal mine drainage were applied the Comet assay and the Micronucleus test in V79 cells. According to the results it was observed that Evaporites 1 and 2 samples induced DNA damage (P<0.05), thus presenting a genotoxic potential when compared to the negative control. Such DNA damage increased can be explained by high concentrations of elements such as aluminium, copper, chromium, iron, nickel and zinc in the samples. These elements are found in large amounts in evaporites and are present as the major mineral components that are highly soluble in water. The secondary minerals toxicity in coal area and the potential influence of emissions from coal-mining have been assessed and characterized for the first time. Thus, this study becomes of great significance to environmental pollution research due to the little knowledge of the toxicity of evaporites when disposed in the environment and/or when in contact with the organisms.

P4 - GENOTOXIC EFFECTS OF BISPHENOL A AND INTERACTIONS WITH DOxorubicin ON IN VITRO MRC-5 HUMAN LUNG FIBROBLASTS

Carina Ramos¹, Edna Ribeiro¹,²,³, Manuel C. Gomes⁴, Carina Ladeira¹,²,⁵
¹ Environment and Health Research Group, Escola Superior de Tecnologia da Saúde de Lisboa – IPL, Lisboa, Portugal
² Research Group in Genetics and Metabolism, Escola Superior de Tecnologia da Saúde de Lisboa – IPL, Lisboa, Portugal
³ Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal
⁴ Laboratory of Cancer Biology, Lutheran University of Brazil, Canoas, RS, Brazil
⁵ Centro de Investigação em Saúde Pública – Escola Nacional de Saúde Pública, (CISP-ENSP), Universidade Nova de Lisboa, Portugal

Nowadays, loads of chemicals are present in food, air, water and consumer products, making unavoidable the human exposure to them daily. Some of these chemicals are endocrine disrupting chemicals (EDCs), these substances have the capacity to change function(s) of the endocrine system by acting on hormone receptors directly or interfering in proteins that control the delivery of hormone. Bisphenol A (BPA) is a commonly utilized EDC, that has been suggested to interfere with cell division mechanisms. The aim of this in vitro study is to evaluate the DNA damage induce by BPA in human lung fibroblasts (MRC5) and its interactions with Doxorubicin (DOX). In this study, the cells were exposed to low concentrations of BPA (4.4 μM, 4.4 nM, 0.44 nM). And some of the samples were also exposed to a therapeutic concentration of Doxorubicin (4 μM). After the exposure, all the samples were cryopreserved and comet assay was performed at equal conditions. Our results showed a decrease in the DNA damage in the samples exposed to BPA and to the both substances being the lowest value referring to BPA 4.4 nM and DOX (9.36±10.28) in comparison to control (20.20±13,52). Nevertheless, the cells exposed only to DOX presented an increase (25.88±13,32). Relative to the oxidative damage, there was an increase of the damage of almost all the samples in relation to the control (5.97±15.02), being the highest value corresponding to the sample exposed to BPA 0.44 nM and DOX (34.10±20.72). However, the sample exposed with BPA 4.4 μM and DOX presented a decrease of the damage (1.74±10.27).

This results suggest that BPA influence the effect of DOX in this cells, decreasing the damage at all concentrations and the oxidative damage at the two highest concentrations.
P5 - DNA damage induced by different brands of e-cigarette refill liquids

Aminah Alotaibi, Saad Almugbel, Abdulrahman Bin Mummer, Tahreer Al-Rajudi, Iman Al-Saleh
1 National Center for Biotechnology and Genomic Research, King Abdulaziz City for Science and Technology, Saudi Arabia
2 College of Medicine, Al-Imam Muhammed Ibn Saud Islamic University, Saudi Arabia
3 Biostatistics, Epidemiology & Scientific Computing Department, Saudi Arabia
4 Environmental Health Program, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia

With the widespread perception that e-cigarettes are less harmful than conventional cigarettes and accessible online marketing and promotional offers, it has become a socially common practice among Saudi youths. Recent studies have shown that e-cigarettes contain carcinogenic compounds and nicotine that makes their long-term safety questionable; however findings on their genotoxic behavior are still limited. An in vitro assay was used to examine genotoxicity of different brands of e-cigarette refill liquids sold in Saudi market by single cell gel electrophoresis (comet) assay. A total of 70 samples contain 1-8 mg nicotine as indicated in the label were collected; mainly manufactured in Germany, Italy, Malaysia, USA and Saudi Arabia. Human lymphoblastoid TK6 cells were exposed to 400 µl of e-cigarette refill liquids, nicotine (4 mg) for 24 hours. Negative (untreated cells) and positive controls (cells treated with 100 mM H2O2) displayed respectively the absence and presence of DNA damage. In this study, the results of only 27 e-cigarette refill liquids were tested. All samples induced strand breaks in DNA as depicted by tail moment that was significantly higher than the negative control, but did not differ from nicotine. Tail moment in 15 samples was higher than the one found in cells induced by nicotine. Nicotine in these samples was in the range of 1.5 to 6 mg. Our results suggest that nicotine in e-cigarette refill liquids may be one of the main ingredients that induced DNA damage, other ingredients with mutagenic/genotoxic properties might have also participated. Further investigation is required confirming our observation.

P6 - Genotoxic and oxidative damage of shape-engineered Titanium dioxide nanoparticles (TiO2-NPs) in bronchial epithelial cells.

Sara Bonetta, Marta Gea, Silvia Bonetta, Luca Innarelli, Andrea M. Rossi, Valter Maurino, Giorgio Gilli, Tiziana Schilirò
1 Department of Public Health and Pediatrics, University of Torino, Torino, Italy
2 Department of Quality of Life, Food Metrology Group, INRiM, Torino, Italy
3 Department of Chemistry, University of Torino, Torino, Italy

Different studies on titanium dioxide nanoparticles (TiO2-NPs) genotoxicity have been conducted but no clear accordance exists among mechanism of action. Many physico-chemical properties of TiO2-NPs (i.e. shape, crystal structure, aggregation and coatings) can influence biological effects. The aim of this study was to evaluate the genotoxic effect of three engineered TiO2-NPs shapes (bipyramids, rods and platelet NPs) in bronchial epithelial cells (BEAS-2B) in comparison with two commercial TiO2-NPs (p25 and food grade). Detailed characteristics of TiO2-NPs dispersions were defined (T-SEM, DLS by SETNanoMetro project). BEAS-2B were exposed to different doses of TiO2-NPs (range 0 - 120 µg/ml) for 24h (1h light, 23h dark). Genotoxic and oxidative damage was assessed by Comet assay (with/without Fpg enzyme). Cytotoxic effects were also evaluated by cell viability assays (WST-1) and membrane damage (LDH assay).

Moderate viability reduction (88-96%, p<0.05) was detected in BEAS-2B exposed to the highest concentration of all TiO2-NPs. No significant membrane damage was observed confirming the low cytotoxic effect. A significant (p<0.05) dose-response DNA damage (direct and oxidative) was induced by food grade NPs, while p25 showed only oxidative damage. Bipyramids and rods TiO2-NPs did not show any genotoxic effect; platelet TiO2-NPs induced direct and oxidative DNA damage at the highest doses (p<0.05) and it could be probably related to the higher aggregation tendency. The commercial TiO2-NPs had a higher genotoxic effect than shape engineered ones, however the shape induced different genotoxic effects. This study suggests higher safety in using shape engineered TiO2-NPs for different technological applications (i.e. paints, foods, cosmetics, pharmaceuticals).
The impact of inorganic nanomaterials on global DNA methylation

Alena Gabelova, Annamaria Srancikova, Monika Sramkova, Katarina Kozics, Bozena Smolkova
Cancer Research Institute, Biomedical Research Center SAS, Dubravska cesta 9, 845 05 Bratislava, Slovakia

DNA methylation is an essential epigenetic modification that, along with other epigenetic mechanisms, allows sophisticated time- and tissue-specific control of gene expression. Aberrant DNA methylation could therefore lead to developmental failure or disease development. Although it is widely accepted that epigenetic mechanisms respond to environmental signals, the functional consequences of nanoparticle-dependent DNA methylation changes are still largely unknown. Nanomaterials (NMs) are becoming widely used in pharmacology, food industry, agriculture, and medicine. Although the benefit of NMs is obvious, their growing manufacturing and wide distribution in numerous commercial products has triggered discussions about their possible adverse health effects. We investigated here the genetic and epigenetic toxicity of inorganic nanoparticles (gold, iron oxide, silica and titan dioxide nanoparticles) in human renal proximal tubule epithelial TH1 cells using the alkaline comet assay modification. To assess the changes in global methylation, Methy-sens Comet [1] that requires enzymatic DNA digestion with two restriction enzymes (HpaII, MspI) was employed. Decitabine (5-aza-2’-deoxycytidine), a hypomethylation agent, was used as a positive control. The results from the Methy-sens Comet were validated by pyrosequencing. High throughput version of the SCGE assay, with 12 minigels on a microscope slide allowing separate incubation of gels with restriction enzymes or reagents was utilized to speed up the hazard assessment of these inorganic NMs. Based on our preliminary results, none of inorganic NMs tested at given concentration range affect the global methylation level in TH1 cells. However, analysis of global DNA methylation may mask the redistribution of methylation patterns between the different genomic loci, where the hypomethylation of one and hypermethylation of others may result in cumulatively unchanged levels of DNA methylation.

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P8 - Toxicity of binary mixtures of metal(oxide) nanoparticles and quantum dots in two human cell lines HepG2 and A549

Lucyna Kapłań-Skrzypczak1,2, Sylwia Meczynska-Wielgosz3, Magdalena Matysiak-Kucharek2, Maria Wojewódzka3, Marcin Kruszewskii1,2,3
1 Department of Molecular Biology and Translational Research, Institute of Rural Health, Jaszczewskiego 2, 20-090 Lublin, Poland
2 Department of Medical Biology and Translational Research, Faculty of Medicine, University of Information Technology and Management, Sucharskiego 2, 35-225 Rzeszów, Poland
3 Institute of Nuclear Chemistry and Technology, Centre for Radiobiology and Biological Dosimetry, Dorodna 16, 03-195 Warszawa, Poland

Due to the abundant presence of the different types of nanoparticles (NPs) in environment, the most likely scenario of environmental exposure to NPs is chronic, multiple exposure to mixture of NPs. However our knowledge about the toxicity of nanomaterials mixtures is very limited. In this work, two human cell lines, A549 and HepG2, were exposed to CdSe QD (6 nm), AgNPs (20 nm), TiO2 NPs (21 nm) or their mixtures ratio. Cytotoxicity was estimated by MTT or NR assay 24 h and 48 h after exposure. Genotoxicity was studied by comet assay after 2 h or 24 h exposure. TiO2 NPs did not induced marked toxicity in both cell lines. AgNPs and QD were remarkably toxic for HepG2 cells, whereas A549 cell line was relatively resistant to NPs treatments. Notably, treatment binary mixtures of NPs had additive or sparing effect depending of NPs type and concentration present in the mixture. Comet assay revealed that QD and AgNPs induced high level of DNA damage in both cell lines, as compared to TiO2 NPs. Each cell line studied showed a different pattern of DNA damage versus NPs concentration and time of treatment. However in majority, treatment with NPs mixture revealed simple additive effect. In some cases a sparing effect was observed. In HepG2 cells longer treatment with AgNPs resulted in decrease of DNA damage, whereas in A549 cells DNA damage observed after both treatment times did not differ markedly.

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P9 - Cytotoxicity and genotoxicity of four reference metal nanomaterials: Towards reliable hazard assessment

Naouale El Yamani2, Andrew Richard Collins2,3, Elise Rundén-Pran1, Sergey Shaposhnikov2, Maria Dusinska1
1 Health Effects Group, MILK, Norwegian Institute for Air Research, Kjeller, Norway
2 NorGenotech AS, Norway
3 Department of Nutrition, University of Oslo, Norway

While nanomaterials (NMs) have many beneficial uses, they also have the potential to cause adverse effects on human, animal health and on the environment. In view of the large number of NMs currently in use, a robust and reliable tests including a high throughput screening methods are needed to predict their toxic effects and assess their safety. We have investigated the possible cytotoxicity and genotoxicity of four representative NMs derived from four different metals: (TiO2), (ZnO), (CeO2) and (Ag). The NMs were characterised in the stock dispersion as well as in appropriate culture media just prior to and after the experiments. Two human cell lines, A549 alveolar epithelial cells, and lymphoblastoid TK6 cells were used. A high throughput version of the comet assay was used to measure DNA strand breaks (SBs) as well as oxidised purines (converted to breaks with the FPG enzyme). In parallel, cytotoxicity was measured with the alamar Blue assay, and the ability of NM-treated cells to survive was assessed by their colony forming efficiency (CFE) assay. TiO2 and CeO2 NMs were only slightly cytotoxic measured by the alamar Blue test, and had no long-term effect on CFE. However, both induced DNA damage at non-cytotoxic concentrations; the damage decreased from 3 h to 24 h exposure, except in the case of CeO2-treated A549 cells. ZnO and Ag NMs induced high levels of DNA damage at cytotoxic concentrations. At lower concentrations, there was significant damage, which tended to persist over 24 h. The all four reference metal NMs tested were genotoxic. The adoption of HTS comet assay for NM hazard assessment allows the testing of numerous materials at different concentrations (especially non-cytotoxic), several times of exposure and on different types of cells; reduces the effect of inter-experimental variation, makes substantial savings in time and cost, and allows to generate large data sets. A test for cell viability should be always performed in parallel. Inclusion of Fpg in the comet assay allows detection of secondary effects of oxidative stress.

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In vivo genotoxicity evaluation of a poly(anhydride) biodistribution study. There are few studies in combination with Fpg morphous silica nanoparticle (SAS/NM-203) is a nanomaterial even if the mechanisms have still to be elucidated. Differences in systemic genotoxicity following SAS sub-chronic exposure, increase of DNA damage in female blood suggests gender-specific alterations in ovary. The significant, although not dose-dependent, increase of estradiol and testosterone levels was present. In conclusion, genotoxic data show no DNA damage and tissue alterations in ovary. The significant, although not dose-dependent, increase of DNA damage in female blood suggests gender-specific differences in systemic genotoxicity following SAS sub-chronic exposure, even if the mechanisms have still to be elucidated.

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P12 - Oxidatively damaged DNA in SH-SY5Y neuronal cells exposed to nicotine and cotinine.

Ana L.H. Garcia1, Daiana Dalberto1, Caroline C.Nicolau1, Adriane P. Nordim1, Ivana Grivicich1, Juliana da Silva1

1 Laboratory of Genetic Toxicology, PPGBioSaúde and PPGGTA, Lutheran University of Brazil (ULBRA), Av. Farroupilha 8001, Prédio 22, Sala 22 (4° andar) 92425-900; Canoas, RS, Brazil.

2 Laboratory of Cancer Biology. Programa de Pós-Graduação em Biologia Celular e Molecular Aplicada à Saúde, Lutheran University of Brazil (ULBRA), Av. Farroupilha 8001, Prédio 22, Sala 22 (5° andar) 92425-900; Canoas, RS, Brazil.

Nicotine is the mainly alkaloid found in tobacco leaves, a native plant from America. Nicotine is the main cause of cigarette addiction and most adverse health effects, and its main metabolite is cotinine. Results on tobacco use point to millions of deaths per year, with 90% of lung cancer death and different studies point to genetic instability caused by nicotine. The present in vitro study assessed the effects of nicotine and cotinine on the viability and DNA damage in SH-SY5Y neuronal cells, as well as cytogenetic alterations related to oxidative stress mechanisms were evaluated. Alkaline comet assay modified by repair endonucleases (FPG, OGG1 and Endo III) was used to detect oxidized nucleobases. SH-SY5Y neuronal cells were cultured under standard conditions and exposed during 3 h to different concentrations (0.125-2.000 µL/mL to nicotine and 0.125-2.000 mg/mL to cotinine). Cytotoxicity was observed at higher doses of nicotine and cotinine in the MTT assay. In the tripan blue assay, cells showed cell viability above 80% for both compounds, thus demonstrating cell membrane integrity and low cytotoxicity. Alkaline comet assay results demonstrated significant increase in damage index and frequency for cells treated with nicotine and cotinine, presenting genotoxicity. The results of the enzyme-modified comet assay suggest oxidative DNA damages induced by nicotine and cotinine. Unlike other studies, our results demonstrate genotoxicity induced by both nicotine and cotinine. Thus, this study points out that the main metabolite of nicotine can also be involved in inducing the harmful effects observed on the health of smokers. The similar effects observed for these two pyridine alkaloids may be due to the similarity of their structures. In addition, the results of the modified comet assay using the enzymes demonstrated oxidized purine and pyrimidine bases suggesting an oxidizing nature in DNA damage in cells treated with nicotine and cotinine.

P13 - Combinations of genotoxic tests for the evaluation of group 1 IARC carcinogens

Jacky Bhagat
Department of Zoology, Goa University, Taleigao Plateau, Goa-403206, India

Many of the known human carcinogens are potent genotoxins that are efficiently detected as carcinogens in human populations but certain types of compounds such as immunosuppressants, sex hormones etc. act via non-genotoxic mechanism. The absence of genotoxicity and the diversity of modes of action of non-genotoxic carcinogens make predicting their carcinogenic potential extremely challenging. There is evidence that combination of different short-term tests provide a better and efficient prediction of human genotoxic and non-genotoxic carcinogens. The purpose of this study is to summarize the in vivo and in vitro comet assay (CMT) results of group 1 carcinogens selected from International Agency for Research on Cancer (IARC) and to discuss the utility of the comet assay along with other genotoxic assays like Ames, in vivo micronucleus (MN), and in vivo chromosomal aberration (CA) test. Out of the 62 agents for which valid genotoxic data were available, 38/61 (62.3%) were Ames test positive, 42/60 (70%) were in vivo MN test positive and 36/45 (80%) were positive in vivo CA test. Higher sensitivity was seen in in vivo CMT (90%) and in vitro CMT (86.9%) assay. Combination of two tests has greater sensitivity than individual tests; in vivo MN + in vivo CA (88.6%), in vivo MN + in vivo CMT (92.5%), and in vivo MN + in vitro CMT (95.6%). Combinations of in vivo or in vitro CMT with other tests provided better sensitivity. In vivo CMT in combination with in vivo CA provided the highest sensitivity (96.7%).
P14 - CELLULAR UPTAKE AND TOXICITY OF POSITIVELY AND NEGATIVELY CHARGED SILICA NANOPARTICLES

Elisabeth Elje1,2, Julia Schölermann2, Mihaela Roxana Cimpan2, Maria Dusinska1
1NILU- Norwegian Institute for Air Research, Department of Environmental Chemistry, Health Effects Laboratory, Kjeller, Norway
2Department of Clinical Dentistry – Biomaterials, University of Bergen, Bergen, Norway

The aim of this study was to investigate the cytotoxicity, genotoxicity and cellular uptake of two red fluorescent amorphous silica NPs (25 nm) with either positive or negative surface charges in human lung carcinoma A549 cells in relation to the surface charge of the NPs.

Induction of cytotoxicity of A549 by the silica NPs was investigated by impedance-based monitoring using the xCELLigence RTCA DP (ACEA Biosciences, San Diego, USA). The genotoxicity was studied by the standard alkaline comet assay for detecting strand breaks and by the modified comet assay with lesion specific enzyme formamidopyrimidin glycosylase for detecting DNA oxidation lesions, while the mutagenic potential of the NPs was investigated by mouse lymphoma assay in L5178Y Tk−/+ cells. The cellular uptake of the positively and negatively charged silica NPs was investigated by flow cytometry (BD Accuri C6, BD Biosciences, San Jose, USA) and live cell microscopy (BioStation IM-Q, Nikon Corporation, Tokyo, Japan).

After the cells were treated in cell culture medium with NPs (1-300 µg/ml) for 2-23 hours, no evidence of cytotoxic, genotoxic or mutagenic effects induced by the NPs on A549 and L5178Y cells was found. In contrast, the cellular uptake and internalization rate in A549 cells was significantly higher for positively than for negatively charged silica NPs.

These results indicate that different mechanisms are involved in the NP uptake depending on the NPs’ surface charges and the biocorona formed around the NP. This study also shows that for the concentrations and times of exposure used, amorphous silica NPs can be internalized in A549 cells without causing significant cytotoxic or genotoxic effects.

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P15 - LOW DOSES OF PESTICIDE EXPOSURE CAUSE OXIDATIVE STRESS AND DNA DAMAGE IN FIBROBLAST CELLS

Murat Dikilitas1, Abdurrahim Kocyigit2, Eray Metin Guler2, Sema Karakas1
1Harran University, Turkey
2Bezmialem Vakif University, Turkey

Pesticides are toxic substances that are widely used in agriculture to control the pest. However, their low doses were regarded safe either in a short or long time period. Even if they have been applied at high doses, due to their short half-time period, they have been advised and considered as environmentally friendly chemicals. In this study, we exposed normal fibroblast cells (WS-1) to increasing concentrations (0-, 5-, 10-, 15-, 25-, 50 and 100 µg ml−1) fungicide (Korsilex T-50 WP), insecticide (HTK GAIA) and herbicide (Granstar No.2) to determine their cytotoxic, genotoxic, apoptotic and reactive oxygen species (ROS) generating activities. First two doses were regarded as non-toxic doses according to advice of companies. The last dose was accepted as acute toxic dose. After 30 min incubation of the cells with the various doses of pesticides, the cell viability was assessed based on luminometric ATP cell viability assay. Genotoxicity was evaluated by alkaline single cell gel electrophoresis assay (Comet Assay). Apoptotic effect was detected by Acridine Orange/Ethidium Bromide (AO/EB) double staining method. Intracellular accumulation of ROS was determined using the fluorescent probes 2,7-dichlorodihydrofluoresceindiacetate (H2DCF-DA). Level of ATP significantly decreased (20% of the non-pesticide exposed group) while ROS activity significantly increased (15-20 % of the non-pesticide exposed group) at low doses, p<0.05. There was a negative correlation between cell viability and ROS level. At low doses, DNA damages were also evident and positively correlated with the increase of ROS activity. Doses above 15 µg ml−1 resulted in higher toxicity including genotoxicity. Our results demonstrated that Korsilex T-50 WP, HTK GAIA and Granstar No.2 pesticides are cytotoxic, genotoxic and apoptotic agents even at low doses and these effects might be dependent on their ROS generating activities.
P16 - TOLERANCE OF CARPOBROTUS ACINACIFORMIS L. HALOPHYTE PLANTS EXPOSED TO HIGH LEVEL OF NaCl STRESS

Sema Karakas¹, Murat Dikilitas²*, Rukiye Tipirdamaz²
¹Harran University, Turkey ²Hacettepe University, Turkey

We used the alkaline protocol of the modified plant comet assay for rapid detection of DNA damage in Carpobrotus acinaciformis L. halophyte plants exposed to a series concentrations of NaCl stress (0-, 50-, 100-, 200-, 300-, 400 and 500 mmol l⁻¹) in hydroponic conditions for 2 weeks. DNA damage was measured as the values of percentage of DNA in tails and tail length. When physiological and biochemical parameters such as proline, chlorophyll a/b and total chlorophyll, peroxidase (POX), catalase (CAT), H₂O₂, malondialdehyde (MDA) contents were examined, oxidant molecules such as H₂O₂ (0.912-3.72 µmol g⁻¹ Fwt) and MDA (7.1-34 nmol g⁻¹ Fwt) gradually increased over a dose of NaCl, p<0.05. On the other hand, antioxidant enzyme POX and an osmolyte molecule proline slightly increased up to 400 mmol l⁻¹ NaCl level then slightly decreased after that. Similar issues were obtained from that of protease enzyme which indicates the power of protein hydrolysis in which a slightly decrease (182-95 Unit mg⁻¹ protein) over a dose of NaCl was evident. Chlorophyll contents and CAT activity were not affected upon increase of NaCl concentrations. The halophyte Carpobrotus acinaciformis L did not show any dose response increase up to 400 mmol l⁻¹ NaCl level in terms of parameters for determining DNA damages. DNA integrity measured via comet assay showed that the halophyte preserved its uniform shape up to 400 mmol l⁻¹ NaCl level. However, the very high concentrations of NaCl (400 and 500 mmol l⁻¹) resulted in depletion of antioxidant enzymes and increased amount of oxidant metabolites. At this dose, slightly in increase in DNA damage was evident. Correlations between parameters were examined with a correlation network analysis to visualize the relations between parameters in a network concept. This study showed that the halophyte Carpobrotus acinaciformis L. could be easily used to remove salt up to 400 mmol l⁻¹ concentrations from the saline soil. Measuring DNA damage could be very useful to find out up to what level of NaCl could be tolerated if the halophyte is aimed to remediate the saline soils.

P17 - THE INVOLVEMENT OF GLUTATHIONE IN THE DNA DAMAGE RESPONSE OF CADMIUM-EXPOSED Arabidopsis thaliana PLANTS

Sophie Hendrix¹, Bertrand Pourrut², Jaco Vangronsveld¹, Els Keunen¹, Ann Cuypers¹
¹Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium ²Laboratoire Génie Civil et géo-Environnement (LGCgE), Groupe ISA, Lille, France

Environmental cadmium (Cd) pollution affects many regions worldwide. When present in soils, Cd is readily uptaken by plants. Although it is not redox-active, Cd increases reactive oxygen species (ROS) production and thereby induces an oxidative challenge. This possibly causes damage to cellular macromolecules including DNA. To prevent oxidative damage, plants possess an extensive antioxidative defense system. In particular, glutathione (GSH) plays an important role in plant defense against Cd stress as antioxidant and by acting as a precursor of Cd-chelating phytochelatins. Furthermore, it might be involved in preventing DNA damage.

The current study investigated the involvement of GSH in the Cd-induced DNA damage response in leaves of Arabidopsis thaliana. The level of DNA damage was assessed using the comet assay in leaves of wild-type (WT) and GSH-deficient cadmium-sensitive 2-1 (cad2-1) mutant plants exposed to Cd for 4, 8, 12, 16 and 20 h. First, the comet assay protocol was validated using plants exposed to different ethyl methanesulfonate (EMS) concentrations for 24 h. Furthermore, the expression of genes involved in the DNA damage response was measured using reverse transcription-quantitative PCR in leaves of both genotypes after 24 and 72 h of Cd exposure.

Results demonstrated that short-term Cd exposure did not significantly increase the extent of DNA damage. However, the percentage of DNA in the comet tail was significantly reduced in leaves of cad2-1 mutants after 12 h of Cd exposure. This response was not observed in leaves of WT plants. Similarly, transcript levels of genes related to DNA damage and repair were significantly upregulated by Cd exposure in the WT, whereas they remained unaffected or even decreased in cad2-1 mutants. Taken together, our data assign a role to GSH in mediating the Cd-induced DNA damage response in A. thaliana leaves.
P18 - Evaluation of genotoxic and cytotoxic risks of untreated and treated municipal wastewater

Rukiye Tipirdamaz1, Ayşe Nihal Gömürgen1, Gökçen Baysal Furtana2, Selim Sanin3
1 Hacettepe University, Science Faculty, Biology Department, Ankara, Turkey
2 Gazi University, Science Faculty, Biology Department, Ankara, Turkey
3 Hacettepe University, Engineering Faculty, Environmental Engineering Department, Ankara, Turkey

Treatment of domestic and industrial wastewaters before discharging into the receiving bodies, is a global approach to reduce the pollutant content. Conventional wastewater treatment plants reduce the organic and nutritional load up to 95-98 %. In recent years it was noticed that the residual “difficult to degrade” pollutants, which are observed in the treated effluents, can be responsible for the long term changes in the ecosystem and have negative effect on human health. These low concentration contaminants mainly effect the reproduction or central nerve systems in almost all living things. Recent studies started to show statistical relationship between priority pollutants and the several diseases.

This study was done to assess cytological parameters as indicators of cytotoxicity and genotoxicity including the induction of micronuclei and chromosome aberrations (CAs). A. cepa test assay was utilized as a short term and cost effective indicator of toxicity in the routine monitoring of water pollution. A. cepa roots were treated with influent and effluent wastewater of Ankara for 24 and 48 h. Mitotic index (MI) and genotoxic markers (CAs) were evaluated and data statistically analysed. A significant decrease in MI and increase in CAs percentage as compared to the control was observed. Several metaphase and anaphase aberrations and micronuclei formation were observed in varying frequencies.

The experiments conducted using, influent and effluent domestic wastewater of Ankara showed decreased MI and increased mitotic aberration in root cells of A. cepa. These results indicate that municipal wastewaters have a genotoxic impact potential.

Our results also demonstrated that toxicity and genotoxicity bioassays desirable for environmental monitoring while they are low cost and short term assays.

P19 - Use of whole blood to measure DNA damage in a group of wildland firefighters.

Ana Abreu1,2, Armanda Teixeira-Gomes1,2, Susana Silva1, Simone Morais1, Maria C. Pereira4, Adília Fernandes3, Solange Costa1,2, João P. Teixeira1,2
1 Environmental Health Department, National Institute of Health, Porto, Portugal
2 Epidemiology Research Unit - Institute of Public Health (EPIUnit), University of Porto, Porto, Portugal
3 Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Porto, Portugal
4 Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal
5 Bragança Health School - Polytechnic Institute of Bragança (IPB), Bragança, Portugal

Genotoxicity evaluation is a valuable tool for studying the most important occupational hazards allowing a reasonable epidemiological evaluation of potential health effects. Comet assay has proven to be a very sensitive tool for the detection of different levels of DNA damage in human biomonitoring. Although lymphocytes are the preferred cells whole blood has been presented as a viable and easier alternative to lymphocytes in the comet assay. Its use avoids additional DNA damage from lymphocyte-isolation steps and loss of cells. Portugal is among the European countries more devastated by forest fires in the summer each year. Firefighters are exposed to many toxic combustion products, including many known, probable or possible carcinogens. There are a limited number of studies evaluating genotoxic effects in firefighters results reported are inconsistent and inconclusive. In this context, the aim of the present study was to assess DNA damage and oxidative stress in whole blood of Portuguese wildland firefighters. Study population consisted of a total of 61 non-smoking male subjects, 30 firefighters and 31 control subjects. Oxidative damage was measured by formamidopyrimidine glycosylase. Basal DNA damage and oxidative DNA damage were increased in firefighters compared to controls. However, only basal DNA damage was significantly higher. The influence of lifestyle factors and work-related variables (duration and recent exposure) was also studied, but no significant effect was found. Results obtained provide new data on the biological impact of wildland firefighting and the need to implement effective measures in order to protect firefighter’s health, including regular monitoring and surveillance activities, good practice campaigns, training programs and implementation of written policies and
procedures.

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P20 - Exposures in the tobacco fields: Genetic damage and oxidative stress in tobacco farmers occupationally exposed during harvest and grading periods

Jodel Alves1, Gabriela Flesch1, Elisiane Lima1, Marina B. Abreu2, Flávia V. Thiesen2, Denise Leal dos Santos2, Mirian Salvador3, Cátia dos Santos Branco3, Guilherme S. Schwengber4, Norma Marroni5, Silvia Bona5, Elizangela Schemitt5, Juliana Da Silva1*.

1Laboratory of Genetic Toxicology, PPGBioSaúde, Lutheran University of Brazil (ULBRA), Canoas, RS, Brazil
2Toxicology Institute, Catholic Pontificie University of Rio Grande do Sul (PUC), Porto Alegre, RS, Brazil
3Biotechnology Institute, University of Caxias do Sul, Caxias do Sul (UCS), RS, Brazil
4Santa Cruz Hospital, Santa Cruz do Sul, RS, Brazil
5Laboratório de Estresse Oxidativo e Antioxidantes, PPGBioSaúde, Lutheran University of Brazil (ULBRA), Canoas, RS, Brazil

Agricultural workers engaged in tobacco cultivation are constantly exposed to large amounts of damaging agents related with carcinogenesis, such as pesticides, nicotine, and tobacco-specific nitrosamines (TSNA). In addition, nicotine absorbed through the skin results in green tobacco sickness (GTS; occupational illness of tobacco farmers). The aim of this study was to evaluate genetic damage and oxidative stress in tobacco workers occupationally exposed during harvest and grading periods. We obtained data on DNA damage detected by the Comet assay in blood cells and Micronucleus test in buccal cells. The serum cotinine levels as well as nitrates were also evaluated. Comet Assay results showed a significant increase for individuals during harvest time, and micronucleus and binucleated cells for the grading group. Significant increases were showed to thiobarbituric acid reactive substances (TBARS) for harvest workers, and trolox equivalent antioxidant capacity (TEAC) for individuals during harvest and grading time. Significant increases were shown for cotinine levels during harvest and grading periods (harvest>grading), and nitrates for the grading period. Our study demonstrated that exposure during harvest time induces genotoxic damage, including oxidative stress mechanisms, that can be repaired (not induced increase of micronucleated cells); and that exposure cured tobacco leaves (grading period) induces an increase of cells with micronucleus, without inducing an increase in comet cells. Nicotine has been reported to be genotoxic, generating free radicals that react with cell
membranes and initiate the process of lipid peroxidation. The accumulation of these radicals can cause oxidative stress, depending on the antioxidant capacity of individuals exposed to these chemical compounds. According to our data, a significant increase for tobacco workers from both harvest and grading period was observed in the serum total antioxidant capacity (TEAC level) in relation to the control group, which measures the cumulative ability of all antioxidants to reduce free radicals.

P21 - Occupational exposure to dust and the genetic damage in ceramic industry

Daniela G. Locks, Lara C. Venturini, Adriani P. Damiani, Tamires P. Macan, Vanessa M. de Andrade, Paula Rohr
Laboratory of Molecular and Cellular Biology, University of Southern Santa Catarina, UNESC. Criciúma. Av. Universitária, 1105, B. Universitário, 88806-000, Brasil

Ceramic is the oldest synthetic man-made, but only in the 50’s that were created new products such as than wall coverings to the high resistance to abrasion floor. In Brazil, it had its origin in Marajo with indigenous and today plays an essential role in the economy, contributing to the gross domestic product (GDP), generating about 310,000 direct and indirect jobs. Santa Catarina is the state that ranks first in ceramic production, mainly in the southern region of the state, with about 710 industries. Despite this, the workers are exposed to many occupational hazards, one of the largest of them, exposure to metals and semi-metals from the manufacture of ceramics and carry them various diseases. Silica is one of the most contaminants recorded in the workplace, including in the ceramic industry and can lead to the development of lung inflammation and diseases - mainly silicosis, which is associated with DNA damage, cancer and apoptosis. The aim of this study was to perform biomonitoring of ceramic coating industry workers exposed to dust. Peripheral blood samples from 100 workers in the ceramics industry (exposed group) and 30 non-exposed individuals (control group) have been analyzed through the comet assay. Our results showed that the exposed group showed a significant difference in the control group when analyzed tail length and tail moment, possibly because the respirable dust ceramic contains more silica than allowed and it leads to DNA damage. However, when comparing the tail length and the tail moment of the exposed group compared to the length of service, age and workplace there was no significant difference between them. Therefore, the results of this study suggest that the dust of the ceramic industry has genotoxic effects on workers of this productive sector, probably because of the high amount of crystallized silica at its composition.
P22 - Genotoxic effects produced by Vinclozolin on the aquatic insect *Chironomus riparius* (Diptera).

Mónica Aquilino¹, Paloma Sánchez-Argüello², Jose Luis Martínez-Guitarte¹
1 Grupo de Biología y Toxicología Ambiental, Facultad de Ciencias, UNED, 28040, Madrid, España
2 Laboratorio de Ecotoxicología, Departamento de Medio Ambiente, INIA, 28040, Madrid, España

The dicarboxiamide Vinclozolin (Vz) is a widely used fungicide for agricultural purpose, which is found as a residue in the environment, including underground water, lakes, rivers, and seawater. The antiandrogenic mechanisms of Vz together with its effects on reproduction are well known in mammals, although other effects including genotoxicity have been described. Vz effects have been less studied on invertebrates, therefore in this work, we studied the genotoxic effects of Vz in the freshwater benthic invertebrate *Chironomus riparius*. DNA damage was evaluated with the Comet assay and the transcriptional levels of different genes involved in DNA repairing (*ATM*, *DECAY*, *NLK* and *XRCC1* genes). Fourth instar larvae of *Chironomus riparius*, a dipteran frequently used as a reference species in Ecotoxicology, were exposed to Vz for 24 hours to two different concentrations (20 and 200 µg/L). The Vz exposure affects the integrity of the DNA in these organisms since significant increases in DNA strand breaks were quantified with the comet assay (%DNA in Tail, Tail length, Tail moment and Olive Tail Moment). Additionally, Vz treatment produced a significant induction of the transcripts of the four gene studied measured by RT-PCR. In contrast, these genes were down-regulated with the highest concentration. These results report for the first time the alterations of Vz on the genome transcription of an insect and confirm the potential genotoxicity of this compound on *C. riparius*.

This work was supported by the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (Spain), grant CTM-2015-64913-R from the Ciencias y Tecnologías Ambientales program. M.A. is the receiver of a predoctoral contract Ministry of Economy and Finance (BES-2013-064041).

P23 - Assessment of DNA damage in the lymphocytes of welding workers

İldeniz Aksu¹, Merve Bacanlı¹, Hatice Gül Anlar¹, Servet İritaş², Engin Tutkun³, Nurşen Başaran¹, Ömer Hınç Yılmaz⁴
¹ Hacettepe University Faculty of Pharmacy Department of Pharmaceutical Toxicology, Ankara, Turkey
² The Council of Forensic Medicine, Branch Office of Ankara, Ankara, Turkey
³ Bozok University Faculty of Medicine Department of Public Health Yozgat, Turkey
⁴ Ankara Occupational Diseases Hospital, Ankara, Turkey

Welding is a widely practiced method in many industrial areas. Welding gas contains heavy metals such as chromium, nickel and manganese. Chromium and nickel are classified as Group 1 carcinogens and source fumes as possible human carcinogens (Group 2A) by IARC. Welders are exposed to many toxic compounds including heavy metals, especially those contained in welding and welding smoke, which are used during welding. This exposure can estimated to cause DNA damage. This study is carried out in 50 welding workers and paired controls in Turkey. A standardised, self-reporting questionare was used. DNA damage was assessed by alkaline comet assay in the lymphocyte samples of workers. The data was compared with controls working in office conditions, and not exposed to harmful chemicals. The DNA damage was significantly higher in workers compared to their controls.

This work was supported by Hacettepe University Research Fund (Contract grant number: THD-2015-7282).
P24 - GENOTOXICITY TESTING OF SURFACE DRINKING WATER FROM THREE RESERVOIRS IN SARDINIA (ITALY) SUBJECTED TO CHLORINATION/CHLORAMINATION

Fatigoni Cristina¹, Posadino Stefano², Vannini Samuele¹, Gianfredi Vincenza², Villarini Milena², Monarca Silvano¹, Azara Antonio², Moretti Massimo¹
¹ Department of Pharmaceutical Sciences, Unit of Public Health; University of Perugia, Perugia, Italy
² Department of Experimental Medicine, Graduate School of Hygiene and Preventive Medicine; University of Perugia, Perugia, Italy

Surface waters collected and regulated in water reservoirs are the main water supplies in Sardinia (Italy). Drinking water disinfection is a need for prevention of diseases caused by water borne pathogens; however, natural organic substances (e.g. humic and fulvic acids) in surface water may also react with disinfectants to produce volatile and non-volatile, potentially harmful disinfection by-products (DBPs). Evidence shows that chlorination, the foremost method for water disinfection, leads to the formation of DBPs with mutagenic and/or carcinogenic activity. Water disinfection may generate complex mixtures of DBPs, depending on contaminants in raw water and chemicals used for water purification. To remove dissolved organic matter and to control DBPs formation, pretreatment with pre-oxidants is usually applied.

We evaluated the genotoxicity of chlorinated drinking water (CDW) processed from raw water of three reservoirs in North-Sardinia. Drinking water treatment plants (DWTP) used different processes for pre-oxidation/disinfection: 1) chlorine dioxide (ClO₂)/chloramine, 2) permanganate/ClO₂ + chloramine, and 3) permanganate/ClO₂. Three sampling points were considered for each DWTP: A) raw water, B) disinfection stage, and C) distribution system (along the pipeline, far from the DWTP).

Genotoxicity was tested on human hepatoma HepG2 cells using the comet assay and the micronucleus test. HepG2 cells were exposed for 4 h (comet assay) or 24 h (micronucleus test) to silica C₁₈ extracts of CDW dissolved in DMSO to have 3 L of water (Leq) in 50 µl DMSO.

In the comet assay, 10 out of 90 CDW extracts caused a significant increase of DNA strand breakage in HepG2 cells. The extent of DNA damage was significantly higher in 3 Leq samples collected at the disinfection stage (B) or at the distribution system (C). Genotoxicity was also found in raw water from reservoirs 2 and 3 (3 Leq). None of the extracts significantly increased the frequency of micronuclei.

P25 - OXIDATIVE DNA DAMAGE IN HORSES NATURALLY INFECTED WITH THEILERIA EQUI

Milena Radaković, Darko Davitkov, Sučica Borozan, Ninoslav Djelić, Ivan Vičić, Jevrosima Stevanović, Zoran Stanimirović
Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia

Theilerosis is an important tick-borne disease caused by parasite Theileria equi in equids, and represents a great concern to the global horse industry. The aim of this study was to investigate the effects of theilerosis on the level of DNA damage and oxidative status in horses infected by T. equi. DNA damage was assessed in the peripheral blood in horses by alkaline single cell gel electrophoresis (Comet) assay, while activity of catalase (CAT), Cu,Zn-superoxide dismutase (SOD1) and level of paraoxonase (PON1), malondialdehyde (MDA) and free thiol groups (–SH) were determined spectrophotometrically. Theilerosis in horses was confirmed with duplex PCR. Thirty horses naturally infected with T. equi were selected in the infected group, and thirty horses free of infection belonged to the control group. The Comet assay analysis demonstrated significantly increased DNA damage in T. equi infected horses compared to healthy ones. Also, we observed a significant alteration in the parameters of oxidative stress between infected and control group; horses infected with T. equi had significantly higher SOD1 activities, MDA level, and significantly lower CAT, PON1 activities, and thiol group level compared to the healthy horses. These results clearly indicate that T. equi increases DNA damage and oxidative stress in infected horses. Therefore, the assessment of basal level of DNA damage with oxidative profile from T. equi infected horses may assist in better understanding of the pathophysiological mechanisms of theileriosis and contribute to selection of adequate treatment in infected animals.
P26 - DNA damage response and biomarkers correlation following occupational exposure to pesticides mixtures

Vivian F. S. Kahl1, Fernanda R. da Silva2, Jodel S. Alves1, Daniel Simon3, Varinderpal S. Dhillon4, Michael Fenech5, Juliana da Silva*1
1 Laboratory of Toxicogenetics, Lutheran University of Brazil (ULBRA), Brazil
2 Postgraduate Program in Environmental Impact Assessment, UniLaSalle, Brazil
3 Laboratory of Human Molecular Genetics, Lutheran University of Brazil (ULBRA), Brazil
4 Health and Biosecurity Flagship, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Adelaide, Australia.

Several reports have shown that occupational exposure to pesticides can induce health hazards to farmers. Tobacco farmers have been the focus of several studies as they are routinely exposed to pesticides mixtures. Various biomarkers are used to evaluate possible damages to their cellular and molecular levels. The aim of this study was to analyze different biomarkers for 308 tobacco farmers and a control group of 226 subjects through Comet Assay (CA), buccal micronucleus cytome assay (BMCyt), lymphocyte micronucleus cytome assay (CBMN) and qPCR for measuring telomere length (TL). Additionally, we investigated correlations between these parameters. Mean (± SD) of age was 41.3 (± 13.2) and 43.3 (± 13.9) for control and exposed groups, respectively. Tobacco farmers were exposed for a mean of 28.3 years (± 15.3; SD). When compared to controls, farmers presented significantly higher DNA damage index (DI), buccal (BMCyt-MN) and lymphocyte (CBMN-MN) micronucleus, buccal buds (BMCyt-buds) and nucleoplasmic bridges (CBMN-NPB). In addition, exposed individuals showed significantly reduced TL related to controls. Farmers also presented an inversely correlation of ID with BMCyt-MN (P= 0.0267) and a positive correlation between TL and CBMN-NPB (P= 0.0393). Control group showed positively correlation of DI with BMCyt-MN (P= 0.0267), BMCyt-buds (P <0.0001) and CBMN-NPB (P= 0.0169). Damages inversely associated with reduced mutagenic effects may be related to greater demand for DNA repair mechanisms for exposed subjects, due to molecular challenges to their cells. Results show the importance of the use of different biomarkers in human biomonitoring studies. Micronucleus are recognized as cancer predictors, while BMCyt, a non-invasive method, proved to be very suitable in these investigations. Nevertheless, it is possible to conclude that DI is a powerful indicator of mutagenic damages. In this aspect, our results demonstrated that CA is a useful tool to biomonitoring populations occupationally exposed to pesticides mixtures.

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P27 - Ex vivo engineering of cornea limbal epithelium: effect of type of medium on levels of DNA damage

Yolanda Lorenzo1, Kristiane Haug Berg1, Goran Petrovski2, Andrew R. Collins2, Bjørn Nicolaisen1
1 Center for Eye Research and 2 The Norwegian Eye Bank, Department of Ophthalmology, Oslo University Hospital and University of Oslo, Kirkeveien 166, N-0407 Oslo, Norway
2 Department of Nutrition, Medical Faculty, University of Oslo, P.O. Box 1046, Blindern, 0316 Oslo, Norway

Cornea limbal stem cell deficiency, secondary to insults and diseases, may be treated by transplantation of ex vivo engineered epithelial grafts. We hereby present data on the levels of cellular DNA damage in grafts produced in two different types of culture medium. Cultures were initiated using corneo-limbal donor tissue after removal of the central area for transplantation purposes. Explants (approx. 2x2 mm) were positioned epithelial side down on tissue culture treated polyester membranes and expanded for four weeks in Dulbecco’s Modified Eagle Medium F12 Nutrient Mixture (Ham) [DMEM/F12 (1:1)] with either (1) Human Serum (HS) medium; 10% HS or (2) Complex (COM) medium; 5% fetal bovine serum (FBS), Epidermal Growth Factor (EGF), insulin-transferrin-sodiumselenzine (ITS), cholera toxin-A, dimethyl sulfoxide (DMSO) and hydrocortisone. Cells were obtained from the central areas of the grafts and dissociated using Trypsin-EDTA (0.05%) for 30 min., then the enzyme activity was inhibited using medium and serum. The cell suspension was transferred to tubes on ice and processed using the Comet Assay. Duplicate samples from each culture were analyzed in each assay by visual scoring. Using a fluorescence microscope, 100 comets (50 from each gel) were classified into five categories, 0-4, representing increasing relative tail intensities. Summing the scores (0-4) of 100 comets, therefore, gave an overall score between 0 and 400 arbitrary units. Data show presence of some levels of DNA damage in cells dissociated from the central areas of the engineered grafts. Specific type of DNA damage was detected using lesion-specific enzyme. Our main finding was that levels of FPG-sensitive sites (8-oxoguanine and ring-opened Fapy bases) were low and similar in cells expanded in COM and HS. Recent studies have shown that medium containing HS equally supports production of grafts containing differentiated as well as undifferentiated cells suitable for clinical transplantation. Our data indicate levels of molecular damage to the DNA do not increase in cells cultured in HS medium despite its lack of complexity. The low levels of DNA damage indicate low levels of oxidative stress and/or efficient damage repair.
**P28 - Comparative analysis of the results of DNA-comet images scoring by commercial and Internet available software.**

Nikolay P. Sirota¹, Alij K. Zhanataev², Sergey Shaposhnikov², Gunnar Brunborg³, Tatyana Sirota¹

¹ Institute Theoretical & Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia
² Zakusov Research Institute of Pharmacology, Moscow, Russia
³ Norgenotech AS, Totenvegen 2049, 2849 Skreia, Norway

In investigations exploiting Comet assay a DNA-comet scoring is usually performed by using commercial or Internet available freeware. The effects of using different software upon the results of interlaboratory study are unclear. There are a few articles where different software was used for comet images scoring. In this study a scoring of microphotographies which was collected in a laboratory of Zakusov Research Institute of Pharmacology was performed. We used CASP, OpenComet, Comet D (Diamorph, Russia), Comet IV (Perceptive Instruments), Comet Imager (Metasystems, Germany) and MAIC (Morphological Analysis of Comet Images, IBC, RAS). All microphotographies were divided onto six sets according to mean value of %TDNA. The set 1 – the mean value %TDNA = 1-5%; set 2 – 5-10%; set 3 – 10-20%; set 4 – 20-30%; set 5 - 30-40% and set 6 - >40% respectively. At first all sets were scored by OpenComet and as results we received copies of initial microphotographies with numbers indicating comet scoring order so as Excel files with results of scoring. These copies were used when original microphotographies were scored by other software. It allowed us to compare not only mean values of measurements but also results obtained on the same comets.

No significant difference was shown in case of results indicated as %TDNA after scoring all comets with all programs. Two times differences were found when results of scoring were presented as tail moment (TM) or Olive tail moment (OTM). These differences were connected with possibilities of program to perform calibration of size before comet scoring. The results of measurements expressed as TM/OTM in pixel were two times higher than it was obtained in micrometers.

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**P29 - The comet assay in human biomonitoring: cryopreservation of whole blood and comparison with isolated mononuclear cells**

Gudrun Koppen¹, Sofie De Prins¹-², Jacobs An³, Nelen Vera³, Schoeters Greet¹-²-⁴, Sabine A.S. Langie¹-³

¹ Health unit, Flemish Institute for Technological Research (VITO), Mol, Belgium
² Department of Biomedical Sciences, University of Antwerp, Wilrijk, Belgium
³ Environment and Health unit, Provincial Institute of Hygiene, Antwerp, Belgium
⁴ University of Southern Denmark, Institute of Public Health, Department of Environmental Medicine, Odense, Denmark
⁵ Faculty of Sciences, Hasselt University, Diepenbeek, Belgium

The comet assay is often applied in human biomonitoring. Most of the time the assay is performed with isolated mononuclear cells from peripheral blood (PBMC). However, using whole blood instead of isolated cells reduces processing time and only 20 µl is sufficient for analysis. In the present study, a cryopreservation protocol for human whole blood for application in the comet assay was optimized by removing excess plasma before adding freezing medium. Cryopreservation of whole blood samples (n=30) did not increase the detected level of strand breaks and oxidative damage (assessed as FPG sensitive sites). Strand breaks detected in frozen whole blood were significantly correlated with breaks measured in frozen PBMC (Pearson correlation r=0.54, P<0.01). This correlation was however not observed for FPG sensitive sites. Since we do not yet know the full extent to which cryopreservation might influence the blood cell population, care should be taken to ensure a similar cell type and storage conditions for all samples in one study.
P30- NOVEL 3D in vitro CELL MODEL FOR GENOTOXICITY ASSESSMENT

Martina Štampar, Metka Filipic, Bojana Žegura
Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Vecna pot 111, 1000 Ljubljana, Slovenia

Hepatic cells cultured in two-dimensional (2D) monolayers are the most often used system for studying the adverse effects of xenobiotics in vitro. However, such models express low levels of drug metabolic enzymes and lack relevant hepatic properties, which makes the prediction of toxicity to humans questionable. Therefore, the demand to establish improved experimental models for toxicological studies, in particular in the field of drug development, is increasing. Lately, three-dimensional (3D) cultures, which better reflect in vivo conditions, are considered as an effective model for such studies. The 3D models have improved cell-cell and cell-matrix interactions and have preserved complex in vivo cell phenotypes. Specifically 3D models of hepatic cells usually exhibit higher level of liver-specific functions including metabolic enzymes compared to 2D models.

In this study 3D in vitro cell model for the assessment of genotoxicity of chemicals was developed from human hepatoma cell line HepG2 using forced floating method. Four days old spheroids were exposed to model indirect acting genotoxic compounds: polycyclic aromatic hydrocarbon [B(a)P], two heterocyclic aromatic amines [PhIP and IQ], mycotoxin aflatoxin B1 [AFB1] and a direct acting etoposide [ET] for 24 hours. After the treatment cytotoxic and genotoxic effects were determined with the MTS assay and the comet assay, respectively. In addition, the expression of selected genes involved in xenobiotic metabolism, immediate-early response/signalling and DNA damage response were studied using the real-time quantitative PCR. At non-cytotoxic concentrations all studied compounds induced dose dependent increase in the amount of DNA strand breaks and deregulated the expression of studied genes, which altogether suggests that in newly developed 3D in vitro model indirect acting genotoxins are metabolically activated. Development of new improved model systems and the application of 3D models for genotoxicity testing may provide a bridge between in vitro and in vivo experiments for hazard assessment.

P31 - COMPARISON OF DNA DAMAGE LEVEL WITH ALKALINE COMET ASSAY ON PERIPHERAL BLOOD MONONUCLEAR CELLS AND PERIPHERAL WHOLE BLOOD THAT CAN BE USED IN HUMAN BIOMONITORING STUDIES

Zsuzsanna Nemeth¹, Gyorgy Thuroczy², Elisa Boutet-Robinet²
¹ National Public Health Institute, Budapest, Hungary
² Toxalim (Research Center in Food Toxicology), Université de Toulouse, INRA, ENVT, INP-Purpan, UPS, Toulouse, France

For environmental and occupational human biomonitoring studies mostly peripheral blood is sampled and examined with comet assay after isolation of PBMCs (Peripheral Blood Mononuclear Cells). Sometimes, the study design and the collection of samples are too long, and requires the freezing of the samples. The purposes of our study were first to examine if there is a difference in the basal DNA damage of a healthy donor between fresh vs. frozen whole blood. Second, basal damage of whole blood (either fresh or frozen) vs. isolated PBMCs (either fresh or frozen) were also compared. Finally, different experimental conditions were performed as we worked with 2 different type of supports (either Glass slides or Gelbond® films), with 2 different DNA dyes (SYBR Gold and GelRed) and different image analysis systems (NikonNiS and LuciaKomet or manual CaspLab). The experiments were performed on samples from the same volunteer in 2 different laboratories in 2 different countries (France and Hungary).

Human peripheral whole blood was used for alkaline comet assay as fresh sample or frozen. PMBCs were isolated by Ficoll gradient on fresh blood and used immediately or frozen for further experiments. For positive control fresh PMBCs were treated with methyl methanesulfonate (MMS) and DNA damage was evaluated immediately or after a freezing step.

This study shows that peripheral whole blood and PBMCs, either fresh or frozen, are suitable cells for alkaline comet assay. This study also shows the influence of some technical differences in the comet assay protocol as it included experiments from two different laboratories. Effects of long term storage still needs to be evaluated.

This study was done with the support of COST action hCOMET during the Short Time Scientific Mission.
P32 - COMPARISON OF THE ALKALINE COMET ASSAY RESULTS ON FRESH AND ONE YEAR FROZEN WHOLE BLOOD

Mirta Milić1,7, Ivana Vinković Vrček2, Marijana Vučić Lovrenčić3, Višnja Oreščanin4, Stefano Bonassi5,6, Emilio Rojas Del Castillo5,6, Andrew Collins7, hCOMET Consortium

1Mutagenesis Unit, Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10 000 Zagreb, Croatia
2Analytical Toxicology and Mineral Metabolism Unit, Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10 000 Zagreb, Croatia
3Department of Laboratory Medicine, Merkur University Hospital, Zajceva 19, 10000 Zagreb, Croatia
4ORESCANIN Ltd., A. Jaksica 30, 10000 Zagreb, Croatia
5Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Pisana, Rome, Italy
6Department of Human Sciences and Quality of Life Promotion, San Raffaele University, Rome, Italy
7Department of Nutrition, University of Oslo, Norway

We have already demonstrated that alkaline comet assay results in blood samples frozen as smal-volume aliquots with no cryoprotectors for short period can be comparable with those in fresh blood samples. This time we wanted to compare the results after one year of freezing (-80°C). Tail length and tail intensity parameters were measured in fresh and thawed whole blood samples taken from 30 healthy volunteers (13 males and 17 females). Medical surveillance was done in Merkur University Hospital Zagreb, Croatia, and all volunteers agreed to fill the questionnaire about their lifestyle habits, health condition and afterwards pass the medical checkup. The medical checkup consisted of the doctors physical examination, talk and blood and urine sampling to measure general laboratory parameters and also to perform the assay on their lymphocytes for DNA stability. Furthermore, due to time consuming analysis of individual slides, we have also compared the results of thawed blood on individual slides with 6-well gel assay (lab-made). The results demonstrated insignificantly higher DNA damage in thawed samples for tail intensity parameter, while the same was not seen for tail length. The damage observed in frozen samples correlated with the DNA damage measured in the freshly prepared samples. However, 6-well gel slide assay results on frozen blood were well correlated with individual slides on frozen blood, but no correlation was seen with the amount of DNA damage in the freshly prepared blood samples. Results indicate that comet assay can be successfully performed also in the frozen whole blood samples, but further clarification for high throughput 6/12/96 gel comet assay are needed not for comparison with frozen samples but with comparison with freshly samples and determination of inter-individual differences that can influence the results of the assay in fresh whole blood samples and the ones frozen for longer period of time.
P33 - THE IMPACT OF CRYOPROTECTANT USE AND WASHING STEPS ON DNA DAMAGE LEVELS ASSESSED BY COMET ASSAY

Alla Tirsina1,2, Ana Inês Silva2,3,4, Solange Costa2,3, João Paulo Teixeira2,3, Carla Costa2,3

1 Department of Chemicals Surveillance, Centre of Chemical Safety and Toxicology, National Center of Public Health, Chisinau, Republic of Moldova
2 Department of Environmental Health, Portuguese National Institute of Health, Porto, Portugal
3 EPIUnit-Institute of Public Health, University of Porto, Porto, Portugal
4 Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

The comet assay was traditionally carried out in fresh samples, but for a matter of logistical reasons, samples are now often cryopreserved. Although there are some previous studies looking at the impact of cryopreservation on DNA damage levels, this matter remains unclear as different protocols continue to be published without a suitable analysis of its effect on results. Herein, we intended to evaluate the effect of DMSO use for cryopreservation and different washing procedures on DNA damage levels assessed in whole blood using the alkaline comet assay. Samples from 20 healthy individuals were collected and aliquoted; one of the aliquots was immediately analyzed, a second one was frozen at -80°C without DMSO and the third one was frozen with 10% DMSO. After thawing, 3 different protocols were followed: (1) no wash, (2) washed with PBS and (3) washed with medium (DMEM with 2% FBS) before analysis with comet assay.

Results show that the levels of DNA damage in samples frozen with DMSO were significantly lower than in those without DMSO. Regarding the washing options, results are dependent of the presence of DMSO: in samples without DMSO, the lowest levels of DNA damage were detected when no washing steps were performed while in samples frozen with DMSO, lower DNA damage levels were observed in samples washed with PBS or medium. To refer that there was a significant increase in DNA damage in samples not washed cryopreserved without DMSO and a significant decrease in those with DMSO and washed.

In conclusion, the freezing process must be analyzed before method implementation and defined according to the purpose of the study.

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P34 - Establishment of genotoxicity testing using the comet assay of metabolically active 3D liver spheroid cultures.

Kristine B Gutzkow1,2, Edel Lilleaas1, Marit Låg1, Ann-Karin Olsen1,2, Birgitte Lindeman1,2

1 Norwegian Institute of Public Health; Infection Control and Environmental Health, Norway
2 Centre for Environmental Radioactivity (CERAD CoE), Norway

Human 3D liver cultures (spheroids) maintain a high level of Phase I and II enzymes in culture and are thus an attractive in vitro model for measuring the induction of DNA damage and DNA repair after exposure to chemicals that depend on proficient liver enzyme activities to form DNA reactive metabolites. Liver spheroids are small (about 1000 cells per spheroid), thus requiring assays that can be adapted to low cell numbers. The modified comet assay [1] requires few cells and is a relevant method for analysing DNA lesions in liver spheroids. The challenge is to isolate single cells from the spheroid with good conservation of DNA integrity and to minimise spurious induction of DNA damage or repair during the preparation of single cells.

In this project, both mechanical and enzymatic procedures for isolating single cells from the 3D spheroids was tested. We exposed the spheroids to X-rays or arsenic trioxide and measured DNA single strand breaks, alkali-labile sites and oxidative damage using the bacterial DNA glycosylase, Formamidopyrimidine-DNA Glycosylase (Fpg). Two spheroids per sample were used to ensure sufficient cell numbers to assess 150 cells both with and without Fpg. Our preliminary results demonstrate a dose response with X-rays, and exposure to arsenic trioxide induced DNA strand breaks at human relevant exposure concentrations. However, the background levels of oxidative DNA damage may be too high and we are currently working to reduce these.

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P35 - Freezing procedure optimization of two human cell lines for comet assay analysis

Maria João Bessa1,2, Fátima Brandão1,2, Micaela Querido1,2, Carla Costa1,2, João Paulo Teixeira1,2, Sónia Fraga1,2

1 EPIUnit-Institute of Public Health, University of Porto, Porto, Portugal; 2 National Institute of Health Dr. Ricardo Jorge, Dept. of Environmental Health, Porto, Portugal

The comet assay is a commonly used method for in vitro and in vivo genotoxicity screening. This versatile assay can be performed in a wide range of tissues and different cell types (Langie S et al. 2015). Most studies use samples immediately processed after collection but some few studies also use frozen biological samples (Recio L et al. 2012, Jackson P et al. 2013).

The present study aimed to optimize a freezing procedure of human cell lines for comet assay analysis. We compared DNA strand break levels measured in human lung epithelial (A549) and brain glioblastoma (A172) cells immediately processed after collection and one or two weeks of frozen storage (-80ºC). Cells were gently harvested with a scraper or by trypsin-EDTA dissociation. Two cryoprotective media were tested: (1) Fetal bovine serum (FBS) and (2) complete culture medium, both supplemented with 10% DMSO. The alkaline comet assay was performed as previously described (Bessa MJ et al. 2017). The percentage of DNA in the tail and the tail moment were used as a measure of the amount of DNA damage.

The presented protocol allows analysis of in vitro cell samples collected and frozen at different locations, and stored over short-term time periods, with minimal interference on the basal DNA strand break levels. However, is important to underline that the freezing/thawing procedures require utmost care regarding sample handling and preparation to avoid increasing DNA damage in comparison with samples processed fresh.

Langie S et al. 2015. Frontiers in Genetics, 6: 266.

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P36 - INFLUENCE OF CAFFEINE ON REPAIR KINETICS OF UVC INDUCED DNA DAMAGE IN HUMAN LYMPHOCYTES

Tamara Bakuradze, Julia Gabriel, Gerhard Eisenbrand, Elke Richling
Department of Chemistry, Division of Food Chemistry and Toxicology, University of Kaiserslautern, Kaiserslautern, Germany.

Caffeine is the most widely consumed purine alkaloid, with numerous physiological effects. In millimolar concentrations caffeine has been reported to inhibit DNA repair in vitro and in vivo and different mechanisms have been discussed [1, 2]. We investigated potential modulating effects of caffeine on kinetics of nucleotide excision repair (NER). DNA lesions were induced by UVC (10 J/m², 3-4 sec) radiation. Human peripheral lymphocytes, phytohaemagglutinin (PHA) stimulated were put on ice and UVC irradiated. Using the comet assay DNA single strand breaks (SSB) were monitored at time zero (on ice) and at 37°C at 1, 2, 4, 6 and 24 h after UVC exposure.

In the absence of caffeine we observed a significant increase of SSB 1 h after UVC exposure, reaching its maximum at 4 h (t max), followed by a rapid decline. In the presence of caffeine (60 µM; 2 mM) t max was reached earlier but the AUCs (areas under the SSB kinetics curves) were not significantly different, irrespective of caffeine concentration (0, 60, 2000 µM).

The results suggest that caffeine, at the concentrations tested, may accelerate NER rate in UV irradiated human lymphocytes. Conversely, they do not support considerations that caffeine may exhibit inhibitory effects on DNA repair when present at concentrations close to plasma concentrations expected after very high coffee intake (60µM).


P37 - THE EFFECT OF EXTRACT CONCENTRATION AND TIME OF INCUBATION IN THE COMET BASED IN VITRO DNA REPAIR ASSAY

Amaya Azqueta1,2, Sabine Langie3, Andrew R Collins4.
1 Department of Pharmacology and Toxicology, University of Navarra, Pamplona, Spain
2 IdiSNA, Navarra Institute for Health Research, Spain
3 Environmental Risk and Health Unit, Flemish Institute of Technological Research (VITO), Mol, Belgium
4 Department of Nutrition, University of Oslo, Oslo, Norway

DNA repair plays a crucial role in maintaining genetic stability and it is seen as a valuable biomarker related to cancer risk. Nevertheless, DNA repair activity is not often included in human biomonitoring studies mainly because it is not easily measured. Evaluation of the DNA repair activity of cell extracts can be easily determined by measured their incision activity on a DNA substrate containing specific lesions, using the comet assay. This assay is commonly referred to as the in vitro comet based DNA repair assay. The assay has been developed to measure both base excision repair (BER) and nucleotide excision repair (NER) activity, by using substrate containing appropriate lesions. Although the assay has been successfully used in in vitro, in vivo, and in human biomonitoring studies, there is no commonly accepted and validated protocol.

In this work we study the BER activity of extracts produced from different number of human lymphocytes (i.e. 1.25, 2.5, 5, 10, 15 and 20 millions). A pool of lymphocytes from different donors was used to produce the cell extracts. Substrate was prepared by irradiating cells incubated with 2 µM Ro 19-8022 (photosensitiser) with visible light for 5 min. Non-treated cells were used as control. In an independent experiment, the effect of 10 and 30 min of incubation of the extracts with the substrate was also studied.

Results showed an increase in the incision activity of extracts as the number of lymphocytes used to make the extract increased from 1.25 to 10 million. However, there was a lack of proportionality. On the other hand, there was a clear effect of the time of incubation on the incision activity of the extracts; longer incubation time gave higher incision activity. In this experiment it was also observed that the incision activity did not increase proportionally with the cell number.

The lack of proportionality between the incision activity and the number of lymphocytes in the extract requires further investigation. Meanwhile, it is advised to use the same number of cells to produce all extracts in an experiment.
P38 - MOSS *Physcomitrella patens* AS A MODEL SYSTEM FOR STUDY OF DNA DAMAGE REPAIR BY COMET ASSAY

Radka Vágnerová, Marcela Holá, Karel J. Angelis

*Institute of experimental botany AS CR, Na Karlovce 1, 160 00 Praha 6, Czech Republic*

Due to its unique features, the moss *Physcomitrella patens* represents an attractive model for study of DNA damage response. High frequency of homologous recombination and haploid phenotype enable easy preparation and identification of homozygote mutants. Due to filamentary growth of moss during the early stages of growth, protonemal cultures with different ratios of dividing apical cells can be established. Simple fragmentation of protonemal tissue by shearing allows to prepare culture with up to 50% of dividing cells and so enable study of DNA damage response in actively growing tissue which is in higher plants limited into apical meristems.

As previously shown DNA repair in plants is extremely rapid, especially double-strand breaks of DNA are removed within several minutes. Combination of easy manipulation with *Physcomitrella* tissue with rapid and simple isolation of nuclei during comet assay protocol enables to process sample in time shorter than 2 minutes and allows measurement of kinetics of the quick phases of DNA repair. In this respect, *Physcomitrella* represents an excellent plant model especially for study of DNA damage induction and repair by comet assay.

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P39 - EFFECTS OF TWO TYPES OF IRON OXIDE NANOPARTICLES ON DNA REPAIR COMPETENCE IN HUMAN ASTROCYTES

Natalia Fernández-Bertólez1,2, Carla Costa3,4, Fátima Brandão3,4, Joao Paulo Teixeira3,4, Eduardo Pásaro1, Vanessa Valdiglesias1,4, Blanca Laffon1

1DICOMOSA Group, Department of Psychology, Area of Psychobiology, Universidad da Coruña, Edificio de Servicios Centrales de Investigación, Campus Elviña s/n, 15071-A Coruña, Spain
2Department of Cell and Molecular Biology, Universidade da Coruña, Facultad de Ciencias, Campus A Zapateira s/n, 15071-A Coruña, Spain
3Department of Environmental Health, Portuguese National Institute of Health, Rua Alexandre Herculano, 321, 4000-055 Porto, Portugal
4EPIUnit - Institute of Public Health, University of Porto, Rua das Taipas, 135, 4050-600 Porto, Portugal

The ability of iron oxide nanoparticles (ION) to cross the blood-brain barrier makes them potentially useful for diagnostic and treatment of nervous system diseases through several biomedical applications, such as magnetic resonance imaging, drug delivery and hyperthermia. Different surface coatings may increase their biocompatibility and reduce toxicity. Although ION have been shown to exhibit in general low neurogenotoxicity, little is known about their effects on the repair ability of human nervous cells. For this purpose, DNA repair competence assay was conducted to evaluate possible alterations in the repair efficacy of human astrocytes (A172 cells) caused by exposure to silica-(S-ION) or oleic acid-coated (O-ION) ION. In this assay, DNA damage detected by the alkaline comet assay, immediately after challenging the cells with H2O2 was compared with residual damage remaining after 30 min incubation. Treatments with both ION were performed independently in three different stages: before inducing DNA damage (phase A), during H2O2 treatment (phase B), or during the repair period (phase C). Results showed that S-ION and O-ION did not induce significant alterations in DNA repair processes of astrocytes, independently on the moment of exposure (phases A, B or C). Presence or absence of serum in culture media did not significantly modify the results obtained. In conclusion, none of the ION tested impaired the ability of A172 astrocytes to repair the DNA damage induced by H2O2, supporting the safety of these ION to be employed in biomedical applications, particularly on nervous system.
This work was supported by Xunta de Galicia (EM 2012/079), the project NanoToxClass (ERA-SIINN/001/2013), and hCOMET CA15132 COST Action. V. Valdiglesias was supported by a Xunta de Galicia postdoctoral fellowship (ED481B 2016/190-0). N. Fernández-Bertólez was supported by INDITEX-UDC fellowships. F. Brandão was supported by the grant SFRH/BD/101060/2014, funded by FCT (subsidized by national fund of MCTES).

Flavonoids have antioxidant properties and can have protective effect against different diseases. This work aims to study the effect of the flavonoid quercetin on DNA damage using peripheral lymphocytes obtained from tuberculosis (TB) patients and comparing the results with lymphocytes and sperm collected from healthy individuals. DNA damage was detected by both the Comet and micronucleus assays. Samples each of lymphocytes and sperm were collected from healthy individuals. These samples were treated with a range of concentrations of hydrogen peroxide; 60µM/ml was selected as the non-toxic positive control. Ten blood samples were collected from both TB patients and healthy individuals, and these samples were used for the Comet assay. Next, three samples were collected from TB patients and healthy individuals to be used in the micronucleus assay. The 60µM/ml of hydrogen peroxide was incubated with the lymphocytes. The 0.75µg/ml of bleomycin was selected as the concentration causing the highest non-cytotoxic damage in micronucleus assay. Lymphocytes and sperm were treated at the same time with different concentrations of quercetin (10µM, 25µM, and 100µM). The results demonstrated that a significant reduction of DNA damage in lymphocytes and sperm ranged from (*) (P ≤ 0.0283) to (***)(P ≤ 0.001) in both assays. The nano forms have a large surface area to volume ratio, and the nano size allowed for the fast penetration of the nucleus, in comparison with the bulk form. The nano size was found to be the most efficient form of quercetin in reducing DNA damage in the Comet and micronucleus assays.
P41 - FRAILTY STATUS IS NOT ASSOCIATED WITH INCREASE IN DNA DAMAGE OR REPAIR ALTERATIONS IN OLDER ADULTS

Diego Marcos-Pérez1, María Sánchez-Flores1, Ana Maseda2, Laura Lorenzo-López2, José C. Millán-Calenti2, Eduardo Pásaro1, Blanca Laffon1, Vanessa Valdигlesias1

1Universidade da Coruña, DICOМOSA Group, Department of Psychology, Area of Psychobiology, A Coruña, Spain
2Universidade da Coruña, Gerontology Research Group, Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), SERGAS, A Coruña, Spain

Frailty is a multidimensional syndrome of loss of reserves (energy, physical ability, cognition, health) that gives rise to vulnerability to stressors leading to a significant increase of the age-related decline of different physiological systems and then to disability, comorbidity, and death risk. Genomic instability has been proposed as a primary cause of the aging phenotype since most age-related diseases and aging signs are associated with unrepaired or erroneously repaired genome damage. Therefore, the implementation of clinical data with genomic instability biomarkers would have the potential of anticipating the recognition of frail individuals and improving outcomes of frailty. On this basis, in the current study DNA primary damage, oxidative damage and DNA repair ability were evaluated in 250 older adults (aged ≥ 65 years) classified in frail, pre-frail and non-frail according Fried’s criteria [1], by means of comet assay-based methodologies (alkaline, OGG1-modified and challenge assays, respectively). Data obtained, corrected by age, sex and smoking, showed no significant influence of frailty status on oxidative DNA damage or DNA repair capacity, and a significant decrease of primary DNA damage in frail subjects as compared with non-frail individuals. This may be related to their less active lives, which may prevent them from DNA damaging environmental exposures. Further research on possible relationship of frailty with genomic instability should explore other different biomarkers.

This work was supported by Xunta de Galicia (grant numbers ED431B 2016/013, GPC2014/082, and FrailNet network IN607C 2016/08) and CA15132 hCOMET COST Action. V. Valдигlesias was supported by a Xunta de Galicia postdoctoral fellowship (reference ED481B 2016/190-0). D. Marcos-Pérez and M. Sánchez-Flores were supported by INDITEX-UDC fellowships.


Support: FULBRA, CNPq, and FAPERGS

P42 - LOBELINE TOXICOLOGICAL AND PHARMACOLOGICAL FINDINGS REINFORCING ITS POTENTIAL USE IN TREATMENT OF ALCOHOL ADDICTION.

Liana Dantas da Costa e Silva1, Patrícia Pereira2, Gabriela Gregory Regner2, Fernanda Brião Menezes Boaretto1, Cleonice Hoffman1, Pricila Pfüger2, Lucas Lima da Silva2, Luiza Reinhardt Steffens3, Ana Moira Morás4, Dinara Jaqueline Moura3, Jaqueline Nascimento Picada1

1 Laboratory of Toxicological Genetics, Lutheran University of Brazil (ULBRA), Canoas, RS, Brazil
2 Laboratory of Neuropharmacology and Preclinical Toxicology, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
3 Laboratory of Genetic Toxicology, Federal University of Health Science of Porto Alegre (UFCSPA), Porto Alegre, RS, Brazil

Lobeline (Lob), an alkaloid originally found in Lobelia inflate, is a brain nonselective nicotinic acetylcholine receptor (nAChR) antagonist with high affinity for α4β2- and α3β2- subtype, which have been associated with Lob antidepressant and anxiolytic-like effects as well as its capacity of decreasing alcohol self-administration in rodents. These properties made Lob a candidate in the treatment of alcohol addiction. The most severe manifestations of Alcohol Withdrawal Syndrome include delirium tremens, hallucinations, and seizures, that is why it is important to evaluate possible anticonvulsant and neuroprotective activities of Lob to obtain new information on properties that could reinforce its use in treating alcohol addiction. The anticonvulsant effect of Lob was evaluated using a pilocarpine-induced seizure model. Neuroprotective effects were investigated measuring DNA damage using the comet assay, assessing free radical levels by dichlorofluorescin diacetate (DCF) oxidation, and measuring the antioxidant potential using the α, α-diphenyl-β-picrylhydrazyl (DPPH) scavenging assay, besides measuring superoxide dismutase (SOD) and catalase (CAT) enzyme activities in brain tissues. Lobeline increased the latency for the first seizure and decreased the percentage of seizures in a similar way as diazepam, used as control. DNA damage induced by pilocarpine (Pil) and hydrogen peroxide were decreased in hippocampus and cerebral cortex from mice treated with Lob. The levels of free radicals and CAT activity increased in cortex and hippocampus, respectively, in mice treated with Pil. Lobeline decreased CAT in hippocampus, leading to similar values as in saline negative control. In conclusion, Lob has anticonvulsant and neuroprotective actions that may be mediated by antioxidant-like mechanisms, indicating its potential as candidate drug in alcoholism therapy.

Support: FULBRA, CNPq, and FAPERGS
P43 – Aerobic exercise reduces DNA damage possibly by increase neurotrophic signaling in hippocampus of old rats

Thais Ceresér Vilela¹, Adriani Paganini Damiani¹, Tamires Pavei Macan¹, Alexandre Pastoris Muller², Ricardo Aurino de Pinho², Vanessa Moraes de Andrade¹

¹ Laboratory of Molecular and Cell Biology, Universidade do Extremo Sul Catarinense – UNESC, Criciúma, SC, Brazil.
² Laboratory of Exercise Biochemistry and Physiology, Universidade do Extremo Sul Catarinense – UNESC, Criciúma, S.C, Brazil

Aging is associated with impaired cognition and memory, and increased susceptibility to neurodegenerative disorders. It is known also that DNA is progressively damaged during aging and it has been observed that Brain-derived neurotropic factor (BDNF) could enhance neuronal DNA repair by increasing the expression of repair enzymes such apurinic/apyrimidinic endonuclease 1 (APE1) possibly through a mechanism involving CREB. In this sense, studies have showed that physical exercise increases the expression of BDNF and this could reduce the DNA damage. Therefore, the aim of the present study was to evaluate the effect of two physical training models on levels of BDNF and their receptors (TrkB and p75NTR), CREB, APE1 and DNA damage in hippocampus of old rats. Eighteen 24-month-old male Wistar rats were divided into untrained, aerobic and strength training. The rats were subjected to strength or treadmill training for 8 weeks. The BDNF, TrkB, p75NTR, CREB and APE1 levels were measured by Western Blotting and the Comet Assay was utilized to assess DNA damage levels. The CREB, BDNF and p75NTR levels increased in the hippocampus in the aerobic and strength groups. However, we failed to observe significant alterations in APE1 levels. Besides, hippocampus analysis of the aerobic group showed decreased TrkB levels and DNA damage. Taken together, these findings suggest a protector effect of aerobic exercise on brain DNA damage, possibly BDNF-dependent manner induced by the exercise aerobic training. The mechanisms involved on these results are unclear and future studies are needed.

Supported by: UNESC, CAPES

P44 – Concentration-dependent genotoxic and apoptotic effects of melatonin in human epidermoid carcinoma cells (A-431) and normal skin fibroblastic cells (CCD-1079SK) by activating the reactive oxygen species-mediated mitochondrial pathway

Abdurrahim Kocyigit, Eray M. Guler, Ersin Karatas, Hifa G. Caglar, Huri Bulut
Bezmialem Vakif University, Medical Faculty, Department of Medical Biochemistry, Istanbul, Turkey

Although the vast majority of experiments have documented that N-acetyl-5-methoxytryptamine (Melatonin) is a powerful antioxidant, some recent in vitro studies demonstrated its cytotoxic and apoptotic effects on various types of cells. However, the precise molecular mechanisms of its action are not fully elucidated. In this study, we examined concentration-dependent cytotoxic, genotoxic, apoptotic and reactive oxygen species (ROS) generating effects of melatonin in human epidermoid carcinoma cells (A-431) and human normal skin fibroblastic cells (CCD-1079Sk). The cells were incubated with different doses of melatonin (0.031 to 5 mM) for 24 h. The cell viability was assessed based on luminometric ATP cell viability assay. Genotoxicity was evaluated by alkaline single cell gel electrophoresis assay (Comet Assay). Apoptotic effect was detected by Annexin V/PI flow cytometric, Acridine Orange/Ethidium Bromide (AO/EB) double staining and Western Blotting methods. Intracellular accumulation of reactive oxygen species (ROS) was determined using the fluorescent probes 2,7-dichlorodihydrofluorescein-diacetate (H2DCF-DA). Although lower doses of melatonin (0.031 to 0.06 mM) increased cell proliferation and decreased ROS generation, higher doses of melatonin (0.125 to 5 mM) markedly inhibited the cell proliferation, induced DNA damage and apoptosis through the generation of ROS. There were close negative relationships between cell viability, ROS and DNA damage levels. In conclusion, melatonin acts either as antioxidant or pro-oxidant in a dose dependent manner and, cytotoxic, genotoxic and apoptotic effects depend on its pro-oxidant potential in both cancer and normal cells. However, cytotoxic, genotoxic and apoptotic effects are higher in cancer cells than in normal cells.
Polyphenolic antioxidants are suggested to play an important role in the prevention of diseases related to reactive oxygen species (ROS) production such as cancer, cardiovascular and neurodegenerative disorders or aging. Also epidemiological studies indicate that populations consuming high levels of plant-derived foods have low incidence rates of various cancers. Galangin (3,5,7-trihydroxyflavone) is a polyphenolic compound, found in the Helichrysum aureonitens, propolis and rhizomes of Alpinia officinarum. It is widely used as a traditional Chinese medicine and food additive. Some studies showed that galangin has antigenotoxic, antioxidant, radical scavenging, antiviral and antibacterial activities and the protective role of galangin against cancer, arthritis and obesity were demonstrated in some recent studies. Ursolic acid (3β-hydroxy-12-urs-12-en-28-oic acid) is a well-known pentacyclic triterpene which is also heavily used in traditional Chinese medicine. In recent years, interest in ursolic acid has increased due to its many beneficial effects and low toxicity. Malus pumila, Ocimum basilicum, Vaccinium spp., Vaccinium macrocarpon, Olea europaea, Origanum vulgare, Rosmarinus officinalis, Salvia and Thymus plants are the main sources of ursolic acid. Ursolic acid has been using against different diseases including osteoarthritis, rheumatoid arthritis, ulcer, cancer and diabetes. In the present study, genotoxic/antigenotoxic effects of galangin and ursolic acid were evaluated by alkaline Comet assay in human peripheral blood lymphocytes and Chinese hamster lung fibroblast cells (V79). Cells were treated with 5, 10, 25, 50 and 100 µM galangin and ursolic acid, hydrogen peroxide (H₂O₂) was used as the positive control. According to the study, galangin and ursolic acid did not cause DNA damage in both cells. DNA damage was significantly lower in the galangin+H₂O₂ and ursolic acid +H₂O₂ treated group when compared to positive control. It seems that galangin and ursolic acid might have a role in the prevention of DNA damage.
Manuka honey protects DNA of human leukocytes from oxidative damage induced by hydrogen peroxide in vitro

Lada Zivkovic¹, Andrea Cabarkapa-Pirkovic¹, Dragana Dekanski², Vladan Bajic², Francesca Giampieri⁴, Massimiliano Gasparrini⁴, Luca Mazzoni⁵, Biljana Spremo-Potparevic¹

¹Department of Biology and Human Genetics, Institute of Physiology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia
²Biomedical Research, R&D Institute, Galenika a.d., Pasterova 2, 11000 Belgrade, Serbia
³The Laboratory for Radiobiology and Molecular Genetics, Institute for Nuclear Research "Vinča", University of Belgrade, Mike Petrovića Alasa 12-14, 11000 Belgrade, Serbia
⁴Department of Clinical Sciences, Faculty of Medicine, Polytechnic University of Marche, Via Ranieri, 65, 60131, Ancona, Italy
⁵Department of Agricultural, Food and Environmental Sciences, Polytechnic University of Marche, Via Ranieri, 65, 60131, Ancona, Italy

Manuka honey is a monofloral honey derived from the manuka tree (Leptospermum scoparium) which has attracted a lot of attention as a strong antimicrobial and antioxidative agent. It is known that manuka honey, thanks to a high amount of phenolic compounds exerts a protective role against oxidative damage in an in vivo experimental model, reducing DNA damage, the malondialdehyde level and glutathione peroxidase activity.

In order to assess antigenotoxic potency as well as genoprotective properties of manuka honey extract against oxidative DNA damage, we performed in vitro comet assay in human peripheral leukocytes in two experimental designs, pre-treatment and post-treatment. No genotoxic effect of six concentrations (25, 50, 100, 250, 500 and 1000 ug/ml) of extracts was found. The same concentrations of manuka honey extract have been tested against DNA damaging effects of H₂O₂. In the pretreatment with the manuka extracts, modest decrease of H₂O₂-induced DNA damage was detected. On the other hand, in the post-treatment, when the extracts were applied after the oxidant for 30 min, manuka honey displayed stronger potential. It significantly attenuated hydrogen peroxide induced-DNA damage at all tested concentrations. In other words, manuka honey exhibited potential to protect the DNA of peripheral leukocytes from oxidative damage induced by hydrogen peroxide, on interventional level. Based on different DNA damage reduction seen in the pretreatment and in the post-treatment, we can presume that the antigenotoxic effect of manuka honey extracts is probably based on stimulation of the cell DNA repair mechanisms.
University of Navarra
Pamplona, Spain
(www.unav.es)

The University of Navarra, established in 1952, has 4 campuses in Spain (Pamplona, San Sebastian, Madrid and Barcelona) one in New York and one in Munich. It has 17 faculties and schools, 16 institutes, 11 research centers and 9 associated centers. It currently has 11,181 undergraduate students, 1,821 master students and 880 doctoral students. It is the 5th Spanish University in the QS World University Ranking and has been considered the best private University in Spain during the last five years (by the well-recognized annual ranking score published by EL MUNDO newspaper). The University has 195 agreements with International Universities and belongs to the European University Consortium and other important Frameworks. Within the University’s health sector are the University Hospital of Navarra, the Applied Medical Research Centre and the Nutrition Research Center.

The School of Pharmacy and Nutrition opened in 1964. Since then, more than 7,000 students have passed through its classrooms; it currently has more than 650 undergraduate students, plus 85 master and 70 doctoral students. It has two main areas of research: Medicines and Health, and Food and Health. It has more than 80 international agreements with universities, health institutions and research centres across America, Europe and Asia.
The Science Assembly Hall is located in the main building of the Science area of the University of Navarra. The special shape of the hall and some of its rooms accounts for its popular name, the Hexagon. Renovated 3 years ago, the hall contains more than 300 seats and is fully equipped with all the technical facilities needed to hold a conference.

**Floorplan**

**Science Building**

Groundfloor

Underground floor

*Ground and underground floor foyers for poster presentations and exhibition areas*
HOW TO GET TO THE VENUE?

The University of Navarra is easily accessible by public transport

29 and 30 August 2017: from the conference room to the lunch area
31 August 2017: from the venue to the lunch place
Tuesday, 29 August 2017: Visit to vineyard and winery OTAZU

Navarra is a very well known province due to its food and wine, among other things. Otazu Winery is located in a beautiful landscape 15 km away from Pamplona. It is an old French-style winery, which was built in 1840 and re-modeled in 1994. Winery Otazu integrates the viniculture tradition of the area and expressions of contemporary art. The old winery contains a ‘Wine Museum’, in which traditional tools, machinery and typical manufacturing conditions are exposed, showing the evolution of wine-making process as well as the economic, cultural and historic impact of the wine in Navarra.

The visit will include a guided tour and a wine tasting accompanied by cheese and ham.

Buses will pick up attendants close to the venue (see the map in the next page), carry them to the vineyard and bring them back to the city center after the visit.
Wednesday, 30 August 2017: Discovering Pamplona during a guided walk followed by the conference dinner

A one-hour guided tour in the old part of Pamplona will be organized to give the attendants a glance of the city. Pamplona is a small city, so everything is relatively close. Throughout the tour unique monuments, squares and hidden corners of the city will be visited, such as the cathedral of Santa Maria, the city hall, fortress-churches, palaces, mediaeval walls and the route of the bull run among others. The old town will whet assistant’s appetite, as even the most demanding palates will feel rewarded with the culinary miniatures (‘pinchos’) that Pamplona’s bars serve every day, made with typical products from Navarra. You will have the opportunity to buy and taste them during our tour.

The tour will end up in the restaurant ‘El Colegio’ where the conference dinner will take place. ‘El Colegio’ is a very well-known restaurant placed in the centre of the city. ‘El Colegio’ restaurant offers excellent traditional food with a touch of innovative cuisine. It is a fancy place located in a splendid classical building in the Half-moon park, at the southern end of the city walls. The Half-moon park is one of the oldest and most beautiful parks in the city, with excellent views over the river, Arga, and the hills beyond.
PARTICIPANTS
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<td>Norway</td>
<td><a href="mailto:ney@nilu.no">ney@nilu.no</a></td>
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<td>Norway</td>
<td><a href="mailto:eel@nilu.no">eel@nilu.no</a></td>
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<tr>
<td>Spain</td>
<td><a href="mailto:jenciso@alumni.unav.es">jenciso@alumni.unav.es</a></td>
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<tr>
<td>Portugal</td>
<td><a href="mailto:teixeirafraga@hotmail.com">teixeirafraga@hotmail.com</a></td>
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<tr>
<td>Slovakia</td>
<td><a href="mailto:alena.gabelova@savba.sk">alena.gabelova@savba.sk</a></td>
<td></td>
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<tr>
<td>Italy</td>
<td><a href="mailto:lisa.giovannelli@unifi.it">lisa.giovannelli@unifi.it</a></td>
<td></td>
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<tr>
<td>The Netherlands</td>
<td><a href="mailto:r.godschalk@maastrichtuniversity.nl">r.godschalk@maastrichtuniversity.nl</a></td>
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<tr>
<td>Norway</td>
<td><a href="mailto:kristine.bjerve.gutzkow@fhi.no">kristine.bjerve.gutzkow@fhi.no</a></td>
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<tr>
<td>UK</td>
<td><a href="mailto:francis.hall@instem.com">francis.hall@instem.com</a></td>
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<tr>
<td>Germany</td>
<td><a href="mailto:ulrike.hemmann@sanofi.com">ulrike.hemmann@sanofi.com</a></td>
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<tr>
<td>Belgium</td>
<td><a href="mailto:sophie.hendrix@uhasselt.be">sophie.hendrix@uhasselt.be</a></td>
<td></td>
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<tr>
<td>Brazil</td>
<td><a href="mailto:analeticia.garcia@terra.com.br">analeticia.garcia@terra.com.br</a></td>
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<tr>
<td>Switzerland</td>
<td><a href="mailto:cordula.hirsch@empa.ch">cordula.hirsch@empa.ch</a></td>
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<tr>
<td>Czech Republic</td>
<td><a href="mailto:hola@ueb.cas.cz">hola@ueb.cas.cz</a></td>
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<tr>
<td>Spain</td>
<td><a href="mailto:ehuarte.1@alumni.unav.es">ehuarte.1@alumni.unav.es</a></td>
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<tr>
<td>Germany</td>
<td><a href="mailto:bernd-wolfgang.igl@bayer.com">bernd-wolfgang.igl@bayer.com</a></td>
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<tr>
<td>Serbia</td>
<td><a href="mailto:bjanovic@chem.bg.ac.rs">bjanovic@chem.bg.ac.rs</a></td>
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<tr>
<td>Poland</td>
<td><a href="mailto:lucynakapka@gmail.com">lucynakapka@gmail.com</a></td>
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<tr>
<td>Turkey</td>
<td><a href="mailto:skarakas@harran.edu.tr">skarakas@harran.edu.tr</a></td>
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<tr>
<td>Switzerland</td>
<td><a href="mailto:amelie.kirchhofer@pmi.com">amelie.kirchhofer@pmi.com</a></td>
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<tr>
<td>UK</td>
<td><a href="mailto:dkirkland@genetoxconsulting.co.uk">dkirkland@genetoxconsulting.co.uk</a></td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td><a href="mailto:siegfried.knasmueller@medunwien.ac.at">siegfried.knasmueller@medunwien.ac.at</a></td>
<td></td>
</tr>
<tr>
<td>Turkey</td>
<td><a href="mailto:abdurrahim.kocyigit@yahoo.com">abdurrahim.kocyigit@yahoo.com</a></td>
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<tr>
<td>Belgium</td>
<td><a href="mailto:gudrun.koppen@vito.be">gudrun.koppen@vito.be</a></td>
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<tr>
<td>Slovakia</td>
<td><a href="mailto:katarina.kozics@savba.sk">katarina.kozics@savba.sk</a></td>
<td></td>
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<tr>
<td>Poland</td>
<td><a href="mailto:marcin.kruszewski@gmail.com">marcin.kruszewski@gmail.com</a></td>
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<tr>
<td>Ladeira Carina</td>
<td><a href="mailto:carina.ladeira@estesl.ipl.pt">carina.ladeira@estesl.ipl.pt</a></td>
<td>Escola Superior de Tecnologia da Saúde de Lisboa</td>
</tr>
<tr>
<td>Laffon Blanca</td>
<td><a href="mailto:blaffon@udc.es">blaffon@udc.es</a></td>
<td>Universidade da Coruña</td>
</tr>
<tr>
<td>Langie Sabine</td>
<td><a href="mailto:sabine.langie@vito.be">sabine.langie@vito.be</a></td>
<td>Flemish Institute for Technological Research (VITO)</td>
</tr>
<tr>
<td>Lorenzo Corrales Yolanda</td>
<td><a href="mailto:y.l.corrales@medisin.uio.no">y.l.corrales@medisin.uio.no</a></td>
<td>Oslo University Hospital and University of Oslo</td>
</tr>
<tr>
<td>Mewes Karsten</td>
<td><a href="mailto:karsten.mewes@henkel.com">karsten.mewes@henkel.com</a></td>
<td>Henkel AG &amp; Co. KGaA</td>
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<tr>
<td>Milić Mirta</td>
<td><a href="mailto:mirtamil@gmail.com">mirtamil@gmail.com</a></td>
<td>Institute for Medical Research and Occupational Health</td>
</tr>
<tr>
<td>Moller Peter</td>
<td><a href="mailto:pemo@sund.ku.dk">pemo@sund.ku.dk</a></td>
<td>University of Copenhagen</td>
</tr>
<tr>
<td>Moretti Massimo</td>
<td><a href="mailto:massimo.moretti@unipg.it">massimo.moretti@unipg.it</a></td>
<td>University of Perugia</td>
</tr>
<tr>
<td>Mukherjee Anita</td>
<td><a href="mailto:anitamukherjee28@gmail.com">anitamukherjee28@gmail.com</a></td>
<td>University of Calcutta</td>
</tr>
<tr>
<td>Muruzabal Damian</td>
<td><a href="mailto:damian.muruzabal@alumni.unav.es">damian.muruzabal@alumni.unav.es</a></td>
<td>University of Navarra</td>
</tr>
<tr>
<td>Narciso Laura</td>
<td><a href="mailto:laura.narciso@iss.it">laura.narciso@iss.it</a></td>
<td>ISS - Istituto Superiore di Sanità</td>
</tr>
<tr>
<td>Nemeth Zsuzsanna</td>
<td><a href="mailto:nemeth.zsuzsanna@osski.hu">nemeth.zsuzsanna@osski.hu</a></td>
<td>National Public Health Institute</td>
</tr>
<tr>
<td>Okeke Kelechi</td>
<td><a href="mailto:Kelechi.Okeke@uwe.ac.uk">Kelechi.Okeke@uwe.ac.uk</a></td>
<td>University of the West of England</td>
</tr>
<tr>
<td>Olsen Ann-Karin</td>
<td><a href="mailto:ann.karin.olsen@fhi.no">ann.karin.olsen@fhi.no</a></td>
<td>Norwegian Institute of Public Health</td>
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<tr>
<td>Picada Jaqueline</td>
<td><a href="mailto:jnpicada@gmail.com">jnpicada@gmail.com</a></td>
<td>Lutheran University of Brazil (ULBRA)</td>
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<td>Pourrut Bertrand</td>
<td><a href="mailto:bertrand.pourrut@yncrea.fr">bertrand.pourrut@yncrea.fr</a></td>
<td>Yncrea Hauts de France - ISA Lille - LGCGE</td>
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<tr>
<td>Rabaioli da Silva</td>
<td><a href="mailto:fer.rabaioli@gmail.com">fer.rabaioli@gmail.com</a></td>
<td>La Salle University</td>
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<tr>
<td>Radakovic Milena</td>
<td><a href="mailto:mradakovic@vet.bg.ac.rs">mradakovic@vet.bg.ac.rs</a></td>
<td>University of Belgrade</td>
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<tr>
<td>Rawlinson Paul</td>
<td><a href="mailto:paul.rawlinson@syngenta.com">paul.rawlinson@syngenta.com</a></td>
<td>Syngenta - Jealotts Hill International Research Centre</td>
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<td>Reisinger Kerstin</td>
<td><a href="mailto:kerstin.reisinger@henkel.com">kerstin.reisinger@henkel.com</a></td>
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<td>Richling Elke</td>
<td><a href="mailto:Richling@chemie.uni-kl.de">Richling@chemie.uni-kl.de</a></td>
<td>University of Kaiserslauern</td>
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<tr>
<td>Rohr Paula</td>
<td><a href="mailto:paularohr@gmail.com">paularohr@gmail.com</a></td>
<td>University of Southern Santa Catarina (UNESC)</td>
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<tr>
<td>Rojas Emilio</td>
<td><a href="mailto:emilior@biomedicas.unam.mx">emilior@biomedicas.unam.mx</a></td>
<td>Universidad Nacional Autonoma de Mexico</td>
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<tr>
<td>Sanz Julen</td>
<td><a href="mailto:jsanz.3@alumni.unav.es">jsanz.3@alumni.unav.es</a></td>
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<td>UK</td>
<td><a href="mailto:Kelechi.Okeke@uwe.ac.uk">Kelechi.Okeke@uwe.ac.uk</a></td>
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<td><a href="mailto:jnpicada@gmail.com">jnpicada@gmail.com</a></td>
<td>Lutheran University of Brazil (ULBRA)</td>
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<td>France</td>
<td><a href="mailto:bertrand.pourrut@yncrea.fr">bertrand.pourrut@yncrea.fr</a></td>
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<td><a href="mailto:fer.rabaioli@gmail.com">fer.rabaioli@gmail.com</a></td>
<td>La Salle University</td>
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<td>Serbia</td>
<td><a href="mailto:mradakovic@vet.bg.ac.rs">mradakovic@vet.bg.ac.rs</a></td>
<td>University of Belgrade</td>
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<td><a href="mailto:paul.rawlinson@syngenta.com">paul.rawlinson@syngenta.com</a></td>
<td>Syngenta - Jealotts Hill International Research Centre</td>
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<td><a href="mailto:kerstin.reisinger@henkel.com">kerstin.reisinger@henkel.com</a></td>
<td>Henkel AG &amp; Co. KGaA</td>
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<tr>
<td>Germany</td>
<td><a href="mailto:Richling@chemie.uni-kl.de">Richling@chemie.uni-kl.de</a></td>
<td>University of Kaiserslauern</td>
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<tr>
<td>Brazil</td>
<td><a href="mailto:paularohr@gmail.com">paularohr@gmail.com</a></td>
<td>University of Southern Santa Catarina (UNESC)</td>
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<tr>
<td>Mexico</td>
<td><a href="mailto:emilior@biomedicas.unam.mx">emilior@biomedicas.unam.mx</a></td>
<td>Universidad Nacional Autonoma de Mexico</td>
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<td>Spain</td>
<td><a href="mailto:jsanz.3@alumni.unav.es">jsanz.3@alumni.unav.es</a></td>
<td>University of Navarra</td>
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<td>Institute Theoretical &amp; Experimental Biophysics RAS</td>
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<td>University of Southern Santa Catarina (UNESC)</td>
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<td>Vodenkova Sona</td>
<td>The Czech Academy of Sciences</td>
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<td>Zegura Bojana</td>
<td>National Institute of Biology</td>
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<td>Zivkovic Lada</td>
<td>University in Belgrade</td>
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<td>López de Cerain Adela</td>
<td>University of Navarra</td>
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<td>Villarroel Maria José</td>
<td>RNB Cosméticos</td>
<td></td>
</tr>
<tr>
<td>Norway <a href="mailto:lena.sareisian@fhi.no">lena.sareisian@fhi.no</a></td>
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