ICOETox

International Conference of Environmental and Occupational Health

ABSTRACT BOOK

iBAMTOX

Ibero-American Meeting on Toxicology and Environmental Health

2016

21-23 June | Porto

FOREWARD

On behalf of the Organising Committee, I would like to cordially welcome you to the *3rd International Conference on Occupational & Environmental Toxicology* (ICOETox 2016), which is held in Porto in conjunction with the *3rd Ibero-American Meeting on Toxicology and Environmental Health International* (IBAMTOX 2016).

This conference is co-organised by the Portuguese National Institute of Health (INSA), the Institute of Public Health- Universidade do Porto (ISPUP) and the Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto (ICETA-UP).

The Organising Committee was successful in inviting a number of outstanding international and local speakers in order to offer you a very attractive scientific programme. The Conference covers most of the current topics of Environmental and Occupational Toxicology; we have tried to achieve a good balance between research and practice and to allow sufficient time for interaction and discussion. This meeting provides a good opportunity for divulging one's work and discussing a great variety of topics that we hope will be reflected in a fruitful interchange of experiences, knowledge and ideas. It is also a chance for renewing old contacts and making many new friends.

The city of Porto, known as *Invicta* (unvanquished) City, has an important historical legacy, although architectural images show its urban renovation process giving valuable testimony of its history and modernity. Indeed, Porto historical centre was designated World Cultural Heritage in 1996 due to the many historical buildings and urban mesh. Porto is divided between the river Douro and the Atlantic Ocean, and boasts of poetic sunsets where the eyes absorb and the soul savours. Downtown is located the busiest commercial area, where typical products are found alongside prestigious designer brands. It is also worth highlighting the world famous Porto Wine, produced exclusively in the Douro Demarcated Region and aged in cellars. And finally, our visitors should not forget to try our local cuisine, as Porto has gone beyond tradition in order to reach the best international standards.

I would like to express my sincere thanks to our collaborating institutions and all those organisations and companies which put their trust in this project and provided sponsorship for the meeting; without their effort, support and collaboration this Conference would not have been possible.

I hope that, despite the tight scientific programme, you will find some time to enjoy our landscapes, typical food, and kind people, and that this meeting will meet all your expectations from the scientific and social points of view. I wish you a productive Conference and a pleasant stay in Porto. Thank you for being here.

Bem-vindos ao Porto!

(João Paulo Teixeira)

ICOETox 2016 | IBAMTOX 2016 Scientific Committe

LOCAL ORGANISING COMMITTEE

JOÃO PAULO TEIXEIRA
CARLA COSTA
SOLANGE COSTA
CRISTIANA PEREIRA
SÓNIA FRAGA
ANA MENDES

SCIENTIFIC COMMITTEE

JOÃO PAULO TEIXEIRA – PORTUGAL
FERNANDO BARBOSA - BRAZIL
ANDREW COLLINS - UK
BLANCA LAFFON - SPAIN
CARLA COSTA - PORTUGAL
CRISTIANA PEREIRA - PORTUGAL
FÉLIX CARVALHO - PORTUGAL
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PETER MOLLER - DENMARK
SAM KACEW - CANADA
SÓNIA FRAGA - PORTUGAL
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STEFANO BONASSI - ITALY
VANESSA ANDRADE - BRAZIL
VANESSA VALDIGLESIAS – SPAIN

SPONSORS

PROGRAMME

TUESDAY, JUNE 21ST

8.30 AM – REGISTRATION

9.30 AM – WELCOME SESSION

10.30 AM - INVITED LECTURE

***In vitro* responses to known *in vivo* genotoxic agents in germ stem cells**

Diana Anderson, University of Bradford, UK

11.00 AM – COFFEE BREAK

11.30 AM – SESSION GENETIC TOXICOLOGY

SESSION CHAIR: PETER MOLLER

Genotoxicity assessment of low dose exposure to glyphosate in HepG2 cell line after 4 and 24 hours

Mirta Milić, Institute for Medical Research and Occupational Health, Croatia

The antimalarial drug lumefantrine interacts with human topoisomerase II beta complexed to DNA by in silico analysis

Carmen Bassi-Branco, University Center of Federal District, Brazil

12.00 AM - INVITED LECTURE

Reduction DNA damage in the neural tissue and peripheral blood in old mice treated with caffeine

Vanessa Andrade, University of Extremo Sul Catarinense (UNESC), Brazil

12.30 AM - INVITED LECTURE

Particularities of acute non-professional poisons of chemical etiology in Republic of Moldova in 2011-2015

Ion Bahnarel, National Centre of Public Health, Republic of Moldova

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1.00 PM – LUNCH AND POSTER SESSION

2.30 PM - INVITED LECTURE

High Throughput comet assay: Applications in genotoxicity testing and biomonitoring

Andrew R. Collins, NorGenotech AS and University of Oslo, Norway

3.00 PM - INVITED LECTURE

H2AX phosphorylation analysis as DNA damage biomarker for human population studies

Vanessa Valdiglesias, Universidade da Coruña, Spain

3.30 PM – RISK AND SUSCEPTIBILITY FACTORS

SESSION CHAIR: DIANA ANDERSON

A child´s spit epigenome can reveal its respiratory allergy risk

Sabine Langie, VITO and Hasselt University, Belgium

Studying estrogenic and androgenic influences on effects of anticancer drugs using the hepatocarcinoma cell line HepG2

Mohamed Hamzawy, Misr University for Science and Technology, Egypt

Analysis of 23 SNPs and association of the *UGT2B7, UGT1A9, ABCG2* and *IL-23R* polymorphisms with rejection risk in kidney transplant patients

Ilce Cólus, State University of Londrina, Brazil

Individual susceptibility to the toxic effects of radiation therapy: a potential role for DNA repair SNPs?

Ana Margarida Marques, Universidade Católica Portuguesa, Portugal

Forgotten Public Health Impacts from Cancer

Susana Viegas, Lisbon School of Health Technology/Polytechnic Institute of Lisbon, Portugal

4.45 PM - KEYNOTE LECTURE

Occupational and Environmental Risk factors for Parkinson´s disease

Stefano Bonassi, Istituto di Ricovero e Cura a Carattere Scientifico San Raffaele Pisana, Italy

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5.15 PM – COCKTAIL

WEDNESDAY, JUNE 22ND

9.30 AM - INVITED LECTURE

Designing nanomaterials towards a sustainable nanotechnology: an ecotoxicological approach
Isabel Lopes, University of Aveiro, Portugal

10.00 AM – SESSION ECOTOXICOLOGY

SESSION CHAIR: SAM KACEW

Assessment of environmental hazards caused by release of selected cytostatics
Siegfried Knasmueller, Medical University of Vienna, Austria

Mercury-resistant bacteria and mercury cycling in sediments of the Tagus estuary
Cristina Carvalho, Universidade de Lisboa, Portugal

Spatial and temporal patterns of ecological risk induced by pesticides in Alqueva reservoir: a case study
Patrícia Palma, Instituto Politécnico de Beja, Portugal

Use of multiple biomarkers to evaluate plant species suitability to manage contaminated areas
Bertrand Pourrut, Laboratoire Génie Civil et géo-Environnement, France

Biochemical and behaviour alterations of zebrafish early life exposed to synthetic and natural dyes
Flavia Renata Abe, University of São Paulo, Brazil

11.15 AM – COFFEE BREAK

11.45 AM - INVITED LECTURE

Mixtures Toxicology: Mycotoxins in food as a case study
Maria João Silva, National Institute of Health Doutor Ricardo Jorge, I.P., Portugal

12.15 AM – SESSION MIXTURES TOXICOLOGY

SESSION CHAIR: SIEGFRIED KNASMUELLER

Fish exposure to sub-lethal metallic-nanoparticles mixtures: genotoxicity and haematological parameters
Ana Teresa Reis, University of Aveiro, Portugal

Oxidative effects of PAH mixtures over the thioredoxin and gluthathione systems
Marta Martins, Faculty of Sciences and Technology, Portugal

Mapping environmental human exposure integrating multipollutants based on biomonitors – a methodological approach
Helena C. Serrano, Universidade de Lisboa, Portugal

1.00 PM – LUNCH AND POSTER SESSION

2.30 PM - INVITED LECTURE

Mutagenic and carcinogenic potential of metal nanoparticles depends on their physicochemical properties

Maria Dusinska, Norwegian Institute for Air Research, Norway

3.00 PM – SESSION NANOTOXICOLOGY

SESSION CHAIR: SÓNIA FRAGA

On the track of the *in vivo* toxicological profile of superparamagnetic colloidal iron oxide nanoparticles in zebrafish embryos

Begoña Espiña, International Iberian Nanotechnology Laboratory, Portugal

Determination of nanoparticle uptake by flow cytometry and atomic absorption spectrometry

Barbora Buliaková, Cancer Research Institute, BMC SAS, Slovakia

Surface-modified TiO_2 nanoparticles with ascorbic acid: antioxidant properties and efficiency against DNA damage

Biljana Spremo-Potparević, University of Belgrade, Serbia

Cytotoxicity assessment on cotton fabrics coated with photocatalytic titanium dioxide nanoparticles

Miruna S. Stan, University of Bucharest, Romania

4.00 PM – COFFEE BREAK

4.30 PM - INVITED LECTURE

Analysis of cellular damage induced by silica-coated iron oxide nanoparticles on neuronal cells

Blanca Laffon, Universidade da Coruña, Spain

5.00 PM – SESSION NANOTOXICOLOGY (CONT.)

SESSION CHAIR: SÓNIA FRAGA

A prospective approach to safe nanotechnologies

Christa Schimpel, BioNanoNet Forschungsgesellschaft mbH, Austria

Manufactured nanomaterials: is there a correlation between toxicological effects and the physicochemical properties?

Henriqueta Louro, National Institute of Health Dr. Ricardo Jorge, I.P., Portugal

Determination of "safe" and "critical" nanoparticles exposure to welders in a workshop

João Gomes, Instituto Superior de Engenharia de Lisboa and Instituto Superior Técnico, Portugal

5.45 PM - INVITED LECTURE

Cardiovascular effects in animals after exposure to particles

Peter Møller, University of Copenhagen, Denmark

6.15 PM – END OF DAY

THURSDAY, JUNE 23RD

9.30 AM – INVITED LECTURE

Indoor air quality: Management challenges and assessment tools
Joana Madureira, Institute of Science and Innovation in Mechanical Engineering and Industrial Management, Portugal

10.00 AM – SESSION INDOOR ENVIRONMENT

SESSION CHAIR: FERNANDO BARBOSA JR.

Assessment of indoor environmental quality in elderly care centers
Ana Nogueira, National Institute of Health Doutor Ricardo Jorge, I.P., Portugal

Polycyclic aromatic hydrocarbons in educational settings: A comparison between pre- and primary schools
Klára Slezáková, Faculdade de Engenharia da Universidade do Porto and Instituto Superior de Engenharia do Porto, Portugal

Inhaled dose of $PM_{2.5}$ and PM_{10} by children in Porto, Portugal
Pedro Branco, Faculty of Engineering, University of Porto, Portugal

Bacterial and fungal exposure in schools may influence asthma and allergy in children
João Rufo, FMUP, INEGI and ISPUP, Portugal

11.00 AM – COFFEE BREAK

11.30 AM – INVITED LECTURE

Occupational exposure to process generated nanoparticles: the case of the ceramic industry
Mar Viana, IDAEA-CSIC, Spain

12.00 AM – SESSION OCCUPATIONAL TOXICOLOGY

SESSION CHAIR: STEFANO BONASSI

Hexavalent chromium, an occupational lung carcinogen, attenuates thermal shock effects and interferes with the stress response in human bronchial epithelial cells
Patrícia Lona Abreu, University of Coimbra, Portugal

Genotoxic and cytotoxic effects in nasal and buccal cells of electroplaters
George Wultsch, Institute of Cancer Research, Austria

Hospital surfaces contamination with antineoplastic drugs: influence of cleaning procedures
Ana Cebola de Oliveira, IPL-ESTeSL, Portugal

Bacterial survivability and transferability on biometric devices
Joana Barreira, USF Viver Mais, ACeS Maia/Valongo, Portugal

1.00 PM – LUNCH AND POSTER SESSION

2.30 PM – INVITED LECTURE

Evaluation of DNA damage and changes in oxidative stress parameters of ceramic workers

Nursen Basaran, Hacettepe University, Turkey

3.00 PM – SESSION ENVIRONMENTAL HEALTH

SESSION CHAIR: PROF. BLANCA LAFFON

Antimicrobials in hospital wastewater: the prediction of related resistance selection hazard in the urban wastewater effluent

Anabela Almeida, Escola Universitária Vasco da Gama,and University of Coimbra, Portugal

Environmental pollutants in passive air samples of Tarragona county, Spain: an analytical multi-component approach

Noelia Domínguez-Morueco, Universitat Rovira i Virgili, Sant Llorenç, Spain

A case study about Media and Glyphosate: between scientific evidence and political (in)decision

Hernâni Zão, University of Porto, Portugal

Aflatoxin M_1 in human breast milk in Portugal: estimation of maternal and infant exposure

Angelina Pena, Coimbra University, Portugal

Changes over time in POPs concentrations in human milk in the Republic of Moldova

Alla Tirsina, National Centre of Public Health, Republic of Moldova

Fungal contamination in coffee samples: a Public Health concern

Carla Viegas, Polytechnic Institute of Lisbon and CISP/ENSP/UNL, Portugal

4.30 PM – INVITED LECTURE

A systematic study of the disposition and metabolism of mercury species in mice after exposure to low levels of thimerosal (ethylmercury)

Fernando Barbosa Jr, University of Sao Paulo, Brazil

5.00 PM – AWARD ATTRIBUTION AND CLOSING SESSION

POSTER SESSIONS

TUESDAY, JUNE 21ST

P01. **Protein oxidative damage and level of acute phase proteins in Wistar rats treated with adrenaline**
Ninoslav Djelić, University of Belgrade, Serbia

P02. ***In vitro*** **study of micronuclei induction due to exposure to a 3T static magnetic field in peripheral lymphocytes**
Carina Ladeira, IPL and Universidade Nova de Lisboa, Portugal

P03. **Assessment of genotoxicity of aflatoxin M_1 and B_1 contaminated milks after in vitro human digestion**
Carina Ladeira, IPL and Universidade Nova de Lisboa, Portugal

P04. **Protective effect of essential oil from *Lavadula angustifolia* against UVA induced DNA damage**
Monika Šramková, Cancer Research Institute, Slovak Republic

P05. **Evaluating cytotoxic and genotoxic effects of microcystin using *Saccharomyces cerevisiae* as eukaryotic cell model**
Sara Barreiros, National Institute of Health Doutor Ricardo Jorge, I.P., Portugal

P06. **Cytotoxic and genotoxic effects of an L-amino acid oxidase from *Bothrops jararacussu* in human cell lines HUVEC and HepG2**
Lusania Maria Greggi Antunes, University of São Paulo, Brazil

P07. **The influence of cryopreservation in sperm DNA damage**
Mariana Magalhães, University of Trás-os-Montes and Alto Douro, Portugal

P08. **Abortion products - DNA damage and repair**
Mariana Magalhães, University of Trás-os-Montes and Alto Douro, Portugal

P09. **Influence of vegetables juices source of lutein and beta carotene on the genotoxicity induced by alkylating agents in mice**
Daniela Dimer Leffa, University of the Extreme South of Santa Catarina, Brazil

P10. ***In vitro*** **and *in vivo* anti-inflammatory activity of anthocyanin-rich *C. icaco* L. fruit**
Vinicius P. Venancio, University of São Paulo, Ribeirão Preto, Brazil

P11. **Development of an automated scoring system for plant comet assay**
Bertrand Pourrut, Laboratoire Génie Civil et géo-Environnement (LGCgE- ISA Lille), France

P12. **Evaluation of *Morinda citrofolia* chemopreventive effects against patulin**
Joana Torrejais, National Institute of Health Doutor Ricardo Jorge, I.P. and Nova Medical School, Portugal

P13. **Bioactive compounds from seaweed with anti-leukemic activity: carotenoids and phlorotannins**
Tânia Almeida, University of Porto, Portugal

P14. **Mutagenicity/Genotoxicity of $PM_{0.5}$ collected during winter 2014-2015 in five Italian cities: MAPEC (Monitoring Air Pollutions Effects on Children for supporting public health policy) study**
Tania Salvatori, University of Perugia, Italy

P15. **DNA binding properties of a series of novel derivatives of 1,4-dihydropyridine**
Nikolajs Sjakste, Latvian Institute of Organic Synthesis and University of Latvia, Latvia

P16. **Modification of genes involved in DNA repair and nitrosative stress and proteasomal system by 1,4-dihydropyridines**
Tatjana Sjakste, University of Latvia, Latvia

P17. **Considering cell type for *in vitro* neurogenotoxicity testing: neuronal *vs.* gial cell sensitivity**
Blanca Laffon, Universidade da Coruña, Spain

P18. **Cytotoxicity and antibacterial activity of polar extracts obtained from saffron (*Crocus sativus* L.) floral bio-residues**
João CM Barreira, University of Porto and Polytechnic Institute of Bragança, Portugal

P19. **Evaluation of isoflavone content and cytotoxic activity of two new Mexican alfalfa-based foodstuffs**
João CM Barreira, University of Porto and Polytechnic Institute of Bragança, Portugal

P20. **Cytotoxic effects of hydro-ethanolic extracts from *Coleostephus myconis* (L.) Rchb.f. flowers and green parts**
Sílvia MF Bessada, University of Porto, Portugal

P21. ***In vitro* and *in vivo* Evaluation of Coffee Silverskin as Cosmetic Ingredient**
Sílvia MF Bessada, University of Porto, Portugal

P22. **Toxicity assessment of convenience products using the ciliated model *Tetrahymena pyriformis***
Alla Tirsina, National Centre of Public Health, Republic of Moldova

P23. **Long-term genotoxic effects of immunosuppressive drugs on lymphocytes of kidney transplant recipients**
Ilce Mara de Syllos Cólus, State University of Londrina, Brazil

P24. **Differential modulation of toxicity pathways by respiratory and cutaneous allergens: interaction with physiopathology**
Isabel Ferreira, University of Coimbra, Portugal

P25. **Impact of physical exercise training on DNA damage and repair: does gender play a role?**
Ana Inês Silva, University of Trás-os-Montes and Alto Douro, Portugal

P26. **Can THE COPD assessment test (CAT) be used to improve communication between clinicians and low literate elderly patients? Results from a cross-sectional study in Cova da Beira, Portugal**
Ana CA Sousa, University of Beira Interior, Covilhã, Portugal

P27. **Immunological biomonitoring of elderly adults – influence of physical activity**
Vanessa Valdiglesias, Universidade da Coruña, Spain

P28. **The impact of an acute exercise challenge on DNA Damage – an Human Intervention**
Ana Duarte, National Institute of Health Doutor Ricardo Jorge, I.P. and University of Porto, Portugal

WEDNESDAY, JUNE 22ND

P29. **Chronic exposure effects of the crustacean *Daphnia magna* to environmental concentrations of the stimulant drug nicotine**
Patrícia Palma, Escola Superior Agrária de Beja and Universidade do Algarve, Portugal

P30. **Decrease of NADPH (P450) reductase: a cause of atrazine toxicity in sea lamprey juveniles in freshwater**
Marta Candeias, Universidade de Évora, Portugal

P31. **Acute toxicity and environmental risk of the malathion formulations used to combat *Aedes aegypti* mosquito for aquatic organisms**
Ana Carla Coleone, USP and UNESP, Brazil

P32. **Biochemical responses of *Perinereis cultrifera* to heavy metal environmental contamination along the east coast of Algeria**
Naoufel Zouheir Belfetmi, Badji Mokhtar University of Annaba, Algeria

P303. **Atrazine caused oxidative stress and decreased biotransformation capacity of sea lamprey juvenile gills**
Marta Candeias, Universidade de Évora, Portugal

P34. **Histological evidence of the adverse effects of formaldehyde on the digestive and reproductive system of *Daphnia***
Piedade Barros, Porto Polytechnic, Portugal

P35. **Assessment of two larvicides used for *Aedes aegypti* control: lethal and sublethal effects to fishes**
Flavia Renata Abe, University of São Paulo, Brazil and University of Aveiro, Portugal

P36. **Water repellents protective garments efficiency used by workers in malathion spraying for mosquito control of dengue *Aedes aegypti***
Angela Aparecida Machado, UNESP, Brazil

P37. **Evaluation of the *in vivo* and *in vitro* embryotoxicity of fixative agents to *Daphnia magna***
Piedade Barros, Porto Polytechnic, Portugal

P38. **Unveiling the reason why freshwater heterothophic bacteria are not affected by microcystins: antioxidant system *vs.* degradation**
André Pinto, National Institute of Health Doutor Ricardo Jorge, I.P., Portugal

P39. **Impacts of the pharmaceutical procainamide and microplastics on the environmental health: effects on the marine microalgae *Tetraselmis chuii***
Beatriz RBOLavorante, ICBAS and CIIMAR/CIMAR, Portugal

P40. **Assessment of thiamethoxam toxicity to *Chironomus riparius* and *Dugesia tigrina***
Althiéris S. Saraiva, University of Aveiro, Portugal and Federal University of Tocantins, Brazil

P41. **Competition under saline stress: a case study with three species of plants**
Isabel Lopes, University of Aveiro, Portugal

P42. **Minimum inhibitory concentration of azoles fungicides for oomycete *Leptolegnia caudata in vitro* conditions**
Maria JT Ranzani-Paiva, Fisheries Institute, Brazil

P43. **Development of a high-throughput multi-parameter biomarker set for plant biomonitoring and ecotoxicological studies**
Clarisse Liné, Ministère du Développement durable, de l'Environnement et de la Lutte contre les changements climatiques, Canada and Laboratoire Génie Civil et géo-Environnement (LGCgE- ISA Lille), France

P44. **Effectiveness and control period of larvae *Aedes aegypti* resistant to temephos bydiflubenzuron in field conditions**
Angela Aparecida Machado, UNESP, Brazil

P45. **Humic acids decrease the toxicity of gemfibrozil at lethal level but not at sublethal level**
Inês Domingues, University of Aveiro, Portugal

P46. **Cypermethrin contamination in Pantanal: comparative sensitivity of the endemic shrimp *Macrobrachium pantanalense* and other aquatic species**
Inês Domingues, University of Aveiro, Portugal

P47. **Optimization of microbial detoxification for an aquatic mercury- contaminated environment**
Cristina Carvalho, Universidade de Lisboa, Portugal

P48. **New findings on mercury neurotoxicity disclosed by oxidative stress profiles in fish brain**
Fátima Brandão, University of Aveiro, Portugal

P49. **Mercury neurotoxicity in wild fish (*Liza aurata*) and the interference of estuarine environmental variables**
Fátima Brandão, University of Aveiro, Portugal

P50. **Endocrine disruptors mixtures: the real scenario of human exposure**
Edna Ribeiro, Lisbon School of Health Technology and Agriculture and Food - Instituto Superior de Agronomia Portugal

P51. **Impact of surface charge and functionalization on uptake and toxicity cells of silver nanoparticles in mammalian cells**
Mirta Milić, Institute for Medical Research and Occupational Health, Croatia

P52. ***In vitro* biocompatibility studies of polyester fabrics coated with photocatalytic titanium dioxide nanoparticles**
Cristina I Nica, University of Bucharest, Romania

P53. **Contribution of macrophage activation to the genotoxic effect of nanofibers in lung epithelium**
Célia Ventura, National Institute of Health Doutor Ricardo Jorge, I.P. and Universidade NOVA de Lisboa, Portugal

P54. **Cyto- and Genotoxicity assessment of cerium dioxide nanomaterials in the A549 cell line**
Maria João Silva, National Institute of Health Doutor Ricardo Jorge, I.P., Portugal

P55. **Exposure to ultrafine particles in mag steel welding: influence of metal transfer modes and shielding gas composition**
João Gomes, Instituto Superior de Engenharia de Lisboa and Instituto Superior Técnico, Portugal

P56. **Asbestos – identification on bulk materials**
Fátima Aguiar, National Institute of Health Doutor Ricardo Jorge, I.P., Portugal

P57. **Safe production and use of nanomaterials in the ceramic industry: the CERASAFE project**
Mar Viana, IDAEA-CSIC, Spain

P58. **The impact of size on tissue distribution and elimination of silver nanoparticles in mice**
Fernanda Rosário, University of Aveiro, Portugal

P59. **Toxicity of Gold Nanorods on Zebrafish (*Danio rerio*) embryos**
Bárbara Mesquita, National Institute of Health Doutor Ricardo Jorge, I.P and ISPUP, Portugal

P60. **How important is the study of assay interference prior to nanotoxicity assessment? A Case study**
Maria João Bessa, National Institute of Health Doutor Ricardo Jorge, I.P, Portugal

P61. **TiO2-NP effects in plants are formulation and species dependent**
Sónia Silva, University of Aveiro, Portugal

THURSDAY, JUNE 23RD

P62. **Children's exposure to volatile organic compounds of health relevance in kindergartens and primary schools**
Joana Madureira, Institute of Science and Innovation in Mechanical Engineering and Industrial Management, Portugal

P63. **A preliminar study of carbon dioxide concentrations and students' activity performance in Portuguese primary schools**
Inês Paciência, INEGI, ISPUP and FMUP, Portugal

P64. **Fungal communities in house dust Samples from patients with asthma – preliminary results**
Raquel Amaro, University of Aveiro and National Institute of Health Doutor Ricardo Jorge, I.P, Portugal

P65. **Unexpected effect of Dry olive Leaf extract (DOLE) before and after $CaNa_2EDTA$ chelation therapy in Comet assay in lead intoxicated workers**
Biljana Spremo-Potparević, University in Belgrade, Serbia

P66. **Assessment of DNA damage on a group of professional dancers**
Filipa Esteves, University of Porto and National Institute of Health Doutor Ricardo Jorge, I.P, Portugal

P67. **Wildland Firefighers: DNA damage and oxidative stress assessment**
Ana Abreu, National Institute of Health Doutor Ricardo Jorge, I.P. and University of Porto, Portugal

P68. **Importance of size-selective particle measuring for assessing occupational exposures – A case study "from field to fork"**
Susana Viegas, Lisbon School of Health Technology/Polytechnic Institute of Lisbon, Portugal

P69. **Occupational toxicology in the agriculture of Moldova**
Ion Bahnarel, National Centre of Public Health, Republic of Moldova

P70. **BPA occupational exposure assessment in Europe: a scientific gap**
Edna Ribeiro, Lisbon School of Health Technology and Agriculture and Food - Instituto Superior de Agronomia Portugal

P71. **Firefighters' occupational exposure to polycyclic aromatic hydrocarbons at the Portuguese fire stations**
Simone Morais, Instituto Politécnico do Porto, Portugal

P72. **Exploratory study: bacterial contamination in Hotel rooms during the cleaning activity**
Ana Monteiro, Polytechnic Institute of Lisbon, Portugal

P73. **Assessment of tributylin effects at the vascular level**
Ana Catarina Sousa, University of Aveiro and University of Beira Interior, Portugal

P74. **Early biological effects (cytome assay) in children exposed to different levels of PM0.5 in five Italian cities during winter 2014-2015: MAPEC (Monitoring Air Pollution Effects on Children for supporting public health policy) study**
Sara Levorato, University of Perugia, Italy

P75. **Fungi distribution in poultry feed**
Carla Viegas, Polytechnic Institute of Lisbon and CISP/ENSP/UNL, Portugal

P76. **Air Microbiology – External Quality Assurance Program**
Ana Nogueira, National Institute of Health Doutor Ricardo Jorge, I.P., Portugal

P77. **The relationship between the imbalance of ecosystems and poverty, a current Public Health situation**
Tammy I Pulido Iriarte, Univ. del Atlántico, Colombia

P78. **Air quality bioindicators in health research: what we have learned from an industrial area**
Manuel Castro Ribeiro, Universidade de Lisboa, Portugal

P79. **Microbiota assessment in optical shops: an ignored concern to public health**
Ana Monteiro, Polytechnic Institute of Lisbon, Portugal

P80. **Air pollution in Lisbon: A local or a global concern?**
Manuel J Matos, 1DEQ, ISEL/IPL, Portugal

P81. **Healths concern about heavy metals contents in Lisbon soils**
Manuel J Matos, 1DEQ, ISEL/IPL, Portugal

P82. **Biomonitoring of arsenic, cadmium and lead in urine of children from the Portuguese region of Tâmega**
Virgínia Cruz Fernandes, Center for Health Technology and Services Research and University of Porto, Portugal

P83. **Evaluation of Human exposure to environmental chemicals: the integrative approach of Bioporto group**
Diogo Pestana, Center for Health Technology and Services Research and University of Porto, Portugal

P84. **The Fogo volcano (Cape Verde) 2014 eruption – Impacts on human health**
Carla Candeias, ISPUP and Universidade de Aveiro, Portugal

P85. **Partition of mercury levels along the maternal-fetal-placental unit**
Ana Catarina Alves, University of Aveiro, Portugal

INVITED LECTURES

IN VITRO RESPONSES TO KNOWN *IN VIVO* GENOTOXIC AGENTS IN GERM STEM CELLS

K. Habas, M. Najafzadeh, A. Baumgartner, M.H. Brinkworth, Diana Anderson*

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Genotoxic environmental agents could induce DNA damage in germline stem cells, and in the work place or elsewhere it is difficult to determine such effects without in vivo studies. These cells are extremely sensitive even at low doses in the testis and may pose reproductive risks with potential exposure-related infertility. DNA strand breaks represent a great threat to the genomic integrity of germline stem cells, which are essential to maintain spermatogenesis and prevent reproduction failure. The Comet assay (pH>13.0) has been used to measure DNA damage in male germ cells. We investigated the effects in vitro of six well-known genotoxins as model compounds on rat germ stem cells separated using STA-PUT unit-gravity velocity sedimentation. N-ethyl-N-nitrosourea (ENU), N-methyl-N-nitrosourea (MNU), 6-mercaptopurine and 5-bromodeoxyuridine, methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS) are potent male rodent germ cell mutagens. All compounds were significantly genotoxic in cultured germ cells. Exposure of the isolated germ cells with ENU and MNU produced a concentration-related increase in DNA damage in spermatogonia; spermatocytes were most sensitive to 6-MP and 5-BrdU with MMS and EMS most damaging in spermatids. Immunocytochemistry and western blot analysis revealed that the purities of the isolated germ cells were 92% with viabilities over 95%. These results indicate that STA-PUT isolated rat testicular germ cells are a suitable model to study the genotoxicity of individual exposures in germ stem cells and could be used as a surrogate system for living humans after environmental exposure. Only spermatozoa can be examined in this way in humans.

REDUCTION DNA DAMAGE IN THE NEURAL TISSUE AND PERIPHERAL BLOOD IN OLD MICE TREATED WITH CAFFEINE

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Biologic aging is a process that starts at birth and continues until the death of the individual, it is a period of changes related to the passage of time that causes deleterious effects in the organism. Aging can be described as a gradual change in the physiology of the cell, which causes a decrease in its normal function. Errors in DNA sequences are regular events throughout the lifetime of any given organism. Together with the aging process, arise degenerative diseases which are characteristic of this phase of life, however, studies using caffeine which is a psychoactive substance, present in various products consumed daily have demonstrated negative correlations in the development of these diseases. The aim of the present study was to evaluate the level of DNA damage in peripheral blood and neural tissue of old mice treated with caffeine. For the present study, 40 albin swiss male mice (20 animals between 3-4 months and 20 animals between 13-16 months) were divided into 4 groups: Young Adults-water, young adults - caffeine, older adults -water and older adults- caffeine. Groups of young and old caffeine received caffeine solution (0.3 g/L) in bottle of water (free access) during four weeks. The others received only water during the experimental time. After the treatments were collected blood samples from animals through an incision at the tail end for performing the Comet Assay and after the animals were for the dissection of the hippocampus and femurs for performing the comet assay and micronucleus test, respectively. The comet assay for blood and hippocampus showed no significant differences between animals young in blood and hippocampus in the Comet assay, demonstrating that caffeine showed no genotoxic activity. However, when comparing young and old animals we observed a significant difference between them in blood and hippocampus. Among adults older animals was observed a significant decrease in the damage index in the group receiving caffeine, showing that caffeine was able to reverse the genotoxic damage caused by aging. In the micronucleus test we observe an increase in the frequency of EPCMNs in old animals that received water compared to the young. The old animals receiving caffeine showed a decrease in the frequency of MN in EPC compared with animals of the same age that did not received this substance, showing that caffeine did not show mutagenic activity and was able to reverse the mutagenic damage caused by aging. With our results we conclude that caffeine was not genotoxic neither mutagenic at the dose tested, still being able to reverse the genotoxicity and mutagenicity caused by aging.

PARTICULARITIES OF ACUTE POISONING CASE OF UNPROFESSIONAL EXOGENOUS CHEMICAL ETIOLOGY IN THE REPUBLIC OF MOLDOVA IN 2011-2015

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No systematic monitoring of acute poisoning of unprofessional exogenous chemical etiology has been performed in the Republic of Moldova to date. After the proclamation of independence of the Republic of Moldova (1989), the quantity and diversity of chemical substances has increased significantly in the national economy and human habitat. The paper makes a retrospective assessment of acute poisoning cases of unprofessional exogenous chemical etiology in the Republic of Moldova in 2011-2015. Their role in the structure of the mortality rate associated to acute poisoning of unprofessional exogenous chemical etiology has been determined. The evaluation of the poisonings indicated in the reference period has shown an essential increase and has established the top poisonings as follows: alcohol poisoning on the first place, medicine poisoning - second and gas poisoning on the third place. The pesticide poisoning ranks fourth. According to age and gender characteristics, the adult category (18 years and over) is the most affected, followed by males under 62 years. The females under 57 rank third and children between 3-18 years of age rank fourth. Chemical substances that are penetrating the human body through the digestive system cause the acute poisoning of unprofessional exogenous chemical etiology most frequently. As a rule, these poisonings take place in the human habitat when the family members use chemical substances, including suicide. Monitoring the acute poisoning of unprofessional exogenous chemical etiology represents one of the weak links in the public health surveillance system of the Republic of Moldova, which has an adverse impact on the prevention, diagnosis, reporting, treatment and rehabilitation of persons poisoned with chemical substances. Population and consumer awareness about the precaution measures in using a large number of chemical substances, many of which have not been subject to a complex toxic and hygiene expertise, especially in habitual conditions, is not sufficient. Hence, it is necessary to develop a regulatory framework on the safe use of chemical substances in the Republic of Moldova, harmonised with the European Directives and International Recommendations.

HIGH THROUGHPUT COMET ASSAY: APPLICATIONS IN GENOTOXICITY TESTING AND BIOMONITORING

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The comet assay for DNA damage is widely used in human biomonitoring and also in genotoxicity testing. A serious practical limitation on its usefulness has been the number of samples that can be run in one experiment; a typical electrophoresis tank can accommodate 20 slides with two gels on each. High throughput versions of the assay have been developed to overcome this; we now routinely run 12 mini-gels on one slide, or use a 12x8 array format on a GelBond film. As well as DNA strand breaks, we measure oxidised purines (using formamidopyrimidine DNA glycosylase to convert them to breaks). In biomonitoring studies, we often also measure resistance to H_2O_2 as an index of antioxidant status.

We have investigated the effects of a range of nanomaterials on cultured cells incubated for 3 or 24 h. Responses are varied, but in all cases damage - strand breaks, oxidised bases or both - is seen. In some cases, damage decreases during the 24 h incubation while in others damage is higher at the later time. Generally, but not always, the response is dose-dependent.

Results of the NewGeneris study of effects of environmental exposure and nutritional factors on mothers and newborns have shown correlations between maternal and cord blood in comet assay measures of DNA damage in peripheral blood mononuclear (PBMN) cells, as well as associations between DNA damage and other biomarkers.

The need to isolate peripheral blood mononuclear cells from blood samples adds to the logistical complexity of biomonitoring studies. A promising recent finding is that white blood cells can be isolated - in a fit state for comet analysis - from small samples of whole blood, simply frozen. In a pilot study, with blood samples stored at -80° for 11 months, there was a good correlation between frequencies of oxidised bases and of H_2O_2-induced breaks - both reflecting cellular antioxidant status.

Finally, the importance of including reference standards in each experiment, and the possibility of reducing inter-experimental variability, will be described.

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H2AX PHOSPHORYLATION ANALYSIS AS DNA DAMAGE BIOMARKER FOR HUMAN POPULATION STUDIES

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As response to the formation of double-strand breaks (DSB) – the most serious form of DNA damage in eukaryotic cells – H2AX flanking the DSB sites are rapidly phosphorylated to become γH2AX, and this process plays a central role in sensing and repairing DNA damage. The analysis of H2AX phosphorylation has a number of advantages that make this assay very suitable to be employed as biomarker of DNA damage in population studies, but there is an important lack of standardization in the methodological procedure that makes it difficult to establish this approach as a routine biomarker in population studies, and also hinders the comparison between studies.

The objective of this study was to address the most critical issues limiting the use of the γH2AX assay as DNA damage biomarker in human population studies. To this aim, differences in γH2AX levels between employing fresh or cryopreserved peripheral blood leukocytes (PBL), as well as the influence of stimulation prior to the analysis, were assessed by flow cytometry. Thereby, PBL were treated with 4 known genotoxic agents with well-characterized H2AX phosphorylation potential (bleomycin [BLM], camptothecin [Campt], actinomycin-D [Act-D], and methyl methanesulfonate [MMS]). All these 4 agents induce DSB by means of different mechanisms, direct or indirect; thus, they were chosen to provide evidence that γH2AX analysis detects DNA damage regardless of the DSB origin or experimental condition tested.

Results showed that either fresh or frozen stimulated cells can be employed to evaluate γH2AX levels, but there are important outcome-related differences to consider when the analysis is made on resting or proliferating cells. Moreover, the approach resulted not reliable when using frozen unstimulated lymphocytes. These findings confirm the use of flow cytometry analysis of γH2AX levels as a rapid screening tool for genotoxicity evaluation, as well as define the optimum protocol conditions, particularly referring to cell culture settings, to be properly employed as DNA damage biomarker in biomonitoring studies.

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OCCUPATIONAL RISK FACTORS AND PARKINSON'S DISEASE: PRELIMINARY RESULTS FROM A LARGE CASE-CONTROL STUDY.

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Parkinson's disease (PD) is the second most common neurodegenerative disorder in adults aged 60 and older. Etiology of PD is likely influenced by both environmental and genetic factors. The higher interest in environmental risk factors reflects the potential for disease prevention, although the evidence accumulated especially concerning occupation-related exposures played a role in driving recent etiologic research. We conducted a case-control study based on the outpatients treated at the IRCCS San Raffaele Pisana between Jan 2012 and October 2015. We identified 550 PD cases during the study period and 444 healthy controls. An extensive questionnaire was administered to study participants to investigate in detail lifelong occupational and job task histories. Major confounding factors, including diet, smoking habit, education, familiarity, were investigated as well. Univariate statistical analysis was performed with parametric and non parametric test, while multiple logistic regression analysis was used to estimate association with PD taking into account confounding variable and to search for interaction. Preliminary results showed, an higher frequency of males (OR=1.49; IC 95% 1.15-1.93), and an increase of ORs with age (2% per year (95%CI 1-4%). As concerns occupation, univariate positive associations were found for a few specific occupations, including medical doctor (OR=2.19), military personnel (1.75), pilots and cabin crew (2.43), agricultural workers (1.46). To provide a comprehensive vision of potential occupational or toxic risks associated to PD etiology, also leisure time activities were considered, and stratified according to potential risk exposures. A borderline significant association was found for painters (OR 4.2, 95% CI 0.9-20.5). After completing the evaluation and classification of diet, smoking habit, and familiarity as potential confounders a more defined evaluation of occupational risk factors will be provided. These preliminary findings provide interesting and original hints about the presence of association between the etiology of PD and selected occupations such as medical doctor, aviator and military personnel. The presence of several suspected exposures in all of these occupations, possibly related to neurodegeneration, calls for further epidemiological and experimental investigation.

DESIGNING NANOMATERIALS TOWARDS A SUSTAINABLE NANOTECHNOLOGY: AN ECOTOXICOLOGICAL APPROACH

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Within the rapid development and innovation of nanotechnology, it is necessary to ensure its sustainable development and the safe use of nanomaterials (NMs) being produced, commercialized and used in the society. In the European Union, the EU2020 strategy launched a new paradigm by requesting “Safe innovation for a competitive and sustainable future” and the European Industry is expected to adopt such clear recommendations. Likewise, the Action Plan on Sustainable Industrial Policy of the European Commission targets improving the overall environmental performance of products and encourages EU industry to innovate towards the leadership in this field. Also, in USA, the Pollution Prevention Act establishes a national policy to prevent or reduce pollution at its source whenever feasible. In fact, such change in values towards more safety, environmental friendliness and sustainability constitutes an open gateway to develop new products, highlighting as well the need for designing chemical products with the desired functionality while minimizing their toxicity and persistence in the environment, among others. Nanotechnology, being an emergent field constitutes an uncommon opportunity to use science and engineering to design novel products that are more ecological friendly, right from the beginning. Actually, the possibility of manipulating several characteristics of NM (e.g. size, shape, surface groups) to enhance their functionalities may also constitute a key opportunity for a rational design of safer NMs, thus meeting the previously mentioned EU strategies. Following this rational, the use of ecotoxicological approaches to support the production of safe-by-design NM and promote the development of a sustainable nanotechnology will be discussed and illustrated with some research examples.

MIXTURES TOXICOLOGY: MYCOTOXINS IN FOOD AS A CASE STUDY

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Historically, the potential health impact associated to human exposure to chemical stressors has been assessed on the basis of single-chemical and single-exposure pathway scenarios. In recent years, the acknowledged co-occurrence of multiple chemical contaminants in food has suggested, however, a change in the paradigm of the hazard identification and characterization, towards understanding the potential combined effects of chemicals.

The most well established and conservative model to predict the combined toxicity of chemicals is based on the concept of additivity, advocating that the joint effect of multiple chemicals is the summation of individual effects. Additivity incorporates the concentration addition (CA) model, assuming the same Mode of Action (MoA) and the independent action (IA) model assuming a dissimilar MoA of chemicals in the mixture. Deviations from these models, i.e., interactive effects include synergism, antagonism and more subtle interactions that depend on the actual doses of the mixture components.

To illustrate the challenges faced when addressing mixtures toxicology, my presentation will be focused on the combined toxic effects of mycotoxins with the potential to co-occur in food. Even though the available data concerning the joint effects of mycotoxins using *in vitro* and *in vivo* models and several endpoints (cytotoxicity, immunotoxicity and genotoxicity) have been steadily increasing, a considerable degree of inconsistency is noted among studies focused on similar mixtures. Our recent studies have been directed to the combined cytotoxic and genotoxic effects of binary mixtures involving ochratoxin A (OTA) and aflatoxin M_1, fumonisin B_1 or patulin, in human cell lines representative of relevant target organs. Data will be presented that point to the possibility of interactions between OTA and the other mycotoxins under study, especially in terms of cytotoxicity and genotoxicity. The added value of applying mathematical models to uncover interactive effects will be highlighted.

The interactive effects identified through *in vitro* bioassays deserve to be further explored at the mechanistic level, in order to identify the key biochemical, cellular or molecular pathways. For this purpose, a toxicogenomic approach can provide useful information about genes expression, proteins or biochemical pathways within a reasonable timeframe from which mechanisms of toxicity can be established.

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MUTAGENIC AND CARCINOGENIC POTENTIAL OF METAL NANOPARTICLES DEPENDS ON THEIR PHYSICOCHEMICAL PROPERTIES

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Nanomaterials (NMs) and nanoparticles (NPs) have been studied intensively for almost two decades and still all the characteristics that might be beneficial or harmful are not explored. Although many studies have investigated the mechanisms of action of NPs in cells, results are not fully understood and in many cases are controversial. Endpoints appropriate for detecting harmful chemicals such as cytotoxicity, oxidative stress, inflammation, immunotoxicity, genotoxicity, and in some cases carcinogenicity, have been addressed. However, there might be also new mechanisms that might lead to NM-induced toxic effects.

We investigated silver (Ag), shaped in 12 different AgNMs: elongated nanorods and 11 AgNMs of spherical shape (15, 20, 50, 80 and 200 nm) [1] with different charge (positive, neutral and negative) and different surface coating [2]. We compared whether the toxicity of AgNMs is shape or size-dependent, by comparing effects of particles with the same chemical composition, charge and coating but with different, well-defined sizes and shapes. Additionally, we studied the effect of charge and surface composition on toxicity, using AgNMs of the same size, shape and specific surface area. AgNM uptake and subcellular localisation and aggregation in protein-rich media were also investigated. Uptake and localisation (by TEM), cytotoxic effects and DNA damage (strand breaks and oxidised DNA lesions by the comet assay) were assessed in A549 cells, and the mutagenic potential of AgNMs (*HPRT* in V79-4 and *Tk* locus in mouse lymphoma L5178Y $Tk^{+/-}$ cells), and cell transforming potential in Bhas 42 cells were also assessed. All AgNMs were cytotoxic and induced DNA damage via a mechanism involving ROS formation. The DNA lesions were transient, likely due to DNA repair. Regarding surface properties, positively charged AgENMs had greater impact on cyto- and genotoxicity than did Ag ENMs with neutral or negative charge. This is likely to be related to their presence in the nucleus, implying a direct interaction with DNA owing to their positive charge. Elongated Ag nanorods with positive charge showed carcinogenic potential. Our results show that NMs of the same chemical composition are not equally harmful and their possible adverse effect and impact on cells depends on their size, shape, charge and surface chemistry.

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ANALYSIS OF CELLULAR DAMAGE INDUCED BY SILICA-COATED IRON OXIDE NANOPARTICLES ON NEURONAL CELLS

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Due to their unique physicochemical features, iron oxide nanoparticles (ION) hold immense potential in a vast variety of applications, particularly in biomedicine and biotechnology. Among these, the utilization of ION as contrast agents in magnetic resonance imaging, as carriers for drug delivery or transfection, and as therapeutic agents in cancer therapy by magnetic field-mediated hyperthermia are the most prevalent. They are claimed as generally biocompatible materials, but they have been reported to induce undesirable hazardous effects in various *in vitro* and *in vivo* studies, although results are not conclusive yet. Nanomaterials can reach the nervous system by crossing the blood-brain barrier and, moreover, ION are intended to be used in the brain in some of their applications, but little is known about their neurotoxicity so far, especially in human neuronal cell systems.

Thus, the objective of this work was to evaluate toxicity induced by silica-coated ION on a human neuronal cell line (SHSY5Y). After physicochemical characterization of the nanoparticles and assessment of their cellular uptake, a set of assays was applied to analyze different aspects related to cytotoxicity and genotoxicity, using a range of concentrations and different exposure times.

Results obtained indicated that, despite being effectively taken up by the neuronal cells, ION induce in general low cytotoxicity; positive results were only observed at the highest concentrations and longest exposure time. Genotoxicity evaluations showed a dose and time-dependent increase in DNA damage not related to the production of double strand breaks or chromosome loss. Further investigations are necessary to figure out the specific mechanisms underlying DNA damage induced by these ION.

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CARDIOVASCULAR EFFECTS IN ANIMALS AFTER EXPOSURE TO PARTICLES

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Exposure to particulate matter (PM) from traffic vehicles is hazardous to the vascular system, leading to clinical manifestations and mortality due to ischemic heart diseases. Nanomaterials may be associated with the same outcomes as suggested in a systematic review of the literature, which has shown that exposure to PM from ambient air, diesel exhaust and certain nanomaterials in animals have similar effects in terms of atherosclerosis and vasomotor dysfunction in arteries [1]. Airway exposure to particles is associated with augmented vasoconstriction and blunted vasorelaxation responses in various types of arteries. However, exposure to particles from indoor sources has not been assessed in atherosclerosis-prone animals. Recent studies from our group have indicated that pulmonary exposure, once a week for 5 consecutive weeks by intratracheal instillation, to particles from burning candles was associated with a modest increase in atherosclerosis in Apolipoprotein E knockout (ApoE-KO) mice on a Western-type diet. Pulmonary exposure to multi-walled carbon nanotubes, once a week for 10 consecutive weeks by intratracheal instillation, was associated with increased level of oxidatively damaged DNA in the lungs (measured by the comet assay) and increased thickness of the aortic wall, whereas there was no effect in terms of aortic atherosclerosis in ApoE-KO mice on a Western-type diet. In addition, pulmonary exposure to nanosized carbon black did not affect progression of atherosclerosis in aorta and brachiocephalic artery in ApoE-KO mice on regular diet, although the exposure caused pulmonary inflammation and serum from exposed mice contained factors that caused constriction of blood vessel segments ex vivo. As vasoconstriction is linked to increased peripheral resistance, the latter finding suggests a mechanistic link between exposure to particle and hypertension.

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INDOOR AIR QUALITY: MANAGEMENT CHALLENGES AND ASSESSMENT TOOLS

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People can be exposed to pollutants through inhalation, ingestion and dermal contact, but for the majority of air pollutants, breathing is the most common pathway to exposure. A given indoor space contains a wide variety of substances which may interact among themselves originating new species or have additive and synergistic effects [1]. This type of interaction, added to the diversity of indoor microenvironments to which European population is routinely exposed, makes difficult the establishment of the causal relationships between exposure to air indoors and health effects.

EC's R&D initiatives on indoor air quality (IAQ) started 25 years with an IAQ Audit of 54 office buildings in 9 EU countries [2]. Since then, attention has been paid on the understanding, methodologies and tools to reliably assess exposure to indoor air pollutants and its impact on health. In 2008, the EnVIE project [3] came out with the source control strategy to tackle the emissions. That approach, already adopted by some Member States, triggered the initiative to start a harmonization procedure within the EU.

Yet the actual IAQ status in the field still requires to be assessed. The PILOT INDOOR AIR MONIT project [4] developed a framework of harmonized criteria, protocols and monitoring techniques for five different identified IAQ objectives: 1) guideline compliance; 2) health and comfort complaints; 3) remediation efficiency; 4) source attribution of indoor air pollution and 5) population exposure survey. Each of these IAQ objectives has its own specific scope, targets and associated operational practices. More recently, AIRLOG project [5] went further by aiming at to operationalize the INDOOR AIR MONIT methods. AIRLOG introduced a sixth objective by exploring the potential of anticipating the IAQ status from the building design phase. That implies some strategic inputs regarding the ventilation levels, matter that was more recently tackled by the HEALTHVENT project [6] which couples source control with health-based ventilation requirements.

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OCCUPATIONAL EXPOSURE TO PROCESS GENERATED NANOPARTICLES: THE CASE OF THE CERAMIC INDUSTRY

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Process-generated nanoparticles are defined as those unintentionally emitted into workplace air during high-energy industrial process (mainly thermal or mechanical) [1]. They may be primary or secondary in nature and, in contrast to engineered nanoparticles, they are characterized by their non-specific and highly variable chemical composition and morphology. Industrial ceramic processes have the potential for occupational exposure to this type of health hazardous airborne particles [2].

This work reviews occupational exposure to process-generated nanoparticles in industrial settings during two types of processes frequently used in the ceramic industry: tile sintering, including an innovative process based on laser irradiation of ceramic materials, and tile ablation to produce engravings. The high-energy nature of both laser processes implies a significant potential for unintentional nanoparticle release, which have so far never been assessed under real-world conditions. The work was carried out in two pilot plants: laboratory and industrial scale. This allowed us to assess nanoparticle emissions and exposure during two stages of the up-scaling process.

The aim was to characterise nanoparticle release and its impact on exposure during tile sintering and ablation in a ceramic furnace. Special attention was paid to new particle formation processes and their dependence on process variables such as temperature or tile chemical composition. Particle concentrations in the range 5 nm – 10 µm were monitored at the emission source, in the worker breathing zone and along the furnace's exhaust system. Offline techniques such as transmission electron microscopy (TEM) and Energy-Dispersive X-ray (EDX) spectroscopy were used. Additionally, major and trace elements were determined by inductively coupled plasma mass spectrometry (ICP-MS) and atomic emission spectroscopy (ICP-AES).

Results evidenced significantly high nanoparticle release and impact on exposure during tile sintering (with exposure concentrations of $>10^5$ cm^{-3}) and ablation ($>10^4$ cm^{-3}). Emission patterns during sintering were strongly linked to temperature and raw tile chemical composition, and seemed to be independent of the laser treatment. New particle formation events (nucleation) were detected during sintering, while nanoparticles were mainly primary during ablation. When transported towards the breathing zone, particles increased in diameter (from 20 nm to 38 nm). TEM images evidenced spherical ultrafine particles, originating from the tile melting processes. Our results evidence the need for risk assessments and strategies to minimize occupational exposures to nanoparticles in the ceramic industrial sector.

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EVALUATION OF DNA DAMAGE AND CHANGES IN OXIDATIVE STRESS PARAMETERS OF CERAMIC WORKERS

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Ceramic workers are known to be exposed to mixture of dust and chemicals especially silica. Crystalline silica (quartz) is a ubiquitous mineral dust found worldwide. Occupational exposure to free silica occurs in several large categories of industry, and may have serious risks in the development of silicosis and cancer. International Agency of Research on Cancer (IARC) classified crystalline silica (silica) as a Group I human Carcinogen (IARC 1997). On the other hand, the possible advers effects of silica exposure in ceramic workers in Turkey have not been examined in detail. In this presantation, the genotoxic changes as assessed by comet assays and changes in oxidative stress parameters such as glutathione, glutathione peroxidase, glutathione reductase, malondialdehyde and superoxide dismutase levels in ceramic workers will be given. The effects of age, smoking, alcohol and protective equipment usage on these parameters will also be discussed.

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A SYSTEMATIC STUDY OF THE DISPOSITION AND METABOLISM OF MERCURY SPECIES IN MICE AFTER EXPOSURE TO LOW LEVELS OF THIMEROSAL (ETHYLMERCURY)

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Thimerosal (TM) is an ethylmercury (etHg)-containing preservative used in some vaccines despite very limited knowledge on the kinetics and direct interaction/effects in mammals' tissues after exposure. Thus, this study aimed to evaluate the kinetics of Hg species in mice in a time course analysis after intramuscular injection of TM, by estimating Hg half-lives in blood and tissues. Also, experiments were made in order to evaluate the conversion of TM and etHg *in vitro* in whole blood, plasma and erythrocytes. The project was approved by the Ethics Committee on Animal Use of USP before its initiation (Opinion number 12.1.1158.53.1). Male Swiss mice weighing approximately 25 g/6th week of life were exposed to one single intramuscular dose of 20 µg of Hg as TM. Blood, brain, heart, kidney and liver were collected at 0.5 hour (h), 1 h, 8 h, 16 h, 144 h, 720 h and 1980 h after TM exposure (n=4). For the *in vitro* study, human blood samples were collected from the cubital median vein from a healthy volunteer. This experiment was divided into three steps: I) Aliquots of whole blood, plasma and erythrocytes, kept in a water bath at 37 °C, were added of TM or etHg solutions (final Hg concentration of 3 mg/l). Twenty four hours after this incubation, samples were analyzed for Hg species determination (n=4), II) Aliquots of whole blood, plasma and erythrocytes, kept in a water bath at 37 °C, were added of solutions of etHg and iron (Fe) chloride ($FeCl_3$) (final concentrations of 3 mg/l of Hg and 400 mg/dl of Fe). Twenty four hours after this incubation, samples were analyzed for Hg species determination (n=4), III) Aliquots of plasma, kept in a water bath at 37 °C, were added of solutions of etHg, H_2O_2 and DMSO (final concentration: (tube a) etHg (3 mg/l); (tube b) etHg (3 mg/l) plus H_2O_2 (10 mM); (tube c) etHg (3 mg/l) plus H_2O_2 (10 mM) plus DMSO (100 mM). Then, 24 h later, Hg species were determined (n=4). Hg species in samples were identified and quantified by speciation analysis via liquid chromatography hyphenated with inductively coupled mass spectrometry (LC–ICP-MS). It was found that the transport of etHg from muscle to tissues and its conversion to inorganic Hg (inoHg) occur rapidly *in vivo*. The conversion extent is modulated in part by the partitioning between etHg in plasma and in whole blood, since etHg is rapidly converted in red cells but not in a plasma compartment. Furthermore, the dealkylation mechanism in red blood cells appears to be mediated by the Fenton reaction (hydroxyl radical formation). Interestingly, after 0.5 h of TM exposure, the highest levels of both etHg and inoHg were found in kidneys (accounting for more than 70% of the total Hg in the animal body), whereas the brain contributed least to the Hg body burden (accounted for <1.0% of total body Hg). Thirty days after TM exposure, most Hg had been excreted while the liver presented the majority of the remaining Hg. Estimated half-lives (in days) were 8.8 for blood, 10.7 for brain, 7.8 for heart, 7.7 for liver and 45.2 for kidney. Taken together, our findings demonstrated that TM (etHg) kinetics more closely approximates Hg^{2+} than methylmercury (meHg) while the kidney must be considered a potential target for etHg toxicity.

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ORAL PRESENTATIONS

GENOTOXICITY ASSESSMENT OF LOW DOSE EXPOSURE TO GLYPHOSATE IN HEPG2 CELL LINE AFTER 4 AND 24 HOURS

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The huge expansion of genetically modified plants designed to tolerate high levels of glyphosate has made it the world's most widely used herbicide. Nevertheless, there are controversies about its use; the WHO and IARC have been unable to reach an agreement with the EFSA regarding its further use and distribution ever since the former proclaimed glyphosate a probably carcinogenic to humans in March 2015.

The aim of this study was to evaluate the genotoxic effects of the glyphosate active compound (Sigma Aldrich) on human liver cell line HepG2 after 4 and 24 hour *in vitro* exposures to low concentrations chosen as representative of everyday exposure to glyphosate and calculated by approximations and extrapolations based on the no observed adverse effect level (NOEL).

The calculation to *in vitro* conditions was based on an average male human with 65 kg of body weight and a total volume of extracellular liquids, simulating the submersion of cultured cells in culture medium with these exact glyphosate concentrations: acceptable daily intake (ADI:0.3mg/kg corresponds to 0.5µg/ml in HepG2 cell line treatment), residential exposure level (REL:1.75 mg/kg corresponds to 2.91 µg/ml) and occupational exposure limit (OEL:2.1 mg/kg corresponds to 3.5 µg/ml). The HepG2 cell model system (EMEM medium with 10% foetal bovine serum and antibiotics) covers a wide spectrum of enzymes represented in metabolism phases I and II and represents cells of the liver, a major detoxification organ.

The alkaline comet assay was chosen for genotoxicity assessment with its two parameters: tail length (TL) and tail intensity (TI). Experiments were done in duplicate with 100 nucleoids counted for each concentration and time period. Positive controls were 50µM H_20_2 and 28µg/ml cyclophosphamide.

After 4h, the TL of the control had higher values (only EMEM medium, 17.20±4.29 (mean±SD)) with significant differences from ADI (14.97±1.91) and REL (14.23±1.23). For TI values, all of the concentrations were significantly lower than the control (1.45±2.43): ADI (0.16±0.27), OEL (0.16±0.26) and REL (0.20±0.33). After 24h, values for ADI, OEL and REL did not significantly differ from the control for both TL (13.91±1.76; 15.35±1.95; 13.97±2.08 *vs.* 14.91±2.41) and TI (0.06±0.10; 0.05±0.09; 0.11±0.16 *vs.* 0.06±0.10).

Since commercial compounds usually produce greater DNA damage than observed in this study, which is due to the mixture of different compounds that also produce damage, the use of only the active compound form of pesticide could have been a limiting factor in the DNA damage assessment. The results following 4h exposure indicate a possibility of DNA crosslinking that diminishes the amount of the observed DNA damage. Further studies should be performed with commercial glyphosate products as well as in order to examine whether there is a real crosslinking effect.

THE ANTIMALARIAL DRUG LUMEFANTRINE INTERACTS WITH HUMAN TOPOISOMERASE II BETA COMPLEXED TO DNA BY *IN SILICO* ANALYSIS

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Lumefantrine (LF) is used in artemisinin-based combination therapies against malaria worldwide. It is genotoxic and mutagenic to human lymphocytes *in vitro* and may interact non-covalently with DNA minor groove surface [1]. Considering that DNA binders are often topoisomerase inhibitors, in this study we investigated the potential noncovalent interaction of LF with human topoisomerase II beta (hTOP2β) complexed to DNA by molecular docking study.

Computer-assisted molecular analyses have been performed for predicting the possible interactions between hTOP2β-DNA complex and LF [2]. The hTOP2β-DNA complex bound to LF was then assessed for interactions, energetic contributions, and for identification of the best correlation between the LF conformations and their associated scores.

The fused-tricyclic 9*H*-fluorene rings in the LF chemical structure promote the intercalative binding into cleaved DNA sites present in hTOP2β-DNA complex. Since this is a polycyclic aromatic moiety it gives the LF molecule the necessary planarity and aromaticity for intercalative binding to DNA base pairs in the cleavage sites, which showed aromatic interactions of -8.6 kcal/mol in the binding computational analysis for predicted binding affinity energy. The *N*-dibutyl moiety and hydroxyl group from LF accommodate into the major groove and hydrogen bond to nitrogen and oxygen atoms on the base-pair in the DNA segment. The *N*-dibutyl moiety also interacts with residues on the major groove side. The (4-chlorophenyl) methylidene moiety protrudes into the DNA minor groove side facing nearby residues from this protein–DNA interface. As a continuation of this study, we intended to use a V79 in vitro micronucleus test assay to verify this hypothetical interaction of LF with topoisomerase II.

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A CHILD'S SPIT EPIGENOME CAN REVEAL ITS RESPIRATORY ALLERGY RISK

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Environmental exposures like parental lifestyle and diet, smoking, obesity and exposure to toxin can modulate disease risk. Epigenetic DNA methylation changes can be part of the underlying molecular mechanisms. Early life exposures can alter DNA methylation patterns, and thereby predispose the child to develop respiratory allergy (RA) later in life. Longitudinal birth cohorts are instrumental to study disease development, but DNA biomarker research is hampered because blood sampling is kept to a minimum for practical and ethical reasons. Saliva is a non-invasive and convenient source of DNA that can be used for biomarker research.

In this study, we aimed at discovery and confirmation of differential methylation regions (DMR) in saliva of children with RA when comparing to controls.

Saliva samples collected in two independent longitudinal birth cohorts in Belgium were analyzed using Illumina Methylation 450K BeadChips. A statistical analysis pipeline was developed in R to identify genome-wide differential methylation. The Illumina Methylation 450K BeadChips revealed 13 DMR in saliva from 11y old allergic children (N=26) vs. controls (N=20). 5 DMR were located in genes involved in IL4 signalling and Th2-response, showing a link with wheezing and other RA phenotypes. The 13 DMR were selected for further biological and technical validation by iPLEX MassArray analysis in the same birth cohort as well as in a second cohort (N=78).

This project is providing novel insights in the molecular mechanisms that may predispose children to RA development. We are among the first to show the utility of saliva to identify DNA methylation marks in children that are relevant for RA.

STUDYING ESTROGENIC AND ANDROGENIC INFLUENCES ON EFFECTS OF ANTICANCER DRUGS USING THE HEPATOCARCINOMA CELL LINE HEPG2

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Hepatocellular carcinoma (HCC) is the fourth and ultimate stage of liver disease after earlier hepatitis, fibrosis, and cirrhosis; actually, it is estimated that 80% of patients with HCC have underlying cirrhosis [1]. Additionally, HCC is the fifth most commonly diagnosed cancer and the second cause of death among other types of cancer [2]. Previous studies reported that gender disparity might play a crucial role in preference of development of HCC, with males having a higher risk [3]. Earlier studies reported that estrogens may act as hepatoprotective by multiple ways, such as inhibitory effects on inflammatory processes and significant promotion of antioxidant enzymes, beside down-regulation of IL-6, which is critical for hepatic lesions [4]. Some estrogenic compounds have a significant role in reversing doxorubicin resistance in human breast cancer [5]. Considering the gender disparity on HCC development and outcome, our aim is to study the potential modeling effects of estrogenic and androgenic compounds, such as 17α-ethinylestradiol (EE2), tributyltin (TBT), and other, alone or in combination with the chemotherapeutic agents, like doxorubicin (DOX) and cisplatin (CISP), using the malignant cell line HepG2 as experimental model. Using incubation of 48 h and a range of concentrations of the tested compounds, we start getting the first results. We noticed that EE2 significantly decreased cell viability (at every of four tested concentrations, from 0.01 µM to 10 µM), but under the assayed conditions the estrogen did not potentiate the cytotoxic effect of Dox. As to TBT, it seemed to have potentiated the cytotoxic effect of both DOX and CISP, in opposition to EE2. Data are being expanded and refined mechanistically, to unveil insights about the influences of estrogenic and androgenic signaling/effects in the development and therapeutics of HCC.

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ANALYSIS OF 23 SNPs AND ASSOCIATION OF THE *UGT2B7, UGT1A9, ABCG2* AND *IL-23R* POLYMORPHISMS WITH REJECTION RISK IN KIDNEY TRANSPLANT PATIENTS

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Despite major advances in new testing compatibility between donor and recipient and the improvements in surgical techniques and immunosuppressive drugs, the rejection process still is a current concern. Single nucleotide polymorphisms (SNPs) that codify altered enzymes of metabolism, drug transport and immune system can contribute to trigger a rejection process in kidney transplant patients. We evaluated the association between SNPs in genes of these processes in 246 kidney transplant patients, of whom 86 (35%) were diagnosed with kidney allograft rejection. The study was approved by Human Research Ethics Committee of the State University of Londrina, Brazil. The DNA was extracted from blood and the genotyping of 23 SNPs on the following genes *CYP3A4*, *CYP3A5*, *CYP2E1*, *POR*, *UGT2B7*, *UGT1A9*, *ABCB1*, *ABCC2*, *ABCG2*, *SLCO1B1*, *TNF-α*, *IL-2*, *IRF-5*, *TGF-β*, *NFKBIA*, IL-10, IL-23R, NFAT and *CCR5* was performed by RT-qPCR using TaqMan probes. The association between rejection episodes and the polymorphic variants was assessed by Odds Ratio (OR) with confidence interval of 95% using SPSS®20 Statistics software. The genes *UGT2B7* and *UGT1A9*, responsible by converts the mycophenolic acid (MPA), the active metabolite of mycophenolate mofetil, to its inactive forms, were associated, respectively, with protection (rs7662029 - OR = 0.54 (0.3-1.0) *p* = 0.05) and risk (rs6714486 - OR = 1.6 (1.0-2.5) p = 0.04) to episodes of rejection. The polymorphic allele in the gene *UGT1A9* was associated with the increased level of hepatic protein, increasing the inactivation of MPA and facilitating the occurrence of episodes of rejection. Among the polymorphisms of drug transporter gene assessed, rs2231142 (*ABCG2*) was associated with decreasing of the risk of rejection episodes (OR = 0.52 (0.28-0.96) p = 0.037), being this the first study to show this association. This probably is due to decreased expression of the transporter, resulting in an increase in the concentration of mycophenolic acid glucuronide, that can be activated and participate of the circulation burial-hepatica, increasing the concentration of MPA. The polymorphism rs10889677 (*IL-23R*) was associated with increased risk of rejection (OR = 1.9 (1.3-2.7) p = 0.00). This SNP disrupts the binding site of the microRNA let-7f, increasing the transcription, the amount of receptors and facilitate activation of cytokines, resulting in an increase of inflammation and increased risk of rejection. Therefore, SNPs in these genes can be used as candidate markers for screening of risk of rejection in renal transplant patients and in the future may help in medical management, administrating smaller doses of drugs to patients who have lower risk of rejection, avoiding adverse effects.

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INDIVIDUAL SUSCEPTIBILITY TO THE TOXIC EFFECTS OF RADIATION THERAPY: A POTENTIAL ROLE FOR DNA REPAIR SNPs?

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Cancer radiotherapy takes advantage of the ability of ionizing radiation (IR) to induce DNA damage hence killing tumor cells. Non-tumor cells are also affected, giving rise to radiation-related toxicity. Single nucleotide polymorphisms (SNPs), through interference with the DNA repair efficiency in normal cells, may alter the safety of radiotherapy. As such, DNA repair SNPs could ultimately modulate susceptibility to the toxicity of such therapy. We aimed to systematically review the impact of DNA repair SNPs on the occurrence of toxic effects in cancer patients submitted to radiotherapy, so that genetic biomarkers potentially relevant for the personalization of cancer management may be identified. The PUBMED database was searched using the following MeSH terms: neoplasms AND Polymorphism, Single Nucleotide AND DNA repair AND radiotherapy. 104 articles, published up to March 1, 2016, were retrieved. On applying predefined inclusion/exclusion criteria through manual curation, 63 articles were excluded. 41 articles were thus eligible for further data extraction and systematic review. A high number of significant associations were observed, between SNPs across the most relevant DNA repair pathways – direct damage reversal, base excision repair, nucleotide excision repair, mismatch repair, homologous recombination, non-homologous end-joining – and multiple toxicity (e.g. IR-related pneumonitis, oral mucositis, skin reaction) endpoints. *APEX1* rs1130409 and *ATM* rs189037 have been found to modulate the risk of IR pneumonitis (risk increase for *APEX1* rs1130409 variant allele carriers [1, 2], risk decrease for *ATM* rs189037 variant allele carriers [3,4]), both through 2 independent studies. An association between the *XRCC1* rs25487 variant allele and an IR-related toxicity risk increase is also frequently suggested, but conflicting results exist. DNA repair SNPs across different DNA repair pathways appear to modulate the individual susceptibility to the toxic effects of radiotherapy. Evidence is stronger for the *APEX1* rs1130409 and *ATM* rs189037. Integration of these toxicogenetic biomarkers into the clinical decision process may, in a near future, allow for the optimization of the therapeutic approach to several types of cancer, maximizing the benefit while minimizing the risk. Such information may also be of relevance in an occupational setting since such DNA repair SNPs may also modulate the long-term risk of IR-induced health problems in workers regularly exposed to low doses of such agent.

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FORGOTTEN PUBLIC HEALTH IMPACTS FROM CANCER

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Cancer is a major public health problem, with more than 200 different types. Is one of the most concerning diseases in developed countries and quite a lot of resources are for develop research to discover an effective treatment and cure. Despite the chemotherapeutic treatments have been improved, cancer is still one of the most harmful diseases worldwide.

The global burden of cancer embraces the financial cost implied, divided in direct costs, such as, expenditures for treatment, and indirect costs, which includes the loss of economic output due to missed work (morbidity costs) and premature death (mortality costs). Hidden costs of cancer comprise, for instance, health insurance premiums and nonmedical expenses.

The present review intends to demonstrate that besides the economic impact of cancer there are commonly public health impacts frequently forgotten. For example, the higher number of workers and family exposed to antineoplastic agents, proved to be mutagens, carcinogens and teratogens, due to the increase in the number of patients to whom it is administered chemotherapeutic treatments. Also related to this, the impact on environment due to the patients excreta at their homes and hospital waste waters still needing a detail assessment and define correct solutions. Additionally, the need of health services reorganization and the impact of this in the services costs and quality is also a concern that needs to be fully assessed. On the patient's perspective it is also important to highlight the increased number of second cancers derived from treatments, which has also a strong impact on previously addressed issues.

This overview will contribute to demonstrate that the investment must be done in the prevention because only in that way the risks for workers and population can be reduced and costs can be truly minimized.

ASSESSMENT OF ENVIRONMENTAL HAZARDS CAUSED BY RELEASE OF SELECTED CYTOSTATICS

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Cytotoxic drugs are used worldwide for the treatment of cancer and are among the most toxic chemicals which are produced. The therapeutic principle of most of these chemicals is based on induction of DNA damage. Between 2011 and 2014 a coordinated EU project (CYTOTHREAT) was realized which concerned the assessment of the environmental risks caused by release of the most widely used drugs, namely 5-fluorouracil (FU), cisplatin (CDDP), etoposide (ET) and imatinib mesylate (IM). The acute toxic and genotoxic as well as reproductive properties of these compounds were investigated in a variety of eucaryotic and procaryotic organisms and indicator cells (i.e. fish, molluscs, crustaceae, algae, higher plants, bacteria and in a panel of human/ mammalian cell lines). The results were used to estimate the consequences of the release of these drugs in the environment by use of a model developed by the European Medical Agency (EMEA) which is based on the determination of the ratios between predicted environmental concentrations (PEC) and predicted non effective environmental concentrations (PNEC) (values > 1 indicate possible risks). On the basis of the use of the NOEC data obtained with the most sensitive species (in long term experiments) we came to conclusion that no elevated risks are likely to occur in the aquatic environment. However, in regard to induction of genetic damage it can be not excluded that adverse effects are caused by FU, CDDP and IM.

Further experimental work addressed the question if synergistic and antagonistic effects occur in mixtures of cytostatics. Indeed such effects were detected; the most relevant finding beeing the observation of synergistic effects of IM in combination with other compounds which was seen in higher plants (MN assay) and also in other test systems.

Overall our findings underline the importance of removal of the cytostatics by adequate sewage treatment strategies in regard to prevention of genetic damage, which may lead to destabilization of ecosystems.

MERCURY-RESISTANT BACTERIA AND MERCURY CYCLING IN SEDIMENTS OF THE TAGUS ESTUARY

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Mercury is a pervasive pollutant well known to cause several disorders in humans and wildlife. The major concern related with mercury pollution is the neurotoxicity associated to methylmercury and its presence in aquatic systems, as it undergoes bioaccumulation and biomagnification in the food chain. In aquatic ecosystems, mercury-resistant microorganisms are the main responsible for methylation of Hg^{2+} and also for processes of detoxification (reduction of Hg^{2+} and demethylation of methylmercury). High levels of mercury, including methylmercury, have been shown to exist in the Tagus Estuary.

This study aims to give an insight about the involvement of bacteria in the cycle of mercury in the Tagus Estuary. To achieve this, mercury-resistant microorganisms were isolated from sediments of four mercury-polluted areas of the Tagus Estuary (Barreiro, Cala do Norte, Rosário and Alcochete) and, after their characterization their potential to transform mercury compounds was evaluated.

To evaluate the involvement of bacteria in the processes of methylation and detoxification of mercury in the Tagus Estuary, isolated bacteria were incubated with $HgCl_2$. The results showed that these microorganisms are able to reduce Hg^{2+} into Hg^0, resulting in the removal of around 50% of the total added mercury. The highest removal rates were observed among isolates of high contaminated areas (Barreiro and Cala do Norte). It was also observed the formation of organomercurials, including methylmercury. The rate of methylation among the isolates ranged between 1-8%. To understand better the conditions promoting methylation and demethylation, three microbial communities (aerobic, anaerobic and sulphate-reducing bacteria communities) were incubated with isotope enriched mercury species ($^{199}HgCl$ and $CH_3{}^{201}HgCl$). The results showed that microbial communities are actively involved in methylation and demethylation processes, being the methylation directly related with sulphate-reducing bacteria communities with rates up to 0.07% (after 48h), while the demethylation process is strongly promoted (rates up to 100%) by aerobic community.

Overall, the results showed that bacteria of the Tagus Estuary are involved in processes that change mercury speciation through reduction and demethylation and formation of methylmercury. The removal is a pathway for detoxification and can be used on the bioremediation strategies. Meanwhile, the formation of methylmercury represents a risk for human health. Thus, this set of data is useful for both risk assessment and bioremediation purposes.

SPATIAL AND TEMPORAL PATTERNS OF ECOLOGICAL RISK INDUCED BY PESTICIDES IN ALQUEVA RESERVOIR: A CASE-STUDY

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The purpose of this study was to evaluate the potential impact of pesticides detected in the Alqueva reservoir (Guadiana Basin, South Iberian Peninsula) on the aquatic organisms belonging to this ecosystem. For this purpose, the occurrence and risk assessment of 25 pesticides, and of a number of their degradation products, were determined in the Alqueva surface waters. The target pesticides, which belonged to the classes of phenylureas, triazines, chloroacetanilides and organophosphorous, were analysed by isotope dilution on-line solid phase extraction-liquid chromatography-tandem mass spectrometry. The aquatic risk assessment, which was based on the risk quotient method (RQ=MEC/PNEC; MEC: measured environmental concentrations; PNEC: predicted no-effect concentration) considered three trophic levels: algae, aquatic invertebrates and fish. The areas (sampling stations) most polluted by pesticides were Sra. Ajuda and Lucefecit, in the northern, and Álamos, in the middle portion of the reservoir. The aquatic risk assessment revealed that, from the various compounds analyzed, terbuthylazine, chlorfenvinphos and diazinon presented non-acceptable risk. With the exception of terbuthylazine, that in two areas (Sra. Ajuda and Lucefecit) exhibited high risk (RQ > 1) under normal hydrological conditions, the high risk was only estimated in specific periods, with particularly high pesticide concentrations in the water column, that occurred after rainfall events during the period of pesticide application. The locations that had more samples with RQ > 1 were Sra. Ajuda followed by Lucefécit. The use of risk assessment allowed us to conclude that, despite that pesticides' concentrations in the water column fulfil the European environmental quality standards, some compounds show a high ecotoxicological risk for aquatic organisms in the Alqueva ecosystem. The results demonstrate that, to have an efficient risk management process, the regulatory authorities of each country must consider an integrative chemical and ecotoxicological approach.

USE OF MULTIPLE BIOMARKERS TO EVALUATE PLANT SPECIES SUITABILITY TO MANAGE CONTAMINATED AREAS

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The use of plants and associated microorganisms has been increasely consider as a sustainable and cost-effective option to manage contaminated areas. Phytoremediation techniques aims at using plant species to alleviate environmental and health risks induced by pollutants, and at restoring ecosystem services.

Suitable plant species must be tolerant to contaminants and reduce their transfer into the food chain. The selection of plant species or cultivars is mainly based on their ability to grow on contaminated soils, to stimulate organic pollutant degradation (rhizodegradation) and/or to reduce pollutants mobility (phytostabilisation) or increase pollutant uptake (phytoextraction). However, despite phytoremediation approaches are long-term techniques, only limited studies have focused on plant long-term ability to survive on contaminated areas.

In this study, we investigated sub-lethal effects of contaminated soil exposure on three plant species which have been described as good candidates for phytomanagement of contaminated areas: ryegrass (*Lolium perenne*), clover (*Trifolium repens*) and miscanthus (*Miscanthus x giganteus*). Plants were grown on soils contaminated with heavy metals by a former lead smelter. We analyzed several biomarkers (oxidative stress, lipid peroxidation, photosynthetic pigments, DNA degradation) to evaluate plant health. Our results clearly demonstrated different metal tolerances among these plants. Despite its potential to stabilize pollutants in soils, ryegrass plants exhibited high level of oxidative stress, lipid degradation and DNA stand breaks. These results challenge the suitability of this plant for a long-term management of contaminated soils. In the other hands, miscanthus plants showed little effects of metals, event at extremely high concentrations. This confirm this plant as good candidate for phytomanagement.

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BIOCHEMICAL AND BEHAVIOR ALTERATIONS OF ZEBRAFISH EARLY LIFE EXPOSED TO SYNTHETIC AND NATURAL DYES

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Eco-friendly dyes are increasingly desired by industries as alternatives to the replacement of azo dyes that are usually precursors of mutagenic compounds. Due to the European Commission concern about environmental health, the number of approved dyes for consumer has been reduced and toxicity tests to non-target organisms became a critical step to dye development. Early-life stages of zebrafish (*Danio rerio*) are important ecotoxicological models to development studies, and according to our previous results, several morphological alterations were induced in embryos by the azo dye Basic Red 51 (BR51), widely used by cosmetic industries, and also by the natural dye erythrostominone (ERY), extracted from microorganisms. In contrast to ERY, its photodegraded product (DERY) did not induce any changes in zebrafish early-life development.

Taking into account previous results, we aimed to investigate different biological levels responses of zebrafish early-life stages that might be induced by ERY, DERY and BR51, such as behavioral and biochemical alterations. Biochemical responses were measured after 96 h exposure using biomarkers of oxidative damage (lipid peroxidation), biotransformation (glutathione S-transferase - GST), energy metabolism (electron transport system), antioxidant system (catalase), and neurotransmission (acetylcholinesterase - AChE). Behavior was assessed by locomotor activity (total swimming time, distance and velocity) after 144 h exposure using ZebraBox tracking (ViewPoint Life Science, Lyon, France). All tests were performed under controlled temperature (26^{o}C±1) with dark incubation in order to avoid ERY and BR51 degradation or further DERY degradation.

Our results showed a significant decrease of locomotor activity concomitantly with low levels of AChE and GST in zebrafish early-life stages exposed to ERY and BR51. Interestingly, DERY did not affect any biomarker response nor locomotor parameter assessed, suggesting that degradation of ERY by light was sufficient to prevent its toxic effects. Therefore, BR51 and ERY are able to impair zebrafish early life, whereas DERY caused no damage to them. Finally, ERY has a potential applicability to industries as an eco-friendly dye considering its degradation into a non-toxic compound.

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FISH EXPOSURE TO SUB-LETHAL METALLIC-NANOPARTICLE MIXTURES: GENOTOXICITY AND HAEMATOLOGICAL PARAMETERS

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Toxicological studies regarding metallic-nanoparticle mixtures are of highest priority due to the continuous and simultaneous release of various contaminants to the environment, and the lack of information on the potential hazards of these mixtures, which might cause adverse effects to organisms. Particularly, there is a lack of information regarding the toxicity of mixtures within the legal effluent discharge thresholds for each contaminant, specifically in marine systems. This knowledge gap reinforces the importance of evaluating the effects resulting from the interaction of "classical" contaminants namely, cadmium- Cd, lead- Pb, mercury- Hg, with emergent contaminants such as silver- Ag and gold-Au nanoparticles (NPs), within their sub-lethal limits. To address this issue, a marine fish (turbot - *Scophthalmus maximus*) was exposed during 7 days to the maximum allowable concentrations for effluents discharges (MAC), and to the concentrations obtained after an efficient remediation process applied to the water (Treated). Both MAC and Treated conditions were tested in the presence and absence of equivalent concentrations of Ag and Au NPs. In order to evaluate MAC genotoxic potential and the effectiveness of the remediation process in decreasing the toxic potential of the mixture, the frequency of the Erythrocytic Nuclear Abnormalities (ENAs) and of immature erythrocytes (IE) were performed in peripheral erythrocytes of turbot. The alterations of the haematological parameters such as red blood cells count (RBC), haemoglobin (Hb), the mean concentration of haemoglobin (MCH) and the Erythrocytic maturity index (EMI) were also assessed. Ag, Au, Cd, Hg and Pb accumulation in blood and kidney was quantified to establish a causal relationship between toxicity and tissue burdens. Accumulation of Cd, Hg and Pb was observed both in blood and kidney and was higher in MAC with and without NPs than in control and Treated conditions. Thus the presence of NPs did not influence the accumulation, while the effectiveness of the remediation process was demonstrated. Pb was the element with the highest accumulation capability, namely in kidney. Regarding the metallic-nanoparticle mixture toxicity, sub-lethal concentrations (MAC) were not able to induce genotoxicity, as shown by the absence of ENAs and IEs. Still, the alteration of RBC and MCH indicated that sub-lethal concentrations affected the synthesis of haemoglobin and induced changes on erythropoiesis, which were avoided by the remediation process. These alterations may indicate impairment on the hemodynamic processes that rely on kidney. The effectiveness of the remediation process was also confirmed by the absence of genotoxicity and by the improvement of fish haematological parameters in Treated conditions.

OXIDATIVE EFFECTS OF PAH MIXTURES OVER THE THIOREDOXIN AND GLUTATHIONE SYSTEMS

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Polycyclic Aromatic Hydrocarbons (PAHs) are organic pollutants that invariably occur in the environment as mixtures. Their metabolism involves the addition of an electrophilic group to the molecule by CYP monoxygenases, yielding highly reactive intermediates (PAH metabolites) and generating reactive oxygen species (ROS) as by-products. These reaction products may cause DNA damage hence their recognised genotoxic, mutagenic and carcinogenic potential. Moreover, PAHs may mediate the induction of CYP enzymes through the aryl hydrocarbon receptor (AhR) pathway. Previous *in vivo* work showed PAH mixtures cause oxidative effects that surpass linear addition, by still unknown mechanisms [1-3].

The aim of the present work was to assess the oxidative effects caused by the exposure of mixtures of PAHs over the thioredoxin and glutathione systems. In *vitro assays* were conducted, exposing human hepatoma cells (HepG2) to individual and binary mixtures of two PAHs: phenanthrene (Phe) and benzo[b]fluoranthene (B[b]F). Cell viability was determined by the MTT assay. Enzyme activities of the thioredoxin system (thioredoxin reductase and thioredoxin) and the levels of gluthathione and related enzymes (glutathione peroxidase, reductase and S-transferases) were assessed. Enzyme expression was determined by Western Blot and ROS production was measured by the dichlorofluorescein assay. Phase I biomarkers, such as EROD, was also surveyed.

Results showed that Phe exposures yielded higher acute toxicity to the HepG2 cells, as shown by the MTT assay with a LC50 of 65 μM, lower than the verified for B[b]F (>100 μm). The higher toxicity of Phe was driven by a stronger ROS production. PAHs mixtures generated complex responses from both antioxidant systems, with glutathione depletion playing a key role in the unfolding of toxicity.

These results stress the importance of considering PAHs in the environment as complex mixtures, for they generate toxic effects with a magnitude that goes beyond the one that is predicted by simply looking at isolated compounds.

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MAPPING ENVIRONMENTAL HUMAN EXPOSURE INTEGRATING MULTIPOLUTANTS BASED ON BIOMONITORS – A METHODOLOGICAL APPROACH

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In industrial areas, many organic compounds are formed as combustion byproducts, such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F). Also released in these processes are metals (e.g. lead) and non-metals (e.g. arsenic), many of which potentially toxic and carcinogenic, even in low concentrations. Humans can be directly exposed by inhalation or contact and indirectly by contaminated soil and food ingestion. Atmosphere is a major pathway for transport and deposition of pollutants in the environment. However, assessing the spatial pattern of this deposition is not easy, due to its high spatial and temporal heterogeneity. Lichens have been extensively used as biomonitors of atmospheric deposition, because they allow more spatial detail than the few existing air monitoring stations and, being living organisms, are able to integrate longer periods of atmospheric deposition.

Based in the results of the lichens pollutant´s concentration in a large area, the main objectives of this work were twofold, a) to map the environmental and human exposure to several atmospheric pollutants (dioxins/furans and toxic chemical elements), and b) to develop a semi-quantitative index that would be derived from risk estimates for several pollutants and will use actual pollutant concentrations measured in the environment.

The accumulation of pollutants was measured during an eight-month period, using lichen transplants, in 25 sampling points with different uses including a diverse industrial area (i.e., chemical, combustion, etc.). The distribution of sampling points allowed the interpolation and mapping of pollutants within a regional area, in which the populations location and density was accessed. Thus, the use of the aforementioned index allows a spatial representation that does not result from the absolute accumulation of the different pollutants, but from the accumulation weighted by the relative risk of the different pollutants. Furthermore the relative risks are previously evaluated by the land use.

The assessment of environmental human exposure to multi-pollutants through atmospheric deposition can be used by industries to improve their mitigation processes and also by health stakeholders to target populations for epidemiological studies and health recommendations, considering not only the polluted areas but also the “clean” background areas.

ON THE TRACK OF THE *IN VIVO* TOXICOLOGICAL PROFILE OF SUPERPARAMAGNETIC COLLOIDAL IRON OXIDE NANOPARTICLES IN ZEBRAFISH EMBRYOS

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Phase-pure Fe_3O_4-based colloidal nanoparticles presenting an overoxidized outermost surface were successfully synthesized in large-scale by our research group, via controlled coprecipitation and hydrothermal synthesis [1]. With a mean size between ca. 13 and 20 nm, these superparamagnetic iron oxide colloids demonstrated high heating efficiency in magnetic hyperthermia [1], making them appealing for cancer therapy, exploiting the local heat generated in an external alternating magnetic field. Importantly, these superparamagnetic aqueous nanocolloids showed excellent protein immobilization capability [2]. A safety profile is a key feature for their validation as proficient nanoagents for biomedical applications. To boost the identification of the limits within which the magnetite-based nanocolloids can be applied safely, zebrafish zygotes were monitored via continuous waterborne exposure to different concentrations, for 80 hours post-fertilization. Mortality, developmental delay signals, phenotypical malformations, spontaneous movements and free-swimming patterns, heart beating and hatching rate were among the experimental endpoints assessed. Zebrafish embryogenesis composed an informative, sensitive and reliable *in vivo* screening tool to fast-track the nanotoxicity of the developed superparamagnetic colloids.

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DETERMINATION OF NANOPARTICLE UPTAKE BY FLOW CYTOMETRY AND ATOMIC ABSORPTION SPECTROMETRY

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The determination of internalized amount of nanoparticles in cells is essential for quantitative particle toxicology and pharmacology. Moreover, understanding of the mechanism(s) underlying nanoparticle`s uptake and factors which influence this process is crucial for development adequate nanocarriers for targeted delivery. The process of internalization is influenced by the physico-chemical properties of nanoparticles such as particle size, morphology, surface area, surface charge, and surface chemistry/coating.

In this study, two analytical methods were used to determine the uptake and mechanism of nanoparticle internalization: flow cytometry and atomic absorption spectroscopy (AAS). The aim of this study was to evaluate the pros and the cons of particular method to estimate the presence of nanoparticles in the cell. The magnetite nanoparticles with 10 nm magnetite core (MNPs) and hydrophilic shells (BSA and PEG) were characterized in-depth by different physico-chemical methods, and their colloidal stability in culture media was determined by DLS. Several inhibitors of endocytosis [1] were used to evaluate the mechanism of MNPs uptake. Two cell lines, the human adenocarcinoma lung cell line (A549) and human keratinocytes (HaCaT) were employed in this study. The cytotoxicity of MNPs and inhibitors was determined by MTT and the viability by InCucyte. The cell morphology was determined by immunofluorescent staining.

Both analytical methods were able to determine differences in MNPs uptake in dependence on inhibitor used. The advantage of flow cytometry is that the uptake is analyzed in individual cells but its limitation is that it cannot provide information about the exact internalized amount of MNPs per cell. The advantage of AAS is that it allows accurate quantification of internalized MNPs (amount and number) per cell but the measurement requires a high number of cells as pooled cell population is analyzed.

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SURFACE-MODIFIED TIO_2 NANOPARTICLES WITH ASCORBIC ACID: ANTIOXIDANT PROPERTIES AND EFFICIENCY AGAINST DNA DAMAGE

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Ascorbic acid (AA) is a standard antioxidant and its radical scavenging properties also appear to be responsible for its antigenotoxic properties. Nanoparticle-mediated delivery of antioxidant compounds is intended to increase their bioavailability while maintaining their effectiveness. Colloids consisting of the 45-Å TiO_2 nanoparticles (NPs) with anatase crystal structure were prepared by acidic hydrolysis of $TiCl_4$. The synthesized TiO_2 NPs were characterized using transmission electron microscopy and X-ray diffraction analysis. The charge transfer (CT) complex formation between surface Ti atoms and AA is indicated by immediate appearance of red color.

The aim of this study was to compare, for the first time, the antioxidant and antigenotoxic effects of AA attached to the surface of TiO_2 NPs with free AA in a wide concentration range. For evaluation of antigenotoxic properties whole blood cells were first treated with 50 μM H_2O_2 to induce DNA damage, and then exposed to 3 different concentrations of free AA (1.3×10^{-2}, 2.6×10^{-2}, and 3.9×10^{-2} M) and the same concentrations of AA attached to TiO_2 NPs (0.05, 0.1, and 0.15 M) for 30 min at 37 ^{0}C. The level of DNA damage was evaluated by comet assay method. For evaluation of antioxidant properties, total antioxidant capacity (TAC), total antioxidative status (TAS) and prooxidative-antioxidative balance (PAB) were determined in human serum pool during 2 and 24 h incubation at 37 °C, without and with terc-buthyl-hydroperoxide (TBH) as exogenously added oxidant. As expected, the results of DNA damage showed that the increase of AA concentration leads to a reduction of DNA damage. The similar concentration dependence was observed for surface-modified TiO_2 NPs with AA. So, no significant differences between the antigenotoxic properties of free AA and AA attached to the TiO_2 NPs were noticed, but only the highest concentrations showed significant effect in both experimental treatments. Regarding short-term oxidative balance in biological material (serum), during 2h, by measuring the TAC we have showed that the complex NP and AA, so as ascorbic acid showed a significant increase in TAC capacity, compared to native serum. This increase of antioxidative capacity couldn't be abrogated even with a powerful oxidant, terc-buthylhydroperoxide presence (TBH). After 24h hour incubation the TAC level in both samples decreased towards the baseline level. About the TOS, which measures all oxidative components in plasma such as hydrogen-peroxide and lipid hydroperoxide, the complex of NP and AA versus AA alone showed inconsistent results. Prooxidative-antioxidative balance (PAB) measuring equilibrium between oxidants and antioxidants remains low, almost imponderable after 2h and after 24h serum incubation with the two substances. To summarize, we suggest that surface-modified TiO2 NPs with AA and/or similar compounds can be used to improve their bioavailability while maintaining its beneficial activities.

CYTOTOXICITY ASSESSMENT ON COTTON FABRICS COATED WITH PHOTOCATALYTIC TITANIUM DIOXIDE NANOPARTICLES

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Taking into account that nanoparticles-treated fabrics are intended to be marketed due to their self-cleaning and antibacterial properties, the biocompatibility of these materials is essential and should be rigorously assessed in order to check any possible risk of harmful effects which could be induced to the skin, such as cytotoxicity or inflammation. To fulfill this aim, the biocompatibility in terms of cell viability, cell membrane integrity and inflammation status of cotton samples, which were coated with 1% Fe and nitrogen doped titanium dioxide nanoparticles (TiO_2 NPs), was tested on CCD-1070Sk normal human dermal fibroblasts. The period of incubation was selected at 4 hours, considered as the average time at which the toxic effects are visible on the skin during clothes wearing or after a contact with such functionalized textiles.

The cell viability assessed by MTT test was not significantly modified after the exposure to NPs-treated samples compared with control and no damage was induced to the cell membrane integrity as established by lactate dehydrogenase-based assay after 4 hours of direct contact with these fabrics. In addition, these data correlated with the level of nitric oxide released in the medium which did not change compared with the control material, highlighting the lack of inflammation after 4 hours of incubation. The human dermal fibroblast cell behavior and morphology as evaluated by F-actin staining using fluorescence microscopy was not significantly influenced in response to the NPs-containing cotton fabrics compared with control. The elongated flattened morphology and numerous focal adhesions between cells were maintained after 4 hours of exposure, with no significant differences between samples.

The *in vitro* biocompatibility evaluation on dermal fibroblasts confirmed the absence of cytotoxicity after the short-term exposure, suporting further *in vivo* investigation of these innovative photocatalytic NPs-coated cotton fabrics with self-cleaning and antimicrobial properties, and afterwards, a possible use for large scale production on self-cleaning clothes market.

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A PROSPECTIVE APPROACH TO SAFE NANOTECHNOLOGIES

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Due to the novel combination of size, structure, and physical/chemical properties (e.g.; optical, electrical, catalytic, magnetic, adhesion properties), nanoscience and nanomaterials can permit remarkable technological advances and innovations in many industrial sectors [1]. However, innovation never comes at zero risk. In order to proactively take actions in assessing the potential health and environmental risks of nanoscale technologies, we developed a risk assessment approach by linking the strategies of hazard assessment [2, 3], life cycle [4-6], and risk analysis [7-9] within the same toolbox. The proposed nanosafety testing strategy is composed of 2 phases: “Phase 1 Hazard & Risk Assessment” and “Phase 2 Risk Estimation”. In Phase 1 all available information and data on physicochemical properties, exposure, toxicokinetics, fate, and hazard of a given nanomaterial was drawn together to build general exposure scenarios (case studies) throughout the life cycle of the nanomaterial. The objective of Phase 2 is to obtain initial exposure estimates on a PROC (process category)-specific basis [10]. For each PROC, exposure values are calculated according to the selected/assigned PROC-class as well as several parameters such as the frequency and duration of exposure, the presence of a local exhaust ventilation (LEV) etc.

The presented approach allows creating a library of critical hotspots associated with initial exposure estimates and thereby help to develop mitigation plans designed to manage, eliminate, or reduce risk to an acceptable level. Moreover, industries that are willing to adopt such safety approaches in their business thinking will be able to responsibly design nanotechnology-enabled products and innovations with adequate attention devoted to understanding the EHS (Environmental, Health and Safety) opportunities and risks of nanomaterials throughout the phases of their life cycle.

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MANUFACTURED NANOMATERIALS: IS THERE A CORRELATION BETWEEN TOXICOLOGICAL EFFECTS AND THE PHYSICOCHEMICAL PROPERTIES?

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Toxicological information on nanomaterials (NMs) is of major importance for safety assessment, since they are already used in many consumer products and promise cutting-edge applications in the future. While the number of different NMs increases exponentially, new strategies for risk assessment are needed to cope with the safety issues, keeping pace with innovation. However, recent studies have suggested that even subtle differences in the physicochemical properties of NMs that are closely related may define different nano-bio interactions, thereby determining their toxic potential. Further research in this field is necessary to allow straightforward grouping strategies leading time-effective risk assessment to enable the safe use of the emerging NMs.

In this presentation the case study of the *in vitro* toxicity testing of a set of multi-walled carbon nanotubes (MWCNTs) in two human cell lines from the respiratory tract will be described. Those MWCNT have been previously characterized in detail, and differ in thickness, length, aspect ratio and morphology. This comprehensive toxicological investigation undertaken in parallel with physicochemical characterization in the cellular moiety showed that the same NM did not display a consistent effect in different cell types, and that, within the same class of NM, different toxic effects could be observed. The correlation of the cytotoxic and genotoxic effects characterized in the two cell lines with their physicochemical properties will be presented and the relevance of considering the NMs properties in the biological context will be discussed.

Overall, this case study suggests that nanotoxicity of closely related MWCNTs depends not only on their primary physicochemical properties, or combinations of these properties, but also on the cellular system, and its context. Challenges posed to toxicologists, risk assessors and regulators when addressing the safety assessment of NMs will be highlighted.

The INSA's team involved in the Nanogenotox Joint Action and in the NANoREG project. The work was co-funded by EU FP7 project NANoREG, grant agreement 310584.

DETERMINATION OF "SAFE" AND "CRITICAL" NANOPARTICLES EXPOSURE TO WELDERS IN A WORKSHOP

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This study confirms that the emission of nanoparticles in the MAG (Metal Active Gas) welding of carbon steel using mixtures of Ar+CO_2, is clearly dependent from the distance to the welding front and also from the main welding parameters, namely the current intensity and the heat input in the welding process. The emission of airborne fine particles increase with the current intensity as fume formation rate does. A marked decay of ultrafine particles with the distance to the weld area is observed. When comparing the tested gas mixtures, higher emissions are observed for more oxidant mixtures, that is, mixtures with higher CO_2 content, which result in higher arc stability. The later mixtures originate higher concentrations of fine particles (as measured by number of particles by cm^3) and higher values of alveolar deposited surface area of particles, thus indicating a severe worker's exposure. However, size distribution of emitted particles does not seem to differ significantly, and morphology analysis shows that fine particles are lower than 10 nm, but form aggregates up to diameters as high as 100 nm or more. Its composition is mainly iron, resulting from projections of the molten material. During welding operations re-suspension and agglomeration of fine particles was also noticed as reflected in the evolution of Alveolar Deposited Surface Area (ADSA) of emitted particles, and its size distribution. Representing measured ADSA values as contour curves, corresponding to certain ADSA range of values, starting from the welding source to crescent distances from this source, a contour map is obtained which allows to: i) understand the evolution of nanoparticles emissions from the source, with time; ii) definition of both "safe" and "critical" zones, in the workshop where welding is taking place, regarding welder's exposure to nanoparticles; iii) definition of zones, within a workshop where welding is taking place, where fume extraction or welding operation containment equipment should be installed in order to obtain a "safe" environment for exposed welders.

ASSESSMENT OF INDOOR ENVIRONMENTAL QUALITY IN ELDERLY CARE CENTERS

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The aim of this study was to characterize indoor environmental quality in a representative sample of Elderly Care Centers (ECC) in order to associate it with ventilation, health and comfort of elderly people. Indoor air quality (IAQ) parameters and thermal comfort were measured twice, during winter and spring/summer seasons, from 18 Elderly Care Centers (ECC) located in Lisbon, with a total of 116 rooms evaluated.

Carbon dioxide and carbon monoxide were monitored during occupation periods using the Indoor Air Quality Meter (TSI, model 7545, USA). Formaldehyde was collected by active sampling on impingers, using personal pumps at an airflow of 1L/min and analyzed according to NIOSH 3500 method, using visible spectrometry (UV4, UNICAM). PM_{10} and $PM_{2,5}$ were collected by active sampling on pre-weighted PTFE filters mounted on PM_{10} and $PM_{2,5}$ collectors (PEM, SKC), using personal pumps operating at 2L/min, followed by gravimetric analysis for particle mass according to the method IP-10A by SKC (2004). Duplicate samples of total volatile organic compounds were collected on TENAX Tubes (Ref. 25054, Supelco) using SKC personal pumps calibrated to 0.05 L/min and analyzed after thermal desorption according to ISO 16000-part 6 using gas chromatography. Duplicate samples of viable airborne bacteria and fungi were collected using the Microbiological Air Sampler (Merck) and TSA, McK and MEA for total bacteria, Gram-negative bacteria and fungi, respectively. Whole-body thermal comfort evaluation was based on PMV (Predicted Mean Vote) and PPD (Predicted Percentage of Dissatisfied) indices, according to the ISO 7730:2005.

Considering the obtained results for environmental indoor quality it is possible to conclude that thermal comfort was not reached in more than 30% of the rooms. In winter, carbon dioxide concentrations were above the reference in 20% of the rooms. PM_{10} and $PM_{2,5}$ mean concentrations were above the reference levels in approximately 25% and 30% of the rooms, respectively. Microbiological contamination (total bacteria and fungi) was above the reference levels in more than 35% of the rooms.

Indoor environmental quality should be improved by controlling contamination sources, ventilation and thermal parameters (or clothing) in order to obtain healthier environments for the elderly.

POLYCYCLIC AROMATIC HYDROCARBONS IN EDUCATIONAL SETTINGS: A COMPARISON BETWEEN PRE- AND PRIMARY SCHOOLS

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Polycyclic aromatic hydrocarbons (PAHs) have been identified as priority indoor air pollutants that strongly affect human health, yet information concerning the respective indoor exposures is still limited. Exposure to PAHs is a public health concern particularly in children who are one of the most susceptible subgroups of the population. Because of the respective health impacts, information concerning PAHs in educational settings (schools, kindergartens) has been slowly emerging but the current understanding is far from comprehensive.

Thus the main aim of this study was to compare levels of particulate–bound ($PM_{2.5}$) PAHs in pre– and primary school environments and to assess the respective risks for 3–5 years old and 8–10 years old children.

Sixteen particulate–bound PAHs (considered by USEPA as priority pollutants, dibenzo[a,l]pyrene and benzo[j]fluoranthene) were concurrently sampled indoors and outdoors during winter-spring 2014 in two preschools and five primary public schools situated in Oporto Metropolitan Area. Sample collection was conducted daily according to USEPA IP-10A method using a personal environmental monitor, i.e. single stage impactor ($PM_{2.5}$) combined with personal air sampling pump. Activity patterns of children were registered during each day as well as information regarding room occupancies, ventilation systems, and potential emission sources and activities.

Indoor levels of PAHs were similar in both educational environments ranging between 0.721–15.9 ng m^{-3} at preschools and 5.03–15.3 ng m^{-3} at primary schools. However, total cancer risks (considering both indoor and outdoor exposure) were negligible for 3–5 years old children according to USEPA, and exceeded (up to 24 times) the recommended WHO health–based guideline of 10^{-5}; for 8–10 years old children the risks exceeded (up to 22 and 52 times, respectively) both USEPA and WHO guidelines. Finally, the risks due to indoor school exposure were higher than for outdoors for both age groups of children, mostly due to long periods that children spent indoors.

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INHALED DOSE OF $PM_{2.5}$ AND PM_{10} BY CHILDREN IN PORTO, PORTUGAL

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Children are more vulnerable to air pollution than adults, being considered a risk group. Besides, their exposure patterns are unlike those of adults, which make their study important. Thus, as part of the INAIRCHILD project, this study aimed to estimate the inhaled dose of particulate matter (PM) in Portuguese children attending nursery and primary schools. Children's daily exposure was estimated based on a microenvironmental modelling approach, which combines time-activity patterns (TAP) and PM concentrations in indoor and outdoor microenvironments (ME). TAP were obtained from a parent-reported daily diary (465 complete diaries considered, 30% response rate), which determined the considered ME (home indoor and outdoor, school indoor and outdoor, in transport and other), and activities and their duration. $PM_{2.5}$ and PM_{10} concentrations were measured inside schools with a TSI DustTrak (30 classrooms and 7 lunch rooms from 7 nursery and 5 primary schools); modelling was used to estimate the other concentrations (homes and transport and outdoor). $PM_{2.5}$ and PM_{10} outdoor concentrations were obtained from the Air Quality Monitoring Network of Porto Metropolitan Area. TAP for a typical day were drawn, and allowing to confirm that studied children spent more than 90% of time indoors: about 25% in school facilities and more than 50% at home. In transport represented 3-5% of the day and the outdoor represented 8-9%. Rest and light activities were the most common (> 90%). Moderate and heavy activities were residual (3-7% and ≤ 2%, respectively). According to the different TAP, inhaled doses of PM_{10} and $PM_{2.5}$ were estimated for each age group of children, and they were respectively 2.2 and 1.5 µg/kg/day for infants (< 3 years old), 2.3 and 1.6 µg/kg/day for preschoolers (3-5 years old) and 1.1 and 0.7 µg/kg/day for primary school children (6-10 years old). Younger children (infants and preschoolers) were exposed to higher concentrations and inhaled higher doses of both PM_{10} and $PM_{2.5}$. The highest exposures and inhaled doses corresponded to periods inside schools, which enhances the importance of these ME for children's respiratory health. Besides, those who are the most vulnerable (infants) inhaled the highest doses of the finer fraction ($PM_{2.5}$), which is the most harmful for health.

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BACTERIAL AND FUNGAL EXPOSURE IN SCHOOLS MAY INFLUENCE ASTHMA AND ALLERGY IN CHILDREN

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Development of allergic diseases may be regulated by microbial exposure. Children spend a lot of their time in schools, under an extensive diversity of biological agents, such as bacteria and fungi. This study aimed to characterize indoor air microbiological exposure in schools as a predictor of allergic sensitization and asthma in children. A total of 860 children aged 8 to 10 years old attending 71 classrooms in 20 primary schools were submitted to spirometry with bronchodilation (with 400µg of Salbutamol) and skin-prick tests (SPT) with *Der p*, mixed weed, mixed grasses, cat, dog and *Alt a* allergens. Asthma was defined by at least a 12% increase in FEV_1 from baseline and atopy was characterized by a positive SPT to at least one of the allergens. Bacteria and fungi air samples were collected in all the participating classrooms using a single-stage microbiological air impactor through TSA and MEA plates at a 100 L/min rate. Quantification was performed by naked eye count and the identification of fungal colonies was based upon phenotypic characteristics and followed standard mycological procedures. Endotoxins were collected using 2 L/min flow control pumps for 4 hours and analysed by limulus amebocyte lysate assay. Mann-Whitney tests and logistic regression models were used to statistically analyse the data. Diversity scores were established as the number of different fungal species found in each classroom.

Prevalence of asthma and atopy was 8.7 and 34.1%, respectively. Classrooms with increased diversity scores showed a significantly lower prevalence of children with atopic sensitization, but not asthma. The risk of sensitization to inhalant allergens increased with increasing endotoxin exposure in classrooms. Similarly, higher concentrations of *Penicillium spp* were associated with a higher risk of positive SPT (1.68 [95%CI: 1.18 - 2.40]) while children in classrooms with higher levels of *Aspergillus fumigatus*, *Aspergillus niger* and *Chaetomium spp* had lower risk of sensitization.

Although the cross sectional approach of the study does not allow to establish causal relationships, the results suggest that current exposure to higher levels of endotoxins and *Penicillium spp* may be associated with increasing odds of allergic sensitization in children. Nevertheless, increased fungal diversity in classrooms was associated with a protective effect in the development of atopy, but not asthma.

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HEXAVALENT CHROMIUM, AN OCCUPATIONAL LUNG CARCINOGEN, ATTENUATES THERMAL SHOCK EFFECTS AND INTERFERES WITH THE STRESS RESPONSE IN HUMAN BRONCHIAL EPITHELIAL CELLS

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It has long been established that, as encountered in certain industries, hexavalent chromium [Cr(VI)] compounds are carcinogenic to humans. Exposure to Cr(VI) triggers several types of cellular stresses, namely oxidative and metabolic stresses. As these stresses are expected to activate the stress response, it is tempting to speculate that cells that survive Cr(VI) exposure become more resistant to further stresses, namely those encountered during neoplastic transformation. To test this hypothesis, we investigated whether Cr(VI)-exposed cells were more resistant to stress than their non-exposed counterparts, using acute thermal shock as a model. Our results show that 48 h exposure of human bronchial epithelial cells (BEAS-2B cells) to 1 μM Cr(VI) attenuates the anti-proliferative effects of both cold and heat shock, giving initial support to our hypothesis. Interestingly, we also observed that the doubling times of the cultures exposed to Cr(VI) were higher than those of their non-exposed counterparts. We then determined the effects of Cr(VI) on the expression of three components of the stress response with a critical role in carcinogenesis: heat shock proteins 90 alpha (Hsp90α) and 70 (Hsp70) and heat shock factor 1 (HSF1). We could confirm that Cr(VI) did interfere with the expression of the two Hsps tested, producing a significant decrease in the protein levels of Hsp90α and in the mRNA levels of Hsp70 (as determined by ELISA and RT-qPCR, respectively). Rather surprisingly, these changes, by themselves, do not support an activation of the stress response. To gain further insight into this apparent contradiction, it will be important to monitor protein and mRNA levels at different time points of the 48 h exposure.

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GENOTOXIC AND CYTOTOXIC EFFECTS IN NASAL AND BUCCAL CELLS OF ELECTROPLATERS

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Several earlier studies showed that metal exposures cause in electroplaters induction of DNA-damage in exfoliated cells from the respiratory tract and it was postulated that genotoxic effects which are induced by chromium, nickel and other metals lead to increased cancer rates in various organs. We studied induction of genetic damage as a consequence of occupational exposure in a group of workers in a plating factory with a high technical standard which produces cobalt and chromium plated products. Buccal and nasal cells were collected from workers (n=42) and controls (n=43, matched for body mass index, alcohol consumption, smoking). The cells were collected, processed and stained as described by Thomas et al. [1] and Knasmueller et al. [2]. The rates of micronuclei (MN), nuclear buds (NB), binucleated cells (BN), cells with condensed chromatin (CC), karyorrhexis (KR), kalyolysis (KL) and pyknosis (PK) were evaluated in cells from both organs. MN reflect structural and numerical chromosomal aberrations; NB are a consequence of gene amplification effects (which are caused by adaptive responses towards toxins); BN reflect mitotic disturbance while all other nuclear changes reflect cytotoxicity. We found no evidence for induction of MN in buccal and nasal cells. However, elevated frequencies of NB and BNs were found in the former cell type. In nasal cells, the rates of all types of nuclear anomalies were increased except MN and PK. Analyses of the impact of working period showed that most markers reflecting cytotoxic effects increase as a function of exposure period. Chemical-analytical measurements showed that chromium and cobalt levels in blood of the workers are below the permitted concentrations and are not higher than in the controls. Taken together, our finding show that no increase of structural and numerical chromosomal aberrations occurs in the workers, however, we found clear evidence for induction of cytotoxic effect (reflected by increased rates of CC, KR and KL) in the nasal epithelium which may cause increased cell divisions and inflammations.

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HOSPITAL SURFACES CONTAMINATION WITH ANTINEOPLASTIC DRUGS: INFLUENCE OF CLEANING PROCEDURES

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The raising frequency of cancer diseases is leading to a widespread application of antineoplastic drugs, thus increasing the probability of workplace surfaces contamination [1]. Most of these drugs are classified by the International Agency for Research on Cancer as known or suspected human carcinogens. Skin absorption is the main route for antineoplastic drugs exposure in occupational settings, therefore cleaning protocols have paramount influence in surfaces contamination and, consequently, in exposure [2]. The aim of this study was to assess surfaces contamination in a Portuguese chemotherapy unit before and during drug administration, in both preparation and administration facilities.

Samples were collected by wipe-sampling from potentially contaminated surfaces selected by previous protocol observation. Samples were analyzed by HPLC-DAD. Cyclophosphamide (CP), 5-fluorouracil (5FU), and paclitaxel (PTX) were used as surrogate markers for surfaces contamination for all cytotoxic drugs.

From the 34 samples collected before any preparation and administration activities, 41.2% were contaminated with 5-FU (4.0-84.7 ng/cm^2) and 23.5% of the samples were contaminated with CP (19.8-139.6 µg/cm^2). Only 2 samples presented contamination by PTX (5.9%) with a maximum value of 3.7 ng/cm^2. Of the 37 samples collected during preparation and administration of antineoplastic drugs, 48.7% were contaminated with 5-FU (1.9-88.7 ng/cm^2) and 24.3% with CP (12.0-93.9 µg/cm^2). None of the samples showed contamination with PTX.

Data showed differences in contamination levels before and after the handling of antineoplastic drugs in preparation and in administration settings. These results point out the importance of cleaning procedures. This is well in accordance to previous studies that showed how the type of cleaning procedures and products used can be determinant for surfaces decontamination [2].

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BACTERIAL SURVIVABILITY AND TRANSFERABILITY ON BIOMETRIC DEVICES

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The prevalence and nosocomial spread of methicillin resistant Staphylococcus aureus (MRSA) in Portugal remains one of the highest in Europe.[1] S. aureus can survive for long periods of time on inanimate objects, which may represent an important reservoir for dissemination. [1] On the other hand, the biometric recognition is being increasingly adopted and mapped to rapidly growing person identification applications. Considering this problem, our aims are: to evaluate the contamination and to identify the species of bacteria (in particular MRSA) existing on the registration biometric devices (RBD) in a public hospital in Portugal and to assess the bacterial recovery after using two types of surfaces disinfectants.

All the biometric devices existing in the hospital were tested. The first phase of the study took place between December 2013 and March 2014 by collecting 2 microbiological samples from the RBD (before and after disinfection with 70% alcohol). The second phase of the study took place between June and October 2014, proceeding in the same way but using the Skin Prep® solution.

In the first phase of the study 18 different microorganisms have been identified (mostly S. epidermidis, S. saprophyticus and S. aureus). The S. epidermidis was eliminated in about 61.5% of the samples after disinfecting with alcohol. Regarding S. saprophyticus and S. aureus were eliminated in 66.7% of the samples. In the second phase of the study 10 different microorganisms were identified (mostly S. epidermidis, S. aureus, S. oryzihabitans and S. hominis). Of the total of samples collected 78.9% became negative (no bacteria found) after disinfection with Skin Prep® solution.

The RBD used in this hospital seem to be safe regardless of the products used for its cleaning. We haven´t found bacteria which may lead to disease in immunocompetent individuals, in particular we haven´t found MRSA. Some of the bacteria found are even commensal skin microorganisms.

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ANTIMICROBIALS IN HOSPITAL WASTEWATER: THE PREDICTION OF RELATED RESISTANCE SELECTION HAZARD IN THE URBAN WASTEWATER EFFLUENT

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Although hospitals wastewater (HWW) represent only a small proportion of the total amount of municipal sewage (below 10%) this fraction could contain distinct molecules from those released by domestic use [1,2], among which antibiotics like piperacillin, meropenem and vancomycin.

Based on data consumption of 46 public Portuguese hospitals, during 2014, the most consumed antibiotic were determined. In order to establish environmental resistance selection hazard of these antibiotics we compared predicted concentrations (PECs) with predicted no effect concentrations (PNECs) calculated from resistance selection based on minimal inhibitory concentration data (MICs) [3]. The predicted environmental concentrations for each compound in the hospital wastewater (PEC_{HW}) were calculated by dividing the consumed amount (considering the excretion factor), with the volume of wastewater (assuming the amount of consumed water in the enrolled hospitals during 2014). In order to predict concentrations in surface wastewaters (PECsw), a dilution factor of 1000 has been applied. Lastly PEC/PNEC ratios (risk quotients - RQs) were calculated for hospital and surface water effluents [4].

The ten most consumed antibiotics, during 2014, in the enrolled public Portuguese hospital centers, were as follows: piperacillin, amoxicillin, cefazoline, ceftriaxone, meropenem, vancomycin, sulfametoxazol, ampicillin, benzilpenicillin and ciprofloxacin. All the RQs of hospital water effluents calculated for these ten evaluated antibiotics surpassed the ratio 1 (ranging from 2.0 to 2274.6). In surface waters the RQ worst-case estimations were observed for piperacillin, meropenem, ceftriaxone and amoxicillin (ranging from 1.6 to 2.3).

Considering the usage pattern, these estimated ratios showed a high selection pressure in the environment.

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ENVIRONMENTAL POLLUTANTS IN PASSIVE AIR SAMPLES OF TARRAGONA COUNTY, SPAIN: AN ANALYTICAL MULTI-COMPONENT APPROACH

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Some semi-volatile organic chemicals (SVOCs) are characterized by their human toxicity, bioaccumulative potential, environmental persistence, as well as long-range atmospheric transport capacity. The need to get more data from air monitoring has led to the development of a wide range of sampling devices and analytical methods. Because of their advantages in front to active air sampling devices, passive air samplers (PAS) has emerged as a viable alternative to monitor SVOCs. The goal of this research was to estimate the air concentrations of SVOCs in Tarragona County, Spain, where the most important chemical/petrochemical industrial complex in Southern Europe is located. Eight PAS containing polyurethane foam (PUF)-disks were deployed for a period of 2 months (November 2014-January 2015) at several areas of Tarragona County, each one characterized by the presence of different potential emission sources (petrochemical, chemical, urban, and background areas). Air levels of five classes of SVOCs (brominated flame-retardants (BFRs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), and synthetic musks fragrances) were simultaneously quantified in each one of the PAS. For that purpose, an innovative multi-component protocol developed by Silva et al. (*Analytica Chimica Acta* 858: 24–31(2015)), was used for the first time for the air samples extraction process. Air extracts were obtained by ultrasonic solvent extraction (USE) and quantified by GC/MS ion trap mass spectrometer operated in electron ionization mode. Total accumulated amounts of SVOCs in air samples were found to range from 1.30 to 2.80 ng/PUF for BFRs, from 2.88 to 120 ng/PUF for PCBs, from 4.65 to 16.4 ng/PUF for OCPs, from 2098 to 4333 ng/PUF for PAHs and from 0.38 to 13.4 ng/PUF for synthetic musks fragrances. Some correlations were observed between pollutant levels and the presence of potential emission sources. In agreement with data previously reported, the highest concentrations of PAHs were found in the petrochemical area (max: 4333 ng/PUF). In turn, while the greatest levels of PCBs were observed in the chemical zone (max: 121 ng/PUF), probably due to the presence of a chlor-alkali plant and various electrical substations in the vicinity. Finally, similar concentrations of OCPs, BFRs and synthetic musks fragrances were observed irrespectively of the sampling area. Future studies will be aimed at monitoring the levels of the same SVOCs in soil and vegetation samples from the same area under study and establishing potential correlations between environmental monitors.

A CASE STUDY ABOUT MEDIA AND GLYPHOSATE: BETWEEN SCIENTIFIC EVIDENCE AND POLITICAL (IN)DECISION

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On March 2015, the International Agency for Research on Cancer (IARC) classified glyphosate as a probable carcinogen to humans (Group 2A). Glyphosate is an herbicide used worldwide to control a variety of plants in agriculture, gardening, grasslands and in aquatic environments. In Portugal, it is also commonly used to control weeds growth in urban areas.

This classification triggered the scientific community. Several international studies were aimed to evaluate the presence of glyphosate in food, environment and humans. The German study "Urinale 2015" detected glyphosate in a mean value of 1.1 ng/mL (n=2009) in urine samples. Another study comprising individuals from 18 European countries (n=182) revealed a mean value of 1.8 ng/mL.

In Portugal, the presence of glyphosate had never been studied, and the Portuguese parliament opposes in 2016 to the ban of the herbicide in the country. This position was in line with the initially favourable decision from Portugal regarding the revalidation of glyphosate license in the European Union for 7 years.

On April 29th, the public television channel (RTP) reported the results of a Portuguese study that for the first time assessed the urinary content of glyphosate in a group of 26 volunteers. Glyphosate was present in all the urine samples with a mean value of 26.2 ng/mL, about 20 times higher than the mean value observed in the German study. Further, the level of glyphosate found in urine was 260 times higher than the limit value established for water. Taking in account the worldwide use of glyphosate and the recent health implications, it is urgent to assess its toxicological profile and to evaluate the impact of the long-term exposure on both public health and environment.

The spread of the news piece with these results put the issue at the centre of the national attention, reflecting the mobilization of civil society and the change of political vision regarding this topic. Portugal, given the new developments, redefined its position and now admits the prohibition of glyphosate, if the indecisiveness of European experts, given the little scientific evidence, persists.

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AFLATOXIN M_1 IN HUMAN BREAST MILK IN PORTUGAL: ESTIMATION OF MATERNAL AND INFANT EXPOSURE

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Aflatoxin (AF) M1 is a metabolite of AFB1 in humans and animals. Human exposure to AFM1, classified as possibly carcinogenic (IARC group 2B), occurs mainly through consumption of AF-contaminated milk, including mother breast milk. Infants are considered a vulnerable population. The occurrence of AFM1 in mother breast milk and the evaluation of the exposure degree to the AFM1 of the mothers and lactating children were studied [1-4].

Breast milk samples were collected from 37 lactating mothers living in coastal regions between February 2015 and February 2016. All participants signed a written informed-consent agreement. Food consumption (recall period 1 month) was assessed through a semi-quantitative food questionnaire. The study was approved by the Scientific Committee of the Faculty of Pharmacy of the University of Coimbra and respected the Helsinki Declaration. Determination of AFM1 was carried out by means of the RIDASCREEN® test kit (R-Biopharm, Germany), a commercial competitive ELISA (detection limit of 5 ng/L), performed in accordance with the enclosed instructions.

Seven samples (18.9%) featured a contamination above the detection limit (mean 8.3±2.1 ng/L). Though incidence of contamination was lower than previous studies, mean AFM1 concentration was higher than the ones recently reported in Mexico [1], Colombia [2], Iran [3] and Turkey [4]. The values ranged between 5.7 and 10.6 ng/L. All positive samples were collected during the summer period and from mothers living in the district of Aveiro. In the collected food frequency questionnaire, it was observed that the positive samples originated from mothers consuming higher quantities of milk and dairy products, cereals and derived products, as well as dried fruits. Estimated infant average dietary exposure to AFM1 through milk consumption was estimated at 0.98 ng/kg bw/day, which is nearly the level (1 ng/kg bw/day considering total aflatoxin) that contribute to the risk of liver cancer [5]. The observed preliminary findings suggest that additional studies should be conducted to further determine the risk of exposure of the breast fed infants included in this work.

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[2] G.J. Diaz, *et al*., *Food Additives & Contaminants: Part A* **32**, 1192-1198 (2015)
[3] F. Maleki, *et al*., *Osong Public Health Res Perspect* **6**, 283-287 (2015)
[4] M. Atasever, *et al*., *Food and Chemical Toxicology* **66**, 147–149 (2014)
[5] JECFA. Geneva: WHO, **47** (2001)

CHANGES OVER TIME IN POPs CONCENTRATIONS IN HUMAN MILK IN THE REPUBLIC OF MOLDOVA

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Mothers´ milk is an important matrix for human biomonitoring studies. Persistent organic pollutants (POPs) in human milk are used as makers of environmental exposure as well as dietary exposure related to different consumption habits. The mothers´ milk is also the main pathway for human exposure to POPs in the early life. The present study was conducted to provide baseline and time trends information in human milk POPs concentrations in the Republic of Moldova in the framework of the Fourth World Health Organisation – Coordinated Survey of Human Milk for Persistent Organic Pollutants in Cooperation with United Nations Environment Programme.

The pooled sample of breast milk was analyzed for PCDDs, PCDFs, dioxin-like PCBs, basic pesticide POPs, marker PCBs, optional and new POPs and their transformation products, by the WHO reference laboratory, the State Institute for Chemical and Veterinary Analysis of Food, Freiburg, Germany, using validated methods. Perfluorinated chemicals were analyzed in a single laboratory at the Man-Technology-Environment Research Centre, Örebro University, Sweden.

According to the obtained results, from the group of basic POPs, aldrin, endrin, endosulfan, hexabrombiphenyl and pentachlorobenzene were not detected. In the first phase of survey was not detected mirex, in the second - gamma-hexachlorocyclohexane (γ-HCH). Almost all POPs concentration levels were decreasing, including alpha-hexachlorocyclohexane (α-HCH) with about 80 %, hexachlorobenzene (HCB) 69 %, beta-hexachlorocyclohexane (β-HCH) and dieldrin with 68% and 60 % respectively. DDTs profiles declined up to 35 %. Concentrations of dioxin-like polychlorinated biphenyls (PCBs) and PCBs indicator are now with 44 % and respectivelly 46 % less than in first phase, while polychlorinated dibenzofurans and dibenzodioxins (PCDF/PCDD) follow the same pattern registering 42 and 30 % less. Exception makes toxaphene group which seems to increase up to 3.22 %.

Substances from the newly listed POPs: sum of polybrominated diphenyl ethers is 1872 pg/g lipid weight. 2,2',4,4'-tetrabromodiphenyl ether (BDE 47), 2,2',4,4',5-pentabromodiphenyl ether (BDE 99) and 2,2',4,4',5,5'−hexabromodiphenyl ether (BDE 153) congeners are the main contributors in this sum. Chlorodecone was not detected. The concentration of hexabromocyclododecane is equal 8.1 ng/g lipid weight.

Results of analysis show a decreasing tendency of POPs concentration over time in the pooled sample of human breast milk comparing to the first phase of the study. Data regarding new POPs determination compounds show the presence of high polybrominated diphenyls ethers concentration in milk sample. However, POPs remain a major public health concern and further data on human exposure is essential for assessment and management in order to protect human health.

FUNGAL CONTAMINATION IN COFFEE SAMPLES: A PUBLIC HEALTH CONCERN

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Fungi are natural coffee contaminants and under certain environmental conditions have the potential to produce toxins. *Aspergillus*, *Penicillium* and *Fusarium* spp. have been reported as the main genera present in coffee samples. *Aspergilli* from the *Circumdati* and *Nigri* sections are known to produce high levels of ochratoxin A, a mycotoxin known as nephrotoxic for animals and humans. By assessing the distribution of fungi present in the coffee beans, prevention and control strategies can be planned, avoiding both the spoilage of the product and preventing the mycotoxin production, ultimately diminishing the risk of exposure trough coffee consumption.

This work aimed to evaluate fungal distribution and also the prevalence of *Aspergillus* sections *Fumigati*, *Flav*i and *Circumdati* in the final product, using 28 coffee samples, from *Coffea arabica* (Arabica coffee) and *Coffea canephora* (Robusta coffee) species from different origins. Twenty grams of coffee beans were suspended in 180 mL of distilled water and homogenized during 20 minutes at 200 rpm. The washed supernatant was plated in malt extract agar (MEA) and dichloran glycerol agar base (DG18) media for morphological identification of the mycobiota present.

The overall contamination of the analyzed samples was 64.3%. Fungal load in the coffee samples analyzed ranged from 0 to 12330 CFU/g.

Aspergillus sections *Nigri* (30.1%), *Circumdati* (20.4%) and *Nigri and Circumdati* concomitantly (17.9%) were the most commonly found in the analyzed samples. In addition to these, *Aspergillus* sections *Fumigati*, *Versicolores*, *Aspergilli* and *Penicillium* genus were also isolated.

Prevalent species found corroborate the fungal burden associated to coffee beans already reported in other studies. The presence of these fungi, which are potential producers of ochratoxin A and several other mycotoxins, can ultimately be considered a real risk since, contrary to fungi, the mycotoxins resist to the roast process and persist in the final product.

POSTER PRESENTATIONS

P01.

PROTEIN OXIDATIVE DAMAGE AND LEVEL OF ACUTE PHASE PROTEINS IN WISTAR RATS TREATED WITH ADRENALINE

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The catecholamines have always attracted interest for investigation due to physiological and pathological effects. This study aims to point on biochemical endpoints of toxic effects after administration of adrenaline. For this purpose the study was carried out on Wistar rats and three experimental doses of adrenaline were used: 0.75 mg/kg, 1.5 mg/kg and 3 mg/kg.

We investigated the effects of adrenaline on total activity of lactate dehydrogenase (LDH), carbonyl groups using spectrophotometric methods, concentrations of nitrite by ELISA test and nitrotyrosine by Western blotting and SDS-PAGE. The level of acute phase proteins (APPs) on alkaline-PAGE, alpha-1-acid glycoprotein and haptoglobulin by Western blot and SDS-PAGE treated with adrenaline was also determined. The obtained results revealed that all doses of adrenaline induced significant rise in total activity of LDH and concentrations of carbonyl groups compared to control. Accordingly, adrenaline exerted significant increase in concentration of nitrite and nitrotyrosine derivate in a dose dependent manner. Further study indicated that adrenaline significantly decreased serum albumine level and albumin-globulin ratio, while the APPs level is increased (alpha-1-acid glycoprotein and haptoglobulin). Based on the results it can be concluded that adrenaline causes significant alterations in APPs and increased protein oxidative damage, which may contribute to better understanding its toxic effects.

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P02.

IN VITRO STUDY OF MICRONUCLEI INDUCTION DUE TO EXPOSURE TO A 3T STATIC MAGNETIC FIELD IN PERIPHERAL LYMPHOCYTES

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In the last decades, Magnetic Resonance Imaging (MRI) has evolved from an experimental innovation to one of the most important medical imaging techniques. This constant evolution resulted in the development of new equipment capable of generating more powerful static and gradient magnetics fields. However, there is limited information about the harmful biological effects caused by exposure to these fields and the published studies analysing their impact on cellular DNA integrity are controversial [1]. The main objective of this *in vitro* study was to analyse if the static magnetic fields used in MRI can induce micronuclei formation.

Peripheral lymphocyte cultures, collected by venipuncture from 3 adult donors, were exposed to a static magnetic field of 3 T from a MRI equipment. The sample from each donor was divided into three sub-samples; two of them were exposed to the static magnetic field for 1 and 6 hours and the last one was used as a control. The sub-samples were placed inside the MRI scanner, close to its isocenter where the static magnetic field can be considered homogeneous. The control was placed in another room of the same building, at room temperature. Subsequently, it was performed the cytokinesis-blocked micronucleus assay and 1000 cells per sample were scored by optical microscopy, with an amplification 1000×.

The results showed there was a 90% increase in the average value of micronuclei in samples exposed during 1 hour (19.0±6.0) relatively to the control group (10.0±6.6). Contrary, the sub-samples exposed during 6 hours presented a decrease (6.2±5.0) of micronuclei compared to the control and to the 1 hour-exposed samples, corresponding to -38% and -67% variations respectively. Otherwise, no significant differences were observed (Mann-Whitney test, $p > 0.05$). The results appear to show an effect of static magnetic field of 3 T on the induction of DNA damage after 1 hour exposure, however, after 6 hours there is a decrease in the number of micronuclei. The overall data strongly suggests the need for further studies.

[1] A. Reddig, et al., *PLoS One* **10(7)**, e0132702 (2015)

P03.

ASSESSMENT OF GENOTOXICITY OF AFLATOXIN M_1 AND B_1 CONTAMINATED MILKS AFTER *IN VITRO* HUMAN DIGESTION

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Milk is considered a complete food from the nutritional point of view. Milk can be exposed to various types of contamination, such as mycotoxins. These metabolites are naturally occurring toxic compounds produced by fungi. Several studies on milk samples have reported the presence of aflatoxin B_1 (AFB_1) and M_1 (AFM_1), due to the high incidence in samples intended for human consumption, carcinogenicity proven AFB_1 and resistance of the contaminants to the process of digestion, making those available for intestinal absorption. Considering these aspects, the objective of this study was to evaluate the genotoxicity of milk samples contaminated by AFB_1 and AFM_1 before and after the action of lactic acid bacteria using Caco-2 intestinal human cells.

The pasteurized milk samples were spiked with AFB_1 (10 $\mu g.mL^{-1}$) and AFM_1 (2 $\mu g.mL^{-1}$) and subjected to fermentation with 4 different lactic acid bacteria (*Lactobacillus delbruecki* subs. *bulgaricus, L. acidophilus, Bifidobacterium animalis* subsp. *lactis* Bb-12 and *Streptococcus salivarius* ssp. *thermophilus*) in separate and in combined forms, totaling twenty four fermentation tests beyond the Positive Control (only milk and mycotoxin) and Negative Control (only milk). The samples were incubated at 37°C and fermented milk products (4.4 to 4.9). The samples were digested. The digestion model a model of in vitro digestion based in an initial saliva processing for 5 min at 37°C to simulate the mouth compartment and the gastric conditions for 2 h, followed by simulated small intestine compartment for 2 h at 37°C. The digested samples were lyophilized to use in cell culture. The human colon adenocarcinoma Caco-2 cells were cultured according to the methodology described by Zhang [1] followed by inoculation into 24-well plates at a density of 1 x 10^6 for cells/well and genotoxicity assessment by the cytokinesis-block micronucleus assay.

Digested samples were reconstituted and prepared for exposure to Caco-2 cells (100 µL sample in 900 µL of medium and cells) for 24 hours. The genotoxicity was evaluated by the quantification of micronuclei in Caco-2 cells in the different samples, being the results ongoing at the present.

[1] Z.J. et al., *Food and Chemical Toxicology* **83**, 54-60 (2015)

P04.

PROTECTIVE EFFECT OF ESSENTIAL OIL FROM LAVANDULA ANGUSTIFOLIA AGAINST UVA-INDUCED DNA DAMAGE

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The use of natural substances in the treatment or prevention of cancer is in the spotlight of intensive research over the past years. Essential oil from lavender (*Lavandula angustifolia;* LO) is a frequent ingredient of moisturizers, face and hand creams, cosmetics and sunscreens as a component helping to reduce the risk of UV-induced skin damage. UVA light has the ability to deeply penetrate the skin up to the derma causing erythremas, inflammation and photoaging, in addition of being a key risk factor in the etiology of skin cancer. However, the chemoprotective and photoprotective effect of LO, as well as the knowledge about mechanism of its action is not yet sufficiently understood.

The goal of this study was to investigate the protective effects of LO against UVA - induced DNA damage, and the levels of antioxidant enzymes (SOD - superoxide dismutase, GPx - glutathione peroxidase, CAT - catalase) in normal human keratinocytes (HaCaT) vs melanoma cell line (HMB-2) cultured *in vitro.*

To determine the effect of LO on the viability of selected cell lines, the MTT assay was used and the IC50 values were calculated for further experiments. The level of DNA breaks was evaluated using the comet assay. Biochemical methods were used to measure the activities of antioxidant enzymes (SOD, GPx and CAT), and the level of protein expression was confirmed by Western blot.

The results from MTT assay showed that the melanoma cell line HMB-2 was more sensitive to the treatment with LO compared to the normal kerationocytes HaCaT (IC50 for HMB-2 was 0.33 µl/ml and for HaCaT – 0.54 µl/ml). Interestingly, the UVA-induced DNA lesions were significantly reduced in both cell lines pre-treated with LO. Also, changes in the expression and activity of antioxidant enzymes SOD, GPx and CAT after treatment with LO and UVA were detected. In HaCaT cells, the increase of the activity of SOD as well as GPx was detected after the pre-treatment with LO. On the contrary, in HMB-2 cells activities of these enzymes were reduced.

Our results confirmed the antioxidant activity *Lavandula angustifolia* essential oil and its possible photoprotective effect against UVA-induced DNA damage.

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P05.

EVALUATING CYTOTOXIC AND GENOTOXIC EFFECTS OF MICROCYSTIN USING *SACCHAROMYCES CEREVISIÆ* AS EUKARYOTIC CELL MODEL

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Microcystins (MC) are one of the most common hepatoxins, produced by cyanobacteria. These toxins are cyclic peptides that have a high affinity for the Serine / Threonine (PPs) protein phosphatases family, namely PP1 / PP2A, acting as their inhibitors. MCs also induce oxidative stress in cells through the production of reactive oxygen species (ROS). Both effects have been associated to cytotoxic and genotoxic effects of MC in animal cells [1]. The effects of MCs on cells of higher eukaryotic organisms, such as animals and plants, have been extensively studied. However, a complete characterization of the effects of these toxins has not yet been achieved [1]. In this study the cytotoxic and genotoxic effects of MC on a eukaryotic cell model (the yeast *Saccharomyces cerevisiœ*) were evaluated. The cytotoxic effects were assessed using an MTT assay and the genotoxic effects evaluated using the comet assay and the expression levels of genes involved in DNA repair systems obtained by Real-Time PCR (RT-qPCR). The results obtained will be discussed.

[1] E. Valério, et al., *Mini Rev Med Chem.***16**, doi: 10.2174/1389557516666160219130553 (2016)

P06.

CYTOTOXIC AND GENOTOXIC EFFECTS OF AN L-AMINO ACID OXIDASE FROM *BOTHROPS JARARACUSSU* IN HUMAN CELL LINES HUVEC AND HEPG2

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Cancer is a chronic disease that is considered the mainly cause of death in developing countries. Due to high rates of morbidity and mortality in patients with cancer, it is mandatory to discover new forms of therapy in order to achieve better response to treatment. Studies have been developed in an attempt to identify new compounds with carcinogenic capacity. Many of these new compounds are derived from animal toxins especially in countries with high biodiversity. Also, L-amino acid oxidases (LAAO) isolated from venom of snakes have shown cytotoxicity in tumor cell lines.

The aim of this study was to assess the cytotoxic and genotoxic effects of a LAAO isolated from *Bothrops jararacussu*. Initially, concentrations from 0.25 - 5.0 µg/mL were tested in endothelial cells from human umbilical vein (HUVEC) and human hepatocellular carcinoma (HepG2) by MTT assay at 24 and 48 hours. After this, the DNA damage was evaluated at the same concentrations by comet assay. Phosphate-Buffered Saline (PBS) was used as negative control and methyl methanesulfonate (MMS – 300 µM) as positive control in both assays.

LAAO showed a significant decrease in cell viability as compared to negative control at all concentrations evaluated at 24 and 48 hours, evidencing its cytotoxic effect in both cell lines. In the comet assay, the two highest concentrations tested (1.0 and 5.0 µg/mL) significantly increased the amount of DNA damage as compared to negative control showing genotoxic effects. MMS also showed cytotoxic and genotoxic effects.

In conclusion, our results showed that all concentrations of LAAO tested reduced cell viability in human lines HUVEC and HepG2. The highest concentrations tested induced damage DNA. The next experiments will elucidate the mechanism of action of cytotoxicity and genotoxicity induced by LAAO.

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P07.

THE INFLUENCE OF CRYOPRESERVATION IN SPERM DNA DAMAGE

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Infertility is a worldwide problem and affects 15% of all couples trying to conceive. In general, the cause of infertility involves the male in one-third of cases, the female in another one third, and in the remaining cases, both the male and female, or no cause can be identified. Sperm DNA damage has been related to male infertility and it is associated with reduced fertilization rates, embryo quality and pregnancy rates, higher rates of spontaneous miscarriage and childhood diseases. Cryopreservation is a common technique used to preserve male sperm. This technique is routinely used in a variety of circumstances including assisted reproduction, pre-radiation or chemotherapy treatment, surgical treatments that may affect fertility and for storage of donor semen. However studies suggest that cryopreservation can cause DNA damage.

In this work it will be compared DNA damage of cryopreserved versus fresh sperm cells. From December 2015 to February 2016 a comet assay was performed in 11 samples from men aged between 28 and 43 years old, followed in the Hospital Center of Trás-os-Montes and Alto Douro fertility consultation. Semen was analyzed according to World Health Organization specifications. The comet assay was used to DNA damage analysis where Total Sperm DNA Damage Count (TDC) was assessed and compared in fresh and cryopreserved samples. From each sample, three aliquots were separated: one aliquot was immediately processed to comet assay and the other two aliquots were stored in liquid nitrogen and processed later. DNA damage was quantified by visual classification of nucleoids into five comet classes, according to the tail intensity and length, from 0 (no tail) to 4 (almost all DNA in tail). 50 comets per duplicate gel were analyzed, on a scale of 0-400 arbitrary units (AU).

We observed that all the cryopreserved samples presented higher DNA damage when compared with fresh samples. The average variation between fresh and cryopreserved samples was 190U. The minimum value obtain was 50AU and a maximum of 370 AU.

Despite the reduced number of samples, the study suggests that cryopreservation increases DNA damage, thus possibly being a rather counterproductive way to preserve male fertility as DNA damage is associated with reduced fertilization rates. However more samples are necessary in order to fully comprehend the role of cryopreservation in DNA damage.

P08.

ABORTION PRODUCTS - DNA DAMAGE AND REPAIR

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Pregnancy loss is the most common obstetric complication and is estimated to affect, at least, one in every four women who tries to conceive. Multiple factors have been associated with recurrent pregnant loss, chromosome abnormalities such as autosomal trisomy, monosomy X and polyploidy, are the most important ones. Identification of the causes of pregnancy loss is very important since may indicate if there is the risk of repetition, in order to reduce recurrence in future pregnancies.

Increased levels of DNA damage and ineffective repair mechanisms are the underlying bio-molecular events in the pathogenesis of most of the life-threatening diseases like cancer and degenerative diseases. Sperm DNA damage has been closely associated with numerous indicators of reproductive health, including, fertilization, embryo quality, implantation and spontaneous abortion. That fact contributes to the interest in analyzing DNA damage in abortion products.

Among the various methods employed in the estimation of DNA damage, alkaline comet assay is proven to be a relatively simple and versatile tool and also in determining the efficacy of DNA repair mechanism.

In this work DNA damage was analyzed in three conception products (PA1, PA2 and PA3). The analysis was made in fresh products and products with 24, 48,72h of culture. The objective was quantified the DNA damage and verified if the cells have the capacity to repair. The quantification was performed by visual classification of nucleoids. Five comet classes were used, according to the tail intensity and length, from 0 (no tail) to 4 (almost all DNA in tail). 100 comets per gel were analyzed, on a scale of 0-400 arbitrary units.

In the fresh samples analysis it was possible to verify that damage differs in the three products. PA1 does not present damage, with only 53UA; instead PA2 and PA3 present a high damage with 368UA and 232UA respectively. At the end of 48h in culture was possible to observe that the cells were capable to repair because the damage decreases to 49, 18 and 17 UA on PA1, PA2 and PA3 respectively. The damage variation at the 24h and at 72h of culture was not relevant.

Despite the small number of samples we could observe that DNA damage was dissimilar on the different products and the capacity to repair was obtained in all three samples. Because of that, this is an innovative approach to assess DNA damage in abortion products and the cell capacity to DNA repair as a line of investigation to know if there is a relationship between DNA damage and the occurrence of spontaneous abortions.

P09.

INFLUENCE OF VEGETABLES JUICES SOURCE OF LUTEIN AND BETA CAROTENE ON THE GENOTOXICITY INDUCED BY ALKYLATING AGENTS IN MICE

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Carotenoids represent one of the most studied bioactive compounds, and are classified into carotenes and xanthophylls, which are represented of beta-carotene and lutein, respectively. Recent studies related roles of these carotenoids in its synthetic form of protection in DNA damage. The aim of present study was to verify the action of juices source these carotenoids (kale juice and green juice), as well as lutein and beta carotene synthetic against DNA damage caused by the action of methyl methanesulfonate and cyclophosphamide, *in vivo*. Moreover, analyze the presence and amount these carotenoids in juices were determined by High Performance Liquid Chromatography. Albinos Swiss male mice were divided into 23 groups with 6 mice per group. After the treatments were collected blood samples from animals through an incision at the tail end for performing of Comet Assay in time 24h and 48h. The groups that received the juices in pre-treatment showed damage reduction greater than 50%, and those received the compounds synthetic showed a reduction of approximately 20%. Similar results to post-treatment. Chromatographic analysis showed that presence of trans β-carotene, cis β-carotene and the xantophylls lutein and zeaxanthin. The juices and carotenoids exercised a protective action and repair in pre and post-treatment, respectively. It was observed beneficial effect most pronounced in the groups that received juices, showing the synergism of the compounds present in the food matrix were more effective compared with single compounds.

P10.

IN VITRO AND *IN VIVO* ANTI-INFLAMMATORY ACTIVITY OF ANTHOCYANIN-RICH *C.ICACO* L. FRUIT

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C. icaco L. (CI) is an anthocyanin-rich fruit found in tropical areas such as the Brazilian Amazon and the Caribbean. Since this fruit contains expressive concentrations of anthocyanins, we hypothesize that CI may possess anti-inflammatory properties. This investigation aimed at assessing the anti-inflammatory activity of CI using both *in vitro* and *in vivo* models. Human colon cell lines CCD-18Co (normal) and HT-29 (cancer) were treated with CI anthocyanins (1.0 – 20.0 mg/L GAE) and the expressions of inflammatory markers IL-1β, IL-6, NF-κB and TNF-α were analyzed at mRNA and protein levels. TNF- α was used as inflammation inducer in CCD-18Co cells. Male Wistar rats were treated for 14 days with CI fruit (400 mg/kg b.w./day, gavage) and injected with doxorubicin (DXR, 15 mg/kg i.p.) 24 hours before euthanasia. The expressions of the same inflammatory markers were analyzed in liver, kidney and heart by real time quantitative PCR (RT-qPCR). 20.0 mg/L GAE CI anthocyanins decreased *IL-1β*, *IL-6*, *NF-κB* and *TNF-α* gene expressions in HT-29 colon cancer cells and downregulated IL-1β, IL-6 and TNF-α proteins in inflamed CCD-18Co cells. *Il-1β* was downregulated in liver, kidney and heart tissues of rats treated with CI fruit, and *Tnf-α* was also modulated in liver and heart. It is possible that the major phytochemicals in *C. icaco fruit* (petunidin-3-glucoside, all-trans-lutein and ellagic acid derivative) are the responsible for the anti-inflammatory activity observed in this investigation. Additional mechanistic studies are necessary to better evaluate the usage of CI as nutraceutical.

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P11.

DEVELOPMENT OF AN AUTOMATED SCORING SYSTEM FOR PLANT COMET ASSAY

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In ten years, the application of the comet assay has been established as one of the most interesting techniques in eco-genotoxicology. It is a rapid, sensitive and relatively inexpensive assay for measuring DNA damages and repairs in individual cells. However, its use in plant studies was rather limited compared to animal studies because of (i) the difficulty to isolate intact nuclei compared to animal systems, (ii) the low throughput of current nucleus extraction, and (iii) the lack of a high throughput comet assay scoring method.

In the frame of the French-Norwegian project ComPack (2014-2017), we first developed a new nucleus extraction technique compatible with the medium-throughput comet assay (12-gel system). This new extraction method appeared to be faster, more reliable and more efficient than the so far used methods. However, the mechanical nuclei extraction generates nonuniform backgrounds with several debris that could potentially induce bias in image analysis with automated scoring systems. Thus, we have optimized our nuclei extraction to reduce the presence of debris and increase background quality.

Meanwhile, we have worked on the automation of the scoring method which represents a technological breakthrough in plant comet assay. We have adapted the automated scoring system Pathfinder™, developed by IMSTAR, which was initially set up for human/animal cells, to score plant nuclei. Our promising results open up the perspective of an automated high-throughput scoring of plant nuclei.

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P12.

EVALUATION OF *MORINDA CITROFOLIA* CHEMOPREVENTIVE EFFECTS AGAINST PATULIN

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Morinda citrofolia (noni), is a tropical plant that has been studied for its content of phenolic compounds, which display antioxidant properties. Although many authors have shown that fruit pulp is the part of noni plant richer in those compounds, all parts (leaves, rods and stem) are being used as traditional medicines in tropical countries. Patulin (PAT) is one of the most common mycotoxins, frequently found in spoiled fruits (apples), moldy feeds and stored cheese, representing a serious health concern because of its genotoxic, carcinogenic, neurotoxic and teratogenic effects that are mainly mediated by the formation of reactive oxygen species. Therefore, it is of utmost importance to identify bioactive natural compounds able to reduce the deleterious effects of patulin and other mycotoxins, in order to protect the human health. This is a particularly relevant concern in tropical regions where food contamination with mycotoxins has a strong negative impact on human health. On the other hand, ethyl methanesulfonate (EMS) is a model alkylating agent inducing mostly point mutations.

This work intended to explore the chemopreventive properties of a noni fruit hexanic extract prepared in Brazil. Its potential capacity to reduce the cytotoxic and genotoxic effects of two compounds with dissimilar modes of action (MoA) - patulin and EMS - was evaluated in a liver-derived human cell line (HepG2 cell line) through the MTT and the Comet assays, respectively. Two different treatment setups were used throughout the cytotoxicity experiments: i) 24h pre-exposure of cells to extract (0.1 mg/ml) followed by 24h exposure to patulin (1.25 and 2.5 µM) or EMS (10 and 20 mM) in the absence of extract and ii) 48h exposure of cells to extract, with patulin or EMS being added for the last 24h. A similar setup was applied for genotoxicity analysis except that exposure to EMS (5 and 10 mM) occurred during 1h. Data analysis was performed using SPSS software Standard Edition.

The results showed that the cytotoxic effect of patulin or EMS was accentuated by the pre-exposure of HepG2 cells to the noni extract, reaching statistical significance for 2.5 µM of patulin. In contrast, cells pre-exposure to the extract followed by co-exposure to patulin or EMS was able to significantly reduce the level of cytotoxicity induced by the toxicants alone, except for the concentration of 10 mM of EMS. Accordingly, cells pre-treatment with the extract seemed to sensitize cells to the genotoxic effect of EMS. Further genotoxicity studies are underway to understand whether co-exposure to the noni extract is able to prevent genotoxicity induction by patulin or EMS. Overall, the noni extract under study has bioactive properties and can either sensitize cells or prevent cell death induced by two different toxicants, depending on the experimental setup.

P13.

BIOACTIVE COMPOUNDS FROM SEAWEED WITH ANTI-LEUKEMIC ACTIVITY: CAROTENOIDS AND PHLOROTANNINS

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Hematologic malignancies are the fourth most frequent cancer in the economic developed countries [1]. Lack of drug specificity, undesirable side effects and drug resistance are considered as important limiting factors for the success of the existing therapies. Seaweeds are an important source of bioactive compounds, with several biological activities described, but few explored in terms of leukemia treatment [2]. In this work were used fucoxanthin (Fx) [0-10 µM] and phloroglucinol (Ph) [0-300 µM], alone and combined with drugs used in leukemia treatment, such as doxorubicin (Dox) [0-1 µM] and imatinib (Imat) [0-10 µM], in order to produce more efficient and less toxic anticancer treatments. To achieve this aim, the effect on cell viability and proliferation was determined in K562 cells, derived from blood of a patient with chronic myeloid leukemia (CML) in terminal blast crisis, and TK6 cells, a human-derived lymphoblastoid cell line. After 24 h of treatment, cells were washed, counted to check cytotoxicity and plate during 48 h (in fresh medium, without the test compounds) in order to evaluate the effect on cell proliferation. For the combination assays, the natural compounds were incubated simultaneously with Dox and Imat (IC_{70}). The results from cytotoxicity/proliferation assay showed that TK6 seem to be more sensitive to Dox than K562, while Imat has anti-proliferation effects more accentuated in K562 cells. When incubated alone the natural compounds affect the viability of both cell lines, however the combinations that showed some improvement in the action of the drug was Fx/Ph with Imat in TK6 cells. To assess possible mechanisms involved in the anticancer effect of natural compounds, DNA damage was assessed by the comet assay in combination with the enzyme formamidopyrimidine DNA glycosilase (24 h of treatment), to detect not only DNA breaks but also oxidized and alkylated bases, and apoptosis was checked by nuclear condensation (48 h of treatment). Our results suggest that both natural compounds potentiate the anticancer activity, mainly with Imat in TK6 cells; however this toxicity is not due to DNA damage. Further studies are necessary to clarify the molecular mechanisms involved.

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P14.

MUTAGENICITY/GENOTOXICITY OF $PM_{0.5}$ COLLECTED DURING WINTER 2014-2015 IN FIVE ITALIAN CITIES: MAPEC (MONITORING AIR POLLUTION EFFECTS ON CHILDREN FOR SUPPORTING PUBLIC HEALTH POLICY) STUDY

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In the recent literature there has been an increased interest in the effects of particulate matter (PM) air pollution on health. The objective of the MAPEC (Monitoring Air Pollutions Effects on Children for supporting public health policy) study is to evaluate the associations between the concentrations of urban air pollutants and biomarkers of early biological effect in oral mucosa of 1,000 children recruited from first grade schools of 5 Italian towns (Brescia, Torino, Pisa, Perugia and Lecce) characterized by different PM levels. Child exposure to urban air pollution was evaluated by collecting ultra-fine PM ($PM_{0.5}$) samples in the school areas on the same day of biological sampling. $PM_{0.5}$ organic extracts were chemically analyzed (PAH, Nitro-PAH) and tested on human pulmonary A549 cell line by comet assay and micronuclei test and on Salmonella (TA100, TA98, TA98NR and YG1021 strains) by Ames test. Chemical analysis showed that $PM_{0.5}$ varied significantly among the five considered towns, with different percentage contribution to PM_{10} (range 19.6-63%). The results of Ames test showed that all $PM_{0.5}$ extracts induced indirect mutagenic effects in TA98 strain (net revertant/m3 range 0.3-1.5) while lower effect was observed with TA100 strain. Ames test with TA98NR and YG1021 strains showed the presence of nitroaromatic compounds. Except for some sporadic samples, no genotoxic or oxidative effect induced by $PM_{0.5}$ extracts was demonstrated using comet assay and micronuclei test. These results could be explained by low level of air pollution observed in this winter sampling (2014-2015) and suggest further studies on biological effects of PM fractions, in particular, of the finest fraction of PM.

P15.

DNA BINDING PROPERTIES OF A SERIES OF NOVEL DERIVATIVES OF 1,4-DIHYDROPYRIDINE

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1,4-dihydropyridines (1,4-DHP) possess important biochemical and pharmacological properties, however data on their interaction with DNA are scarce.

In the present study we have determined mode of interactions with DNA of a series of 1,4-dihydropyridines with different biological activities synthesized in the Latvian Institute of Organic Synthesis. UV-VIS spectra of the tested compounds were recorded on a UV/VIS spectrophotometer in absence of DNA and presence of increasing amounts of rat liver or plasmid DNA. Observation of hyperchromic or hypochromic effects produced by DNA indicated interactions of the substance with minor grove of the DNA or intercalation of the substance between the DNA strands.

In a series of water-soluble monocyclic derivatives of 1,4-dihydropyridine with carboxylate groups in position-4 manifested different affinity to DNA determined mainly by substituents in positions 3 and 5. Compounds with cyano group or acetyl groups in position 3 and 5 (J-3-183 and AV-154 correspondingly) did not interact with DNA. Replacement of 3,5-acetyl groups (AV-154) with ethoxycarbonyl groups (AV-153) made the compound capable to interact with DNA: a pronounced hyperchromic and bathochromic effects were observed. Further addition of glutamic acid in position-4 as amide of 2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydroisonicotinic acid (glutapyrone) decreased affinity of DNA, but addition of taurine in the same position (tauropirone) abolished ability of the compound to interact with DNA. Unsubstituted in position-4 of 1,4-DHP, derivative carbatone [disodium-2,6-dimethyl-1,4-dihydropyridine-3,5-bis(carbonyloxyacetate)] manifested hyperchromic effect in the presence of DNA without any spectral band position shifts. Similar, but stronger effect were observed in spectra of tricyclic fused 1,4-DHP derivatives – decahydroacridine-1,8-diones (PP-150-Na and PP-544-NH_4). Thus DNA-intercalating activities of 1,4-DHP are evidently determined by groups in positions 3 and 5. Ability to interact with DNA does not correlate with other effects produced by the compounds: radical scavenging or peroxynitrite binding.

P16.

MODIFICATION OF GENES INVOLVED IN DNA REPAIR AND NITROSATIVE STRESS AND PROTEASOMAL SYSTEM BY 1,4-DIHYDROPYRIDINES

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One of the novel treatment strategies of diabetes mellitus and its complications can be aimed on decrease of production of reactive oxygen species with consequent DNA damage prevention, normalization of NO production and DNA repair enhancement. 1,4-dihydropyridine (1,4-DHP) derivatives appear to be prospective as novel drugs with above spectrum of effects. Aim of our work was to evaluate impact of a group of novel 1,4-DHP on expression of genes involved in nitric oxide production and DNA repair.

Induction of diabetes mellitus model by injection of streptozotocin to rats increased expression of *iNos* gene in kidneys and blood cells, decreased expression of *eNos* in kidneys cells, DNA-repair related gene *Parp1* was up-regulated in blood cells, DNA breakage level was increased in nucleated blood cells. AV-153, metcarbatone and etcarbatone increased *eNos* gene expression in kidneys of diabetic animals, the effect seems to be favourable in DM conditions. Etcarbatone, metcarbatone and glutapyrone normalized *iNos* expression in blood cells of diabetic animals. Surprisingly, we have observed increased DNA breakage in animals treated with AV-153, known to be antimutagenic and DNA-repair enhancing compound, it also enhanced effects of DM on DNA integrity and expression of DNA repair-coupled gene *Parp1*. Etcarbatone decreased expression of these genes in blood of diabetic animals, although it even increased the DNA breakage induced by diabetes. Glutapyrone *per se* increased expression of *Parp1* in kidneys, but decreased in blood. J-9-125 and metcarbatone increased expression of DNA repair genes in kidneys of both intact and diabetic animals, however metacarbatone was the only compound able to reduce DNA breakage in diabetic animals. Conclusions: 1,4-DHP can interfere with expression of genes involved in DNA-repair and nitrosative stress. Effects of the compounds are dependent on structure and organ-specific.

P17.

CONSIDERING CELL TYPE FOR *IN VITRO* NEUROGENOTOXICITY TESTING: NEURONAL *VS.* GLIAL CELL SENSITIVITY

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In vitro models may be useful for the rapid toxicological screening of large numbers of agents regarding their potential to produce toxicity. Cell cultures derived from nervous system tissue have proven to be powerful tools for elucidating cellular and molecular mechanisms of nervous system development and function. They are also frequently used to understand the mechanism of action of neurotoxic agents and to assess critical cellular events of neurodevelopment, including neural differentiation and neurite growth. Different cells of neural origin, including neurons, astrocytes, oligodendrocytes or microglial cells, are commonly used in the literature as neurotoxicity models to evaluate *in vitro* the possible effects of chemicals and drugs to the cells and physiology of the nervous system. However, these cells have been shown to respond to neurotoxic insults in a disparate way, leading to non-consistent results from different studies.

The main objective of this study was to compare the sensitivity of two cellular models of neural origin commonly employed in *in vitro* neurotoxicity screening. To achieve this aim, neurons (SHSY5Y) and glial cells (astrocytes A172) were treated with a set of genotoxic agents (i.e. bleomycin [BLM], griseofulvin [GF], actinomycin-D [Act-D], mytomycin C [MMC] and methyl methanesulfonate [MMS]). All these five agents induce DNA damage by means of different well-known mechanisms; thus, they were chosen to cover all main types of genetic damage. After discarding cytotoxicity by means of MTT assay, genotoxicity induced by these compounds in neuronal and glial cells was evaluated by a battery of assays encompassing detection of a range of genetic lesions.

The five compounds chosen to induce DNA damage, together with the different genotoxicity tests performed, provide a complete vision of the different sensitivity of the nervous system cells to the genetic damage induced, as well as offer the basis to properly employ these approaches as neurotoxicity *in vitro* models for DNA damage evaluation.

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P18.

CYTOTOXICITY AND ANTIBACTERIAL ACTIVITY OF POLAR EXTRACTS OBTAINED FROM SAFFRON (*Crocus sativus* L.) FLORAL BIO-RESIDUES

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Saffron (*Crocus sativus* L.) flower comprises six purple tepals, three yellow stamens and a white filiform style ending in a stigma with three threads, which represents less than 10% (w/w) of the flower weight. However, the stigma is the dominant reason to farm/harvest saffron, since it represents the most valued spice (after being dried) [1]. Interesting large amounts of floral bio-residues are produced and wasted from this action, considering that a single kg of saffron spice generates ~63 kg of floral bio-residues. This scenario creates opportunities for the extended use of this discarded material, particularly owing to its important bioactive compounds and related health promoting properties, such as antioxidant, antityrosinase, antidepressant, antifungal, antinociceptive, anti-inflammatory and arterial pressure reduction [2]. The effectiveness of bioactive compounds extraction from plants, as well as their bioactivity, is dependent on factors such as solvent polarity or solvent-to-solid ratio [3]. Herein the cytotoxic effects of different polar extracts (ethanol, ethanol:water 1:1 v/v, and water) of saffron were evaluated in Caco-2 (ATCC® HTB-37™) cultures by using LDH (lactate dehydrogenase) and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) assays. In addition, the antibacterial activity was also evaluated against a panel of indicator strains and selected clinically and food relevant human pathogens using the agar well diffusion and broth dilution methods. Saffron extracts induced dose-dependent decreases in viability percentages of Caco-2 cells, particularly pronounced at 24 h treatments (but also observable after 1h of exposure to hydroethanolic and ethanolic extracts). The aqueous was the less toxic to Caco-2 cells. Moreover, no inhibition zones were observed and no significant differences were noticed between the OD values of the control strains and the treated cells at all concentration tested. Although our results suggested that there was no apparent effect on the panel of pathogen and indicator strains tested in the presence of floral bio-residues extracts, further investigations need to be done for a better comprehension of the biochemical characterization and concentration of the bioactive compounds present in the extracts. However, the bioactivity results indicate that saffron flowers might have different applications in the development of food supplements and/or ingredients or pharmaceutic related products.

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P19.

EVALUATION OF ISOFLAVONE CONTENT AND CYTOTOXIC ACTIVITY OF TWO NEW MEXICAN ALFALFA-BASED FOODSTUFFS

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Alfalfa (*Medicago sativa*) has been used as traditional medicine for the treatment of some diseases, being a source of phytoestrogens (mainly isoflavones), with beneficial properties for humans. Furthermore, this plant is widely consumed, either directly ingested as a fresh food or as in the form of derived foodstuffs. Therefore, the comprehensive chemical characterization and the evaluation of different types of bioactivity is of utmost importance. Herein the isoflavone profiles and the potential cytotoxicity Caco-2 cells were evaluated in Mexican alfalfa-based foodstuffs (dehydrated powder and freeze-dried juice) prepared from plants harvested in different periods (batch 1: May; batch 2: July). Isoflavones were extracted with water and their analyses were carried out by HPLC-DAD [1]. The membrane permeability and mitochondrial activity of Caco-2 (ATCC® HTB-37™) cells treated with alfalfa products extracts at different concentrations (ranging from 1 mg/mL to 100 mg/mL) at two distinct exposure periods (3 and 24 hours) were measured by LDH (lactate dehydrogenase) and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) assays, respectively.

Among the assayed isoflavones (genistein, genistin, daidzein, daidzin, formononetin, biochanin A and glycitein), freeze dried juices from batch 1 contained 43.3 mg of daidzein and 4.6 mg of genistein/100 g, while batch 2 presented 27.3 mg daidzin and 11.7 mg daidzein/100 g of sample. Only daidzein was detected in dehydrated powders, having been verified significant differences among the two analyzed batches: 36.0 mg/100 g, for batch 1; 28.3 mg/100 g for batch 2).

Regarding the potential cytotoxicity of the extracts, the results obtained for each of the assayed extracts were also different. A significant decrease in cell viability (at 3 hours of exposure) was observed in cells exposed to the extracts prepared at 100 mg/mL of alfalfa products in the MTT assay, while a similar effect was observed in cells exposed to extracts prepared at 50 mg/mL, after 24 h of exposure. In general, the effects on cell viability were modulated by exposure time and extract concentration. A concentration-dependent increase in LDH release was observed for both exposure periods, particularly in the case of freeze-dried juice samples. The recovery assay demonstrated that the cytotoxic effects in Caco-2 cells were irreversible. Accordingly, the potential uses of alfalfa-derived products should be considered carefully, particularly those prepared with concentrations above a determined threshold value.

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P20.

CYTOTOXIC EFFECTS OF HYDRO-ETHANOLIC EXTRACTS FROM *Coleostephus myconis* (L.) Rchb.f. FLOWERS AND GREEN PARTS

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The species *Coleostephus myconis* (L.) Rchb.f. is considered as being a harmful and persistent weed, with constant presence in abandoned farmland. Prevailing in the Portuguese flora, *C. myconis* belongs to the Asteraceae family, which is recognized for its global medicinal value and high antioxidant power, as described in different research works [1]. Nevertheless, before considering incorporating this species in food of pharmaceutical applications, it is imperative to evaluate their potential toxicity. Herein, the cytotoxic effects of the stems and leaves and the senescent flowers of *C. myconis* were evaluated in the HepG2 human tumor cell line. MTT and NRU assays were used to test the potential cytotoxicity of the hydro-ethanolic extracts in different concentrations. Two sequential exposure times (24 and 48 h) were evaluated. In addition, to determine whether cells treated with *C. myconis* extracts recovered their proliferative capacity after removing the extracts, the cell recovery assay was used. A parallel set of experiments conducted without cells was carried out to exclude the potential interaction of the extracts with the dyes used in MTT and NRU assays.

The results for the minimum assayed concentration (corresponding to the EC_{25} obtained in previous antioxidant assays) were nearly the same as those obtained for the negative control for both plant parts. Hence, the $EC_{37.5}$ was considered as the minimal assayed concentration, while the EC_{75} corresponded to the maximal. In both cytotoxic assays, cells exposed to stems and leaves extracts (for 24 h and 48 h), showed a statistically significant dose-dependent decrease in viability. However, the mitochondrial damage (MTT assay) seemed to be irreversible, while the lysosomal damage (NRU assay) was reversible, as cell viability was found to be much higher than the one observed immediately after exposure. The results for the senescent flowers were basically the same, except for the lysosomal activity, which could not be restored in the 24 h exposure assay. Our results showed lower viability detected by MTT assay, in comparison with the NRU assay, suggesting that mitochondria is particularly sensitive to the *C. myconis* extracts studied herein. Comparing the effects of the assayed botanical parts, the extracts obtained from stems and leaves allowed higher viability of HepG2 cells, as evidenced by the IC_{50} values obtained in each case (except for the NRU assay under 48 h of exposure). In general, mitochondrial (MTT assay) and lysosomal (NRU assay) damage were only considerable when exposing HepG2 cells to the concentrations corresponding to the EC_{75} for both plant botanical parts.

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P21.

IN VITRO AND *IN VIVO* EVALUATION OF COFFEE SILVERSKIN AS COSMETIC INGREDIENT

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Coffee Silverskin (CS) is a main by-product of the coffee roasting procedure and has no commercial value, being discarded as a solid waste [1]. Recent advances in industrial biotechnology led to potential opportunities for economic valorization of this by-product [2]. Some work has been performed on the properties of CS, in particular its antioxidant behavior, which reveals a good potential [3-5]. These compounds are believed to provide *in vivo* protection against free radical damage. The assessment of irritation is one of the primary procedures to evaluate and classify the potential hazard of a substance, particularly, in cosmetics or pharmaceuticals [6]. A number of *in vitro* tests to assess potential skin or eye irritants has been developed. The study presented herein was conducted to evaluate the *in vitro* and *in vivo* irritation potential of three CS polar extracts.

Extracts were prepared according to procedure described by Rodrigues *et al.* [3]. Three different extracts of CS were evaluated. For both EpiSkin™ and SkinEthic HCE™ assays, MTT and IL-1α were used as endpoints. After the extract contact with the model, the histology of the model was evaluated. To ensure the possible content of caffeine, chlorogenic acid (CQA) and 5-hydroxymethylfurfural (HMF) that pass through the model, an HPLC assay was developed. *In vivo* skin irritation potential observed after single application under occlusion was assessed along with sodium lauryl sulfate (SLS) solution (2%, w/v) as irritant model (positive control) and water as non-irritant (negative control).

In vitro studies revealed that CS extracts are safe regarding skin and ocular irritancy as cell viability was equal to the negative control in both assays and the IL-1α was under 50%. The histological analysis demonstrated that extracts did not affect the skin neither the ocular model. Quantitative chromatographic investigations revealed that the three extracts contained caffeine and HMF, but there were no traces of chlorogenic acid. The *in vivo* patch tests proved that the hydro-alcoholic extract did not cause skin irritation.

The next steps to evaluate the safety of the extract would undergo sensitization and tolerance studies in normal condition of use.

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P22.

TOXICITY ASSESSMENT OF CONVENIENCE PRODUCTS USING THE CILIATED MODEL *TETRAHYMENA PYRIFORMIS*

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Understanding the possible toxicity of various convenience products used daily is essential to protect human health. *In vitro* tests present many advantages as they are usually quicker, less expensive, are carried out under controlled experimental conditions and allow the reduction on the use of animals for testing purposes. The protozoan *Tetrahymena pyriformis* is a well-recognized standard for toxicity testing frequently used as model for studies of environmental and industrial pollutants and biological toxins.

The present work aims to assess the toxic effect of different convenience products for human health using one of the most common protozoan model *Tetrahymena pyriformis*. Studied products included pharmaceutical products, polymer/plastic and textile/toys articles, hygiene items, paper and materials used with household and construction purposes. *T pyroformis* were exposed to extracts of these products for 3 h (acute) and 24 h (sub-acute). The median lethal dose (LD_{50}) of *T.Pyriformis* and the coefficient of accumulation on the lethal level (K_{kum}) were calculated. According to obtained K_{kum} values, each product was assigned to a certain class of toxicity [1, 2].

Results showed that K_{kum} ranged from 0.37 – 2.80 mg/mL in polymer/plastic samples (14 samples), from 0.73 – 3.03 mg/mL in textile/toys samples (6 samples), from 0.64 – 1.94 mg/mL in construction mixtures (7 samples) and from 0.66 – 1.09 mg/mL in the remaining samples (hygiene items, paper and materials). All studied products were referred to the fourth class of toxicity.

All investigated products present a low hazard to human health. This work reinforce previous studies results, which reports that *T. pyriformis* proved to be fast and reliable bio-test for estimation of toxic effects of products of human use.

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P23.

LONG-TERM GENOTOXIC EFFECTS OF IMMUNOSUPPRESSIVE DRUGS ON LYMPHOCYTES OF KIDNEY TRANSPLANT RECIPIENTS

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Immunosuppressive therapy is essential for patients after transplantation due to their ability to reduce rejection rates by suppressing the immune system activity. However, after long exposure time, they decrease quality of life and survival of the patients due to their high level of cellular toxicity. One of major serious complication of the use of immunosuppressive drugs is the risk of developing cancer. Micronucleus and Comet assays are established biomarkers to detect this risk. Therefore, the aim of this study was to evaluate if the prolonged use of immunosuppressive can induce mutagenic effects contributing to increase cancer risk and also associate the DNA damage with allograft function. The degree of DNA instability was assessed in blood lymphocytes of 76 kidney transplant patients from Kidney Institute of Londrina (PR – Brazil) using comet and micronucleus assays. The group of patients was constituted by 43 men and 33 women with mean age of 46 ± 13 years. Time after transplantation ranged from 1 month to 28 years and the estimative of the glomerular filtration rate (eGFR) ranged from 12.4 to 110 mL min-1 per 1.73 m2. Preliminary analysis of CBMN assay showed that the cells presented a very low proliferation index (NDI). This result made impossible the analysis of micronucleus in binucleated cells. For this reason, MN analysis was performed only in mononucleated cells without cytochalasin-B, that indicates the presence of *in vivo* DNA damage before the start of culture. No association between mononucleated micronucleated cells and the time elapsed after transplant was observed. On the other hand, Comet assay results indicated that the induction of DNA damage was associated with exposure time, i.e., the longer the time of transplantation, the higher DNA damage observed. It also was observed that the eGFR of patients did not influence the incidence of DNA damage. Despite the association between DNA damage and time post-transplant, it is not yet possible to indicate the score of damage as a marker to be used in clinical practice to identify patients with cancer risk after long time of kidney transplant and immunosuppressive therapy. In conclusion, the analyses of transplanted patients in the present study showed that the longer the time of transplant, lower the level of glomerular filtration rate and higher DNA damage, which is possibly due to prolonged therapy with immunosuppressive drugs.

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P24.

DIFFERENTIAL MODULATION OF TOXICITY PATHWAYS BY RESPIRATORY AND CUTANEOUS ALLERGENS: INTERACTION WITH PHYSIOPATHOLOGY

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The massification of the use of toiletry products as well as the exposure to environmental and industrial chemicals strongly increased the incidence of allergic contact dermatitis (ACD) and chemical respiratory allergies. Skin sensitizers and chemical respiratory allergens share numerous characteristics and both trigger T cell-dependent responses, however the respective physiopathologies are orchestrated by different T effector subsets. Therefore, is reasonable to speculate that contact and respiratory sensitizers use different signal transduction pathways to induce dendritic cell (DC) maturation and cytokine/chemokine polarizing profiles. The aim of this study was to investigate, on the human DC-like cell line THP-1, the intracellular toxicity pathways elicited by 1-fluoro-2,4-dinitrobenzene (DNFB) and trimellitic anhydride chloride (TMAC), two golden standards of contact and chemical respiratory allergy, respectively. To achieve our goal we addressed the modifications evoked by chemicals in several toxicity pathways, for instance on the mitochondrial membrane potential, on oxidative stress parameters, on the modulation of MAPKs signaling pathways and on the transcription of physiopathological relevant genes. According to our data, the strong oxidative stress rapidly caused by DNFB mainly results from the direct depletion of intracellular GSH, while the most delayed stress induced by TMAC is a consequence of increased xanthine oxidase activity with consequent cytoplasmic ROS production. This temporal and intensity differences result in distinct MAPK activation profiles and in a differential modulation of genes involved in cytoprotection, DC maturation and T cell polarizing profile such as HMOX1, CD86, IL1B and IL8.

P25.

IMPACT OF PHYSICAL EXERCISE TRAINING ON DNA DAMAGE AND REPAIR: DOES GENDER PLAY A ROLE?

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Acute physical exercise is associated with an enhanced aerobic metabolism, which can result in an increased formation of reactive oxygen species (ROS). ROS can react with DNA, causing strand breaks and modified bases, namely 8-oxoguanine, one of the most common products of oxidative DNA damage, which is repaired by 8-oxoguanine DNA glycosylase 1 (OGG1). Regular physical exercise is considered as a key component of a healthy lifestyle, and its preventive effect, at least in part, is due to oxidative stress-induced adaptation, which has been related with an increase in antioxidant activity and in oxidative damage repair enzymes [1]. Gender-related differences concerning DNA damage and DNA repair have been reported [2]. Therefore, the main purpose of this study was to analyse the effects of 16 weeks of combined physical exercise training on DNA damage and repair, in 26 healthy Caucasian individuals, 14 males and 12 females. The comet assay was carried out using lymphocytes and enabled the evaluation of DNA damage, both DNA strand breaks (DNA SBs) and FPG-sensitive sites, and also of DNA repair, evaluated by OGG1 activity. Regarding differences between pre and post-training, the results showed a significant decrease in DNA SBs in male group ($p=0.004$) and female group ($p=0.028$). A significant decrease was also observed in FPG-sensitive sites in both men ($p=0.028$) and women ($p=0.003$). A significant increase in total antioxidant capacity, evaluated by ABTS, was observed in male ($p=0.041$) and also in female ($p=0.002$). There were no significant differences in DNA repair activity in both groups. Regarding differences between male and female groups, the results showed significant differences in ABTS ($p<0.001$) only in pre-training, being higher in male than in female. Significant differences in FPG-sensitive sites post-training ($p=0.031$), with also high values in male, were observed. This pilot study showed that physical exercise training has similar effects in both men and women, increasing total antioxidant capacity and decreasing DNA damage, with a more evident reduction in oxidative DNA damage in women.

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P26.

CAN THE COPD ASSESSMENT TEST (CAT) BE USED TO IMPROVE COMMUNICATION BETWEEN CLINICIANS AND LOW LITERATE ELDERLY PATIENTS? RESULTS FROM A CROSS-SECTIONAL STUDY IN COVA DA BEIRA, PORTUGAL

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Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease characterized by progressive airflow obstruction and destruction of lung parenchyma. In 2002 it was the fifth most common cause of death worldwide and it expected to rise to the 3rd place by 2030. COPD greatly affects the quality of life (QoL) of the individuals, the majority of whom are elderly and frail. In Portugal, and in particular at Cova da Beira region, the patients only visit their pulmonologist once or twice a year, and therefore the disease management is mainly performed at primary care by general practitioners with local pharmacies also playing an important role. The present study was aimed at evaluating if the COPD assessment test (CAT) could be used as a communication aid between patients with COPD and their clinicians in a population mainly composed of elderly illiterate patients. A total of 84 patients with stable COPD were recruited by a medical doctor from the Pulmonology Department of Centro Hospitalar Cova da Beira. The disease severity was assessed and classified according to the Global Initiative for Obstructive Lung Disease (GOLD) by the pulmonology specialist. The patients were then interviewed by a pharmacist that also applied the COPD assessment test (CAT) to determine the impact of COPD in the quality of life (QoL). The obtained results disclose that the majority of the respondents were male (93%) with a mean age of 64 years old and at stage II of COPD. The vast majority (88%) of COPD was related with tobacco smoke, with 63% of the patients being former smokers and 25% current smokers. Almost half of the recruited patients (48.8%) exhibited other comorbidities, including hypertension, congestive heart failure and arrhythmia. CAT scores ranged from 6 to 37 revealing that in 56% of the patients the impact of COPD in their QoL is medium, in 27.4% is high and in 7.1% is very high. Elderly individuals, those with comorbidities and those with recent exacerbations and hospitalizations showed higher CAT scores. Our results suggest that the CAT has good discriminative properties and can be a useful instrument to be applied at home and/or primary care as an integrated measure of QoL in patients with COPD. CAT thus provide a redundancy mechanism that is able to send the general practitioner an alert sign on the requirement of a specialist intervention, when the patients' communicational skills fail to do so.

P27.

IMMUNOLOGICAL BIOMONITORING OF ELDERLY ADULTS – INFLUENCE OF PHYSICAL ACTIVITY

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The increase in life expectancy in developed countries is accompanied by concern about the possible raise in the incidence of aging related diseases in the elderly. Increase in the basal levels of inflammatory response during aging is associated with alterations in innate and acquired immunity. Inflammation also arises in response to the continuous antigenic charge from subclinical infections, atherosclerosis and other chronic diseases. This involves a chronic inflammation condition, which has been related to many harmful effects and may significantly contribute to the increase in morbidity and mortality in elderly.

Initiatives to encourage healthy aging have been triggered by adopting lifestyles aimed to reduce the incidence of common chronic diseases and to improve the quality of life, including environmental, physical, psychological and social factors. These initiatives include promoting physical activity, which is known to be related to good health and physical and psychological wellbeing. Benefits of physical activity are particularly relevant in the elderly, since it contributes to decrease risk factors and prevent diseases.

The objective of this work was to determine whether immunological biomarkers are related to physical activity in a population of elderly adults, by means of a cross-sectional study including 259 individuals aged 65 and over. Data on physical activity and several clinical markers (nutritional status, functional status, cognitive dysfunction, and depressive symptoms) were obtained by means of appropriate questionnaires. Percentages of different lymphocyte subsets, and serum concentrations of neopterin, tryptophan and kynurenine were determined as immunological biomarkers.

Results obtained showed that several lymphocyte subsets and serum levels of neopterin, tryptophan and the ratio kynurenine/tryptophan (indicative of indoleamine 2,3-dioxygenase enzyme activity) were significantly different in the group of low physical activity with regard to the group performing normal physical activity, and some immunological biomarkers were associated with cognitive impairment and functional status. These data contribute to reinforce the belief that physical activity supports healthy aging, particularly by helping protect the immunological system from aging-related changes.

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P28.

THE IMPACT OF AN ACUTE EXERCISE CHALLENGE ON DNA DAMAGE – AN HUMAN INTERVENTION

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Even though it is commonly accepted that a regular physical activity along with a healthy diet brings positive health-related outcomes, it also seems to be related to several alterations in the human metabolism, organs, cells and deoxyribonucleic acid (DNA). Indeed, there is consistent evidence supporting that above a certain level of intensity and duration, exercise may induce an increase in the generation of reactive oxygen species (ROS).

The aim of this study was to analyse the immediate impact of an acute exercise challenge on human's DNA damage, taking into account their individual characteristics and fitness levels. The study population consisted of 22 females and 15 males, totalising 37 subjects, between 18 and 35 years old. The participants had their Body Mass Index calculated, their waist circumference measured and some relevant individual information was collected using questionnaires. Baecke's and International Physical Activity Questionnaire (IPAQ) were also used to characterise the participants' usual physical activity. Participants underwent a progressive multistage exercise test, Bruce Protocol, performed in a treadmill, until exhaustion, and alkaline and oxidative versions of the comet assay were performed to detect DNA damage.

Results attained from the comet assay suggested a decrease of DNA damage after the treadmill exercise, which was consistent both with primary and oxidative damage, even though only the primary damage was statistically significant. Other than exercise, gender, age, asthma, disease risk or regular physical activity level, did not seem to significantly influence the levels of DNA damage.

Data obtained suggest that a single bout of exercise was no sufficient to induce immediate DNA damage; to refer, however, that sampling timing immediately after exercise may have also influenced this result. In this context, more in-depth research should follow.

P29.

CHRONIC EXPOSURE EFFECTS OF THE CRUSTACEAN *DAPHNIA MAGNA* TO ENVIRONMENTAL CONCENTRATIONS OF THE STIMULANT DRUG NICOTINE

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Stimulant drugs, as nicotine, can represent interesting indicators of anthropogenic contamination as a consequence of their widespread detection in the environment and their potential relationship with water contamination levels, giving an indication of the presence of other chemicals related to human activities. Nonetheless, there are fewer studies evaluating the ecotoxicological effects of nicotine on aquatic communities. Consequently, the aim of the present study was assess the chronic effects on *Daphnia magna* of nicotine environmental concentrations. Exposure of maternal dapnhids was performed using the concentrations of 0.5, 1.0, 10.0, 100.0 μg L^{-1}, during 21 days. The endpoints used were molting, growth, number of offspring per female and abnormalities on offspring. The nicotine exhibited no significant effects on survival, molting frequency, growth, and time to first brood at any test concentration. Nonetheless, it showed significant effects on number of offspring per female at the highest concentration tested (100 μg L^{-1}) with a decline of 15% of the reproduction rate. This effect was accompanied by the sporadic appearance of neonates with abnormalities (0.3-0.6%) at all concentrations tested, as well as a small production of males at the two highest concentrations tested. The results suggested that the environmental concentrations of nicotine presented a slight action on the reproductive performance of *Daphnia magna*.

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P30.

DECREASE OF NADPH (P450) REDUCTASE: A CAUSE OF ATRAZINE TOXICITY IN SEA LAMPREY JUVENILES IN FRESHWATER

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The sea lamprey, *Petromyzon marinus* L., 1758, is a cyclostome widely distributed along North Atlantic which is present in several rivers basins of Iberian Peninsula. Nowadays, in Portugal this specie is considered as "Vulnerable" because of the population decrease along the last decade.

The aim of this study was to determine the effects of atrazine in the hepatic biotransformation markers of sea lamprey juveniles from Lima river basin, Portugal.

Sampling occurred at the beginning of the sea lamprey downstream migration in the Lima river basin, Portugal. The macrophthalmia were transported alive to the laboratory and maintained in 200 L tanks with LSS life support system. The body and liver weight were determined after euthanasia of the animals. The specimens were separated in six groups of three pools (#8): i and ii) macrophthalmia kept in freshwater for 7 or 30 days; iii-vi) macrophthalmia, kept in freshwater and exposed to 1, 10, 50 or 100 µg/L atrazine for 30 days. Microsomes obtained from liver homogenates by differential centrifugation were used for determination of heme content and the enzyme activities NADPH (P450) reductase (CPR), UDP-glucuronosyltransferase (UDPGT), glutathione *S*-transferase (mGST) by UV-Vis spectrophotometry and ethoxycoumarin *O*-deethylase (ECOD) by fluorimetry. Statistical analysis includes ANOVA I and Duncan test.

The results showed a decrease in the body weight, HSI, and hepatic CPR activity of macrophtalmia exposed to 100 µg/L atrazine as well as an increase in the hepatic UDPGT (1 µg/L) and mGST activities (correlated with the atrazine level) ($p < 0.05$). The heme content, and ECOD activity of hepatic microsomes were not affected by either concentrations of atrazine used in this study ($p < 0.05$). This response suggests that atrazine caused toxic effects in macrophtalmia kept in freshwater environment, probably due to the partial blockage of the electrons flow from NADPH to CYP1A, needed for deealkylation. The conjugation of atrazine with glutathione or glucuronic acid, probably due a monofunctional induction of mGST or UDPGT may have only compensated the failures in Phase I reactions of the animals exposed to the lower levels of the herbicide.

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[1] O. Arellano-Aguilara, et. al., *Reviews in Fisheries Science* **17**, 541–556 (2009)

P31.

ACUTE TOXICITY AND ENVIRONMENTAL RISK OF THE MALATHION FORMULATIONS USED TO COMBAT *AEDES AEGYPTI* MOSQUITO FOR AQUATIC ORGANISMS

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To control *Aedes aegypti* adult mosquito, the transmitter of dengue, Zika, Chikungunya and yellow fever virus, the Ministry of Health (MH) of Brazil indicates the application of malathion insecticide spraying in the ultra-low volume peridomiciliary. The malathion formulation diluted in soy oil has been used in the recent years, but the World Health Organization has recently approved a diluted formulation in water which decreases application costs. Although, both sprays can contaminate the environment in general and can reach non-target organisms, featuring a potential harmful agent to the environment.

This study aimed to quantify the acute toxicity of two malathion formulations used by MH to combat *Ae. aegypti* using the median effective concentration (EC50) that causes 50% of effect on *Daphnia magna, Lemna minor* and *Hyphessobrycon eques*, classifying them by the acute toxicity and also by the risk of environmental poisoning classes.

Definitive tests were performed with malathion water-soluble formulation (Komvektor® EW 44 %) and soy oil soluble formulation (Fyfanon® ULV 96.5 %). The Fyfanon® was diluted in methanol-water mixture. The insecticides were classified into acute toxicity and environmental toxicity risk classes, according to the EC50. The risk of environmental poisoning was calculated for three scenarios with different surface water depths (0.3, 1.0 and 2.0 m), and malathion doses were 146 $g.ha^{-1}$ to Fyfanon® and 150 $g.ha^{-1}$ to Komvektor®.

Our results showed that both malathion formulations are classified as highly toxic and have a high environmental risk to daphnia in all depths (EC50;48h = 0.0016 to 0.027 $\mu g.L^{-1}$), except to 2.0 m depth of the aqueous formulation, classified as medium environmental risk. Moreover, malathion is slightly toxic to lemna (IC50;7d = 14.75 to 24.39 $mg.L^{-1}$) and moderately toxic to the fish (CL50;48h = 3.05 $mg.l^{-1}$), with low environmental risk to both test organisms.

The toxic effects of malathion were visually observed for all organisms. *D. magna* is the most sensitive specie to acute intoxication by malathion and has a high risk of environmental poisoning due to the peridomiciliary use. On the other hand, the environmental risk of poisoning to fish and lemna is low. The aqueous formulation is less toxic than the oil formulation, but both are classified in the same acute toxicity and risk of environmental poisoning classes.

P32.

BIOCHEMICAL RESPONSES OF THE ANNELIDAE POLYCHAETE PERINEREIS CULTRIFERA TO HEAVY METAL ENVIRONMENTAL CONTAMINATION ALONG THE EAST COAST OF ALGERIA

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During the last twenty years, Algeria's population has increased by 50%. About 45% of this population is concentrated on a very narrow strip of the littoral, especially in the industrial and harbor zones. Coastal waters and sediments of Algerian coast showed high levels of heavy metals.

This work was carried out during 2014 in two sites of east cost of algeria : Skikda as a contaminated and El-Kala a reference site, in order to assess the level of contamination by Heavy metals: Iron (Fe), zinc (Zn), copper (Cu), lead (Pb) and cadmium (Cd) in sediment and in a biondicator *Perinereis cultrifera* (annelid polychaete). This assay was carried out according to the AFNOR Standards [1] .Also, biochemical analysis of biomarkers, the glutahtion-S-transferase (GST) and Acetylcholinesterase (AChE). Total protein content in the homogenate was measured following the method of Bradford [2].

The levels of zinc and cadmium were higher in the tissue than those in sediments at both sites with respective averages of 0.654 ± 0.004 mg / g and 0.0039 ± 0.0002 mg / g in Skikda and 0.519 ± 0.0149 and 0.0025 ± 0.0001 mg / g in El Kala. As for the levels in sediments, they vary from 0.144 ± 0.006 mg / g Zn and 0.004 ± 0.000 mg / g of Cd in Skikda and 0.074 ± 0.017 mg / g Zn and 0.0029 ± 0.000 mg / g Cd in El Kala.

The enzymatic activity of GST in individuals Skikda reveals a peak infection rate in the automn season with 10,92 ± 0.73 µM / min / mg protein.in the other hand the enzymatic activity of AchE decreased in the spring with 10,3 ± 1.53 µM / min / mg protein in Skikda.

The establishment of an inventory of Polychaeta allowed the identification of several species of annelids (*Neries falsa, Platyneries dumerillii, Perineries marionii, Lepidonotus clava*, ...), at two study sites, with the exception of *Platyneries dumerilli* virtually absent at the site of Skikda, unlike *Lepidonotus clava* and *Perineries marionii* which were abundant at the site of Skikda.

These results reflect the direct impact of this pollution on animal biodiversity in the coastal eastern Algeria.

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[2] M. Bradford, *Anal. Biochem.* **72**, 248–254 (1976)

P33.

ATRAZINE CAUSED OXIDATIVE STRESS AND DECREASED BIOTRANSFORMATION CAPACITY OF SEA LAMPREY JUVENILES GILLS

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The use of triazines herbicides, like atrazine contributed to increase the chemical contamination of soils and surface water of Portugal to levels which may cause oxidative stress in aquatic migratory species as sea lamprey (*Petromyzon marinus* L.). The contact of this specie with this pollutant may disturb the success of its trophic migration toward the sea and contribute to decrease its number of effectives in Portugal.

The aim of this study was to determine the effect of atrazine in the content of reactive oxygen species (ROS) and enzyme activities UDP-glucuronosyltransferase (UDPGT), microsomal and cytosolic glutathione *S*-transferases (mGST and cGST), glucose-6-phosphate dehydrogenase (G6PD) and catalase (CTT1) of gills of sea lamprey juveniles (macrophtalmia) from Lima river basin of Portugal.

Sampling occurred at the beginning of the *P. marinus* downstream migration in the Lima river basin, Portugal. The juveniles were transported alive to the laboratory and maintained in 200 L tanks with LSS life support system. The body and gills weight were determined after euthanasia of the animals. The specimens were separated in six groups of three pools (#8): i and ii) juveniles kept in freshwater for 7 or 30 days; iii-vi) juveniles kept in freshwater and exposed to 1, 10, 50 or 100 µg/L atrazine for 30 days. Microsomes obtained by differential centrifugation of gills homogenates were used for determination of enzyme activities UDPGT and mGST. The remaining cytosols were used for determination of ROS content by fluorimetry and enzyme activities cGST, G6PD and CTT1 by spectrophotometry. Statistics includes ANOVA I and Duncan test.

The results showed an increase of ROS content and CTT1 activity as well as a decrease of cGST and G6PD activities of macrophtalmia exposed to 50 and 100 µg/L atrazine ($p < 0.05$). The mGST and UDPGT activities of gills microsomes were not affected by either concentrations of atrazine used in this study ($p < 0.05$). Although the GSI index has not been changed, a decrease in cytoplasmic biotransformation capacity and in availability of reducing equivalents as NADPH, via G6PD may have contributed to cause oxidative stress in macrophtalmia gills exposed to the highest levels of atrazine that was not prevented by antioxidant response of catalase.

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[1] J. González, et al., *Marine Environmental Research*, **67**, 183-188 (2008)

P34.

HISTOLOGICAL EVIDENCE OF THE ADVERSE EFFECTS OF FORMALDEHYDE ON THE DIGESTIVE AND REPRODUCTIVE SYSTEM OF *DAPHNIA*

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Formaldehyde is an aldehyde and is an important precursor to many other materials and chemical compounds. It is used as a disinfectant, embalming agent and tissue fixative in medical applications; in industry is used to make resins, paints and glues. It is used in building materials and many household products. It is very toxic both for humans and aquatic organisms and it was classified as "carcinogenic to humans" based on higher risks of nasopharyngeal cancer and leukemia. Formaldehyde is toxic to several aquatic species both producers and consumers and have toxic effects on *Daphnia* survival growth and reproduction.

In this work, following a 21 days chronic exposure to sub-lethal concentrations of formaldehyde, we studied the histological effects of formaldehyde on the digestive and reproductive system of *Daphnia magna*.

Chronic toxicity test was conducted accordingly to the OECD (2012), Test No. 211: *Daphnia magna* Reproduction. Reproductive parameters were determined and at 10, 14 and 21 days of toxic exposure, organisms were collected for histologic procedure. The digestive and reproductive organs were studied in histological definitive slides: Ovaries dimension, germarium morphology and germinative cells alterations; gut epithelial cells morphology at medial and caudal region and cells microvilli.

Daphnia, even at the lowest exposed concentration, 0.01% formaldehyde, showed quantitative and qualitative negative alterations. Formaldehyde exposure caused a reduction in the structural and reproductive development of the juveniles, delay at age at first reproduction, reduction of juvenile number, ovaries tissue disorganization and abnormal egg formation. The gut epithelium directly exposed to formaldehyde solutions throw ingestion showed loss of tissue architecture, microvilli and nuclear alterations. Cells of the gut epithelium showed nuclear alterations, prominent nucleolus and tissue disorganization.

Formaldehyde has quantitative and qualitative histological negative effects in both feeding and reproduction of *Daphnia magna*.

P35.

ASSESSMENT OF TWO LARVICIDES USED FOR *AEDES AEGYPTI* CONTROL: LETHAL AND SUBLETHAL EFFECTS TO FISHES

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Brazil faces an epidemic of dengue, chikungunya and zika virus with more than millions cases, becoming a worldwide concern. The main transmitter is the *Aedes aegypti* vector and pesticides have been widely applied to eliminate or control their mosquitoes and larvae. Once the intensive use of insecticides becomes environmentally hostile and ecologically unsafe to aquatic ecosystems through the runoff water or directly application, these pesticides provide a risk of environmental intoxication of several non-target organisms.

A sensitive tool to detect adverse effects of pesticides to freshwater fish is histopathological investigations of highly sensitive organs, such as liver and gills. Here, we aimed to assess and compare toxic effects of the organophosphate temephos and the chitin synthesis inhibitor diflubenzuron (DFB) using *Hyphessobrycon eques* and *Oreochromis niloticus* fish models. Acute (48 h) and short-term (7 d) effects were recorded and histopathological changes were assessed in liver and gills of fish adults and fingerlings.

Our results showed that both DFB and temephos insecticides impaired gills and liver tissues such as vascular congestion and edemas of lamellar cells and hypertrophy of hepatocytes, even at concentrations with no effect in acute toxicity test (LOEC=3 and 5 mg/L respectively). Sub-lethal effects of DFB on decreased body weight were also found at concentrations as low as 130 µg/L, whereas temephos caused mortality even at concentrations supposedly sub-lethal, precluding the body weight.

We conclude that histopathological analyses are a sensitize tool to assess sub-lethal effects, useful for assessing environmental risk to fish species. Both insecticides induced changes in liver and gills tissues of fishes under exposure, and adverse effects were also identified at low concentrations in prolonged exposure. Thus, DFB and temephos must be used with caution, once low concentrations of them are able to impair non-target fishes.

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P36.

WATER REPELLENTS PROTECTIVE GARMENTS EFFICIENCY USED BY WORKERS IN MALATHION SPRAYING FOR MOSQUITO CONTROL OF DENGUE *AEDES AEGYPTI*

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Personal protective equipment (PPE) must have a Certificate of Approval (CA) issued by the Ministry of Labor and Employment (MTE) of Brazil to be marketed. The CA is issued according to the protection requirements ISO 27065: 2011, based on tests under laboratory conditions. Therefore, the initial approval of PPE of water-repellent materials and its garments seams must have a percentage of chemicals penetration < 5% (ISO 22608: 2004 norm). The effectiveness of water-repellent PPE can be assessed by the difference between the quantified dermal exposure on garments in the field conditions (DEon) and under garments (DEunder) and also by the calculation of margins security (MS) with these two exposures.

This study aimed to evaluate the protection of water-repellent PPE and its seams and the effectiveness of the PPE after used and washed 10 and 20 times. These PPE were used by workers during spraying the insecticide malathion to the peridomicilary control of dengue mosquito *Aedes aegypti*.

The insecticide formulation used was the Konvektor®, aqueous emulsion, 44% malathion applied outside houses with motorized knapsack sprayer turbo. The water-repellent material of PPE was composed 50/50% cotton/polyester. Washes were performed by the own applicators under heavy mechanical stirring machine Colormaq brand with neutral coconut soap for 20 minutes. Samples of the sets were collected at 0 and after 10 and 20 times used and washed to perform the repellency percentages, retention and penetration assessments (ISO 22608: 2004 norm). The effectiveness of all parts (hooded lapel for neck protection and shoulders, long-sleeved shirt and long pants) was assessed by calculating the safety margins (MS) by the formula [SD / (0.1x DE) x 10], wherein SD is the safe dose of malathion [DS = NOEL (5 mg / kg / day) x 70 kg]. The calculation compares the SD with the DEon and DEunder, estimated by the percentage of penetration assessed on the material and seams (ISO 22608: 2004).

According to the results, only the PPE material 0 and 10 times used and washed met the protection requirement (<5% penetration). The material 20 times used and washed and also the simple seams batting 0, 10 and 20 times used and washed were reproved, according to the requirements to obtain the CA.On the other hand, the PPE was effective in the skin exposure protection evaluated under field conditions (DEon = 76.544 µg / day); with MS > 1 for materials and simple seams and batting 0, 10 and 20 times used and washed.

PPE does not meet the requirements of ISO 22608: 2004 (penetration > 5%), however, based on the value found in the margin of safety, working conditions were considered safe (MS> 1), and the PPE test can be used.

P37.

EVALUATION OF THE *IN VIVO* AND *IN VITRO* EMBRIOTOXICITY OF FIXATIVE AGENTS TO *DAPHNIA MAGNA*

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Fixation is a crucial step in the histological procedure. In order to find an ecological alternative to formaldehyde several new fixatives have been commercially developed: *RCL2*, *FineFix* and *Greenfix*. The effect of chemical agents on the developmental stages of daphnids and the embryo test with parthenogenetic eggs, both *in vivo* and *in vitro*, are commonly used in ecotoxicological research. Acute and chronic toxicity of formaldehyde is well documented however the toxic effects of these three alternative fixative agents in the embryonic developmental stages of the freshwater *Daphnia magna* are not reported.

The objective of this study was to evaluate the acute toxicity, and the effects in reproduction and embryonic development of the test model organism *Daphnia magna*.

The toxicity of the fixatives was evaluated accordingly to the OECD standard guidelines for *Daphnia* acute and reproduction test, and was adapted for the embryo test. In the embryo test there were five solutions concentrations for each fixative. After release of the third brood, females were observed until the passage of the next embryos from the ovaries to the brood chamber. Females were placed under a dissecting microscope and embryos were removed with a Pasteur pipette creating a gentle flow. Eggs/embryos were placed in individual wells of tissue culture plates with 3 mL of control M4 medium or with the fixative solutions. Embryo development was observed at 24 hours intervals. Embryo stage development and development abnormalities were recorded.

The 48h-LC_{50} for *RCL2* was 0,86mL/L, for *Greenfix* was 7,35 ML/L and *FineFix* was 9,07 ML/L. There was a delay in the duration of the embryonic developmental stages with increasing fixative solutions concentration. At higher concentrations we found aborted eggs and deformities in the tail spine and in the sensory bristles.

There was also some slightly differences between *in vivo* and *in vitro* embryo development. The alternative fixatives although less toxic than formaldehyde all caused mortality, quantitative and qualitative alterations on the reproduction and abnormalities on the embryo.

P38.

UNVEILING THE REASON WHY FRESHWATER HETEROTHOPHIC BACTERIA ARE NO AFFECTED BY MICROCYSTINS: ANTIOXIDANT SYSTEM *VS*. DEGRADATION

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Microcystins are the class of hepatotoxins most commonly produced by cyanobacteria. These are found in aquatic ecosystems where they coexist with other microorganisms such as *Aeromonas hydrophila* and *Flavobacterium sp*. There are already a fair amount of studies on the effect of microcystins in eukaryotic organisms, but there are only a few studies about the effects of these toxins in microorganisms. The scarce existing studies show that microcystins can cause a decrease of the microbial growth, even though not being fully inhibited by it [1]. In this study the aim is to determine the reason why aquatic heterobacteria are not very affected by the presence of microcystins in their growth medium. Therefore, the activity of some enzymes of the antioxidant system was evaluated and the presence of genes responsible for microcystin degradation (*mlr*A-D) was assessed in isolates recovered from freshwaters where microcystins are commonly found. These results were compared with the pre-existing bases on the literature to evaluate the relevance of the presence or absence of the genes responsible for microcystin degradation with the natural resistance found in heterobacteria from aquatic environments. Moreover, the activity of the enzymes from the antioxidant system also was accounted to explain this behavior.

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P39.

IMPACTS OF THE PHARMACEUTICAL PROCAINAMIDE AND MICROPLASTICS ON ENVIRONMENTAL HEALTH: EFFECTS ON THE MARINE MICROALGAE *TETRASELMIS CHUII*

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The improvement of human health is closed associated to the increasing use of pharmaceuticals. These substances, their metabolites and their environmental degradation products are not completely removed by sewage treatment plants, and in several regions of the world such treatments do not exist. Pharmaceuticals, as well as several of their metabolites and environmental degradation products have biological activity and several of them also have a considerable environmental persistence. Thus, they are able to cause adverse effects on wild organisms and may also affect ecosystem health and functioning. Microplastics are another class of persistent environmental contaminants (air, water, soil), widely and intensively used, and are now considered ubiquitous pollutants of high concern, including in relation to human health. Microalgae are most important primary producers in aquatic ecosystems. In general, their populations are fundamental components of the phytoplankton community and indispensable for ecosystem function and the services provided by these systems to the human society. Microalgae are sensitive to a wide range of environmental contaminants, including pharmaceuticals and some types of microplastics. The main goals of this study were to investigate the toxic effects of the pharmaceutical procainamide to the marine microalgae *Tetraselmis chuii*, alone and in mixture with microplastics. *T. chuii* cultures were exposed for 96h to different concentrations of each environmental contaminant alone, binary mixtures and controls treatments. The effect criterion was the inhibition of *T. chuii* population growth rate. Overall, the results indicate negative effects of both substances on the microalgae, and the findings are discussed in relation to procainamide-microplastic interactions and the effects of these pollutants on ecosystem health.

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P40.

ASSESSMENT OF THIAMETHOXAM TOXICITY TO *Chironomus riparius* AND *Dugesia tigrina*

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Thiamethoxam (TMX) is a systemic neonicotinoid insecticide widely used for pest control in agriculture. As all neonicoinoids, TMX mimics the action of acetylcholine causing uncontrolled muscular contraction eventually leading to death. TMX is being found in freshwaters ecosystems at concentrations up to 63.4 µg/L [1].

This study aimed to evaluate the effects of TMX on two freshwater invertebrates, the dipteran *Chironomus riparius* and the flatworm *Dugesia tigrina*. Chironomids and planarians, two model species in ecotoxicology, are widely distributed and abundant in freshwater ecosystems. The endpoints chosen for the assessment of effects of thiamethoxan exposure included larval survival, growth and emergence of *C. riparius,* and survival, locomotion (evaluated through an automated video record system) and head regeneration of *D. tigrina*.

The estimated TMX 48-h LC_{50} (95% CI) for *C. riparius* was 68.31 µg/L (55.73 to 83.72 µg/L). Exposure to sub-lethal concentrations of TMX reduced *C. riparius* larval growth LOEC of 18 µg/L and also impaired *C. riparius* emergence rate (LOEC values of 10.5 µg/L). The estimated TMX 96-h LC_{50} (95% CI) for *D. tigrina* was > 60 mg/L. TMX exposure did not cause any effects on planarians locomotion and head regeneration at the concentrations tested (0.005 to 5 mg/L).

The results from the present study confirm that environmental concentrations of TMX are acutely toxic to aquatic insects such as *C. riparius*. Moreover as other neonicotinooids, sub-lethal effects of thiamethoxan are related to reduced activity and uncontrolled muscular contractions, which can limit foraging activity of aquatic insects and consequently impair feeding, and development rates [2]. The planarian *D. tigrina* on the other hand showed to be tolerant to environmentally relevant concentrations of this insecticide. Since planarians are predators of dipteran larvae in freshwaters, this suggests an even greater ecological risk of thiamethoxan via indirect effects and supports the use of multispecies experiments for the assessments of potential deleterious effects of pesticides in natural aquatic ecosystems.

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[2] J. L. T. Pestana, et al., *Aquatic Toxicology* **93**, 138-149 (2009)

P41.

COMPETITION UNDER SALINE STRESS: A CASE STUDY WITH THREE SPECIES OF PLANTS

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Severe and frequent extreme weather events allied with secondary salinisation events constitute a major environmental problem for the terrestrial compartment, compromising some of the services provided by soils (e.g. support for several organisms, support for crops for human consumption). Thus, it is important to understand how terrestrial species deal with salinity while dealing with other natural stressors (e.g. competition). Accordingly, the present work intended to assess the influence of exposure to increased salinity in the competitive outcome of three species of terrestrial plants, used widely for forage and livestock feeding (*Trifolium pratense*, *Vicia sativa* and *Lolium perenne*). Aerial and root biomasses were assessed after 28 days of exposure in pairwise competition assays, with two salt treatments: no salt and 4.0 $mScm^{-1}$ (threshold above which a soil is considered saline). For each treatment, three scenarios were tested: i) sp. A solely; ii) sp. B solely and iii) sp. A x sp. B. Preliminary results showed that, under control conditions and exposed solely, *T. pratense* and *L. perenne* invested in root biomass while *V. sativa* in aerial biomass. When exposed solely to saline stress, *T. pratense* increased aerial biomass and *V. sativa* and *L. perenne* increased root biomass. Under competition, when exposing *T. pratense* x *V. sativa* to control conditions, both species presented similar investments: approximately 40% on root growth and 60% on aerial biomass. However, under salt stress, *T. pratense* invested in aerial growth while *V. sativa* invested in root growth. Regarding the results obtained for *T. pratense* x *L. perenne*, results showed that *T. pratense* invests in aerial biomass and *L. perenne* on root biomass. For *V. sativa* x *L.perenne* results have shown that, when competing under salt stress, both species loses root biomass and increase aerial biomass, relatively to control.

These results highlight that salinisation of soils may impair species growth and interaction, which can cause a decrease of soils productivity. Also, long-term studies allow detecting effects that are not immediately perceptible and scenarios of competition under salt stress may unveil effects that are neglected by standard assays.

P42.

MINIMUM INHIBITORY CONCENTRATION OF AZOLES FUNGICIDES FOR OOMYCETE *LEPTOLEGNIA CAUDATA IN VITRO* CONDITIONS

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Leptolegnia caudata sp. is oomycete that cause mycotic diseases in fish, frogs and their eggs in the winter. This fungi can infect by biflagellate zoospores that are produced by specialized hyphal tips called zoosporangia. Thus, it is emergent the study of molecules to control this pathogen in aquaculture. It was evaluated the minimum inhibitory concentration (MIC100) of itraconazole (ITR); ketoconazole (KET) and fluconazole (FLU) for *Leptolegnia caudata in vitro* conditions. *L. caudata* strain was isolated from fish farming water and it was identified according to molecular technique and deposited in GenBank as KP941577 code. In the *in vitro* assays, one sample of fungi strain was disposed in petri dishes with potato dextrose agar medium (PDA), kept during three days at 25 ^{0}C, and used as source of fungus samples. The concentrations of ITR used in the MIC100 assays were: 170.0; 200.0; 230.0; 260.0 mg L^{-1}; of KET were 70.0; 90.0; 110.0; 130.0 mg L^{-1} and of FLU were 3900.0; 4200.0; 4500.0; 48000 mg L^{-1} and a control, with five repetitions. A fungus piece of 6 mm was disposed in the central position of the plate with fungicides and medium and kept at 25 ^{0}C by 72 hours. It was evaluated the diameter growth halo daily and in the end of 72 hours it was quantified the minor concentration which causes 100% inhibition. MIC100 of ITR was 260 mg L^{-1} because there was 100% inhibition, in 170, 62.44%; 200, 76.44% and 230, 93,11% inhibition. MIC100 of KET was 130 mg L^{-1} because there was 100% inhibition, 70, 82.22%; 90, 83.33% and 110, 95.33% inhibition. MIC100 of FLU was 5100 mg L^{-1} because there was 100% inhibition; 3900, 80.67%; 4200, 82.22%; 4500, 88.89% and 4800, 94.44% % inhibition. Fluconazole isn't viable to follow studies because it has a MIC very high. However ketoconazole and itraconazole should be tested *in vivo* conditions to control *Saprolegnia* sp. and theirs toxicity for the fish because they have potential to be used in the fish fungus control.

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P43.

DEVELOPMENT OF A HIGH-THROUGHPUT MULTI-PARAMETER BIOMARKER SET FOR PLANT BIOMONITORING AND ECOTOXICOLOGICAL STUDIES

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During the last decade plants have been increasingly used in ecotoxicological studies and environmental biomonitoring. However, despite an obvious interest, their uses have been mainly limited by a time-consuming and operator-dependent extraction step. Moreover, most of the studies have been limited to the evaluation of few biomarkers limiting their sensitivity and relevance. Thus, it appears there is a need to develop some alternative, fast, cost-efficient and reliable protocols able to evaluate plant health on hundreds to thousands of samples.

In this frame, we have developed of a fully automatic extraction technique which increases drastically its speed and efficiency, while also enhancing reproducibility and reliability. Indeed, this fully automatic extraction step totally abolishes the operator-related variability. Moreover, this extraction step, performed in 96 deep well plates, is directly compatible with assays using 96 well microplates.

Meanwhile, we have worked on the miniaturization in 96 well microplates of the so far routinely used biomarker assays: antioxidant enzymes activities, lipid peroxidation, photosynthetic pigments content. In order to have a better overview of plant health, we have developed complementary miniaturised biomarker assays: RUBISCO activity, sucrose metabolism enzyme activities, secondary metabolites contents (phenols, flavonoids).

So far, the high-throughput multi-parameter biomarker set we have developed enables to analyze of dozen of biomarkers on 384 plant samples per day, allowing large biomonitoring campaigns.

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P44.

EFFECTIVENESS AND CONTROL PERIOD OF LARVAE *AEDES AEGYPTI* RESISTANT TO TEMEPHOS BY DIFLUBENZURON IN FIELD CONDITIONS

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The temephos is the most used larvicide in Brazil to control *Aedes aegypti*, however there are many mosquito populations resistant to this insecticide in several locations in Brazil. Therefore, there is a need for replacing this larvicide, and diflubenzuron (DFB), a chitin synthesis inhibitor, has a great potential to replace it. Besides dengue, this mosquito also is associated with transmission of two other arboviruses (Zika and Chikungunya), which explains the importance of blocking action with larvicides.

This study aimed to evaluate the effectiveness of DFB to control larvae from two *A. aegypti* populations resistant to temephos. Tests were performed in plastic, glass and rubber containers filled with water, in field conditions.

The resistance ratios (RR95) were calculated by dividing the lethal concentration (LC95) of the resistant populations by the LC95 of the strain susceptible Rockefeller, used as a reference standard in all assays. *A. aegypti* larvae were obtained from eggs collected in Vila Velha city - ES (VVE), with RR95 of 7.5, and also in Santarém city - PA (STR) with RR95 of 4.0. The larval control period (number of days after the larvicide dilution in water) was based on the DFB efficacy criterion of 80% or more mortality of the larvae. The averages of larvae mortalities were compared with the statistical test Scott-Knott ($p < 0.05$).

According to the results, DFB is effective for VVE population for five weeks in the three containers. Regarding STR population, DFB is effective for three weeks in plastic and glass containers and for seven weeks in rubber container.

In conclusion, the DFB controls *A. aegypti* populations resistant to temephos with control periods between three and seven weeks. Moreover, the larvae control period by DFB is longer in rubber than in glass and plastic containers.

P45.

HUMIC ACIDS DECREASE THE TOXICITY OF GEMFIBROZIL AT LETHAL LEVEL BUT NOT AT SUBLETHAL LEVEL

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Climate changes may increase the occurrence of floods and droughts, leading to consequent changes in water quality parameters such as alterations in dissolved organic carbon (DOC) concentration. DOC levels are an important parameter to aquatic communities playing a critical role as a transport vector for metals and organic chemicals, influencing their bioavailability and also interfering in the toxicity of existing pollutants. Hence, this study aims to evaluate the effects of DOC, particularly humic acids (HA), in the toxicity of gemfibrozil (GEM) - one of the most frequently detected pharmaceuticals in wastewaters. Effects were evaluated in zebrafish (*Danio rerio*) embryos at lethal and sub-lethal levels (biochemical and behavioral). GEM concentrations ranging from 0.0016 to 5 mg/L and three HA levels (0, 10 and 20 mg/L) were used in a full factorial design. Our results showed that the presence of HA significantly increased the GEM-LC_{50} values, however this reduction in toxicity was not verified at sublethal levels. At the biochemical level, HA alone seem to elicit a significant decrease in the levels of cholinesterase, glutathione S-transferase and catalase. Their presence seemed to change the pattern of response to GEM only in the case of catalase. Regarding to behavior, effects of HA alone seemed to surpass effects of GEM, by reducing the total distance moved by larvae in the locomotor assay.

This study highlights the importance of attending to DOC in the prediction of pharmaceuticals toxicity to aquatic organisms and reinforces the importance of understanding the effects of climate changes to aquatic biota. In addition, due to its high sensitivity, sub-lethal assays should be taken in consideration/performed in environmental risk assessment.

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P46.

CYPERMETHRIN CONTAMINATION IN PANTANAL: COMPARATIVE SENSITIVITY OF THE ENDEMIC *SHRIMP MACROBRACHIUM PANTANALENSE* AND OTHER AQUATIC SPECIES

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Pantanal (Western Brazil and Bolivia) is a biome characterized by an extraordinary diversity and abundance of wildlife, constituting a Biosphere reserve. It is the refuge of many rare species and also houses several endemic species such as the shrimp *Macrobrachium pantanalense*. However, the increase in agriculture and husbandry activities may increase the risk of contamination with pesticides. Given the importance of using local species in risk evaluation, the main objective of this study was to assess the sensitivity of the endemic freshwater shrimp species *M. pantanalense* compared with other aquatic species: the freshwater shrimp *M. amazonicum* (also found in Pantanal), the crustacean *Daphnia similis* and the fish *Danio rerio*. The sensitivity of these organisms was assessed through acute exposure to cypermethrin using the formulation Barrage®, which is widely applied in the region. The species sensitivity decreased in the following order: *M. pantanalense* (96h-LC_{50} 0.066 µg/L) > *M. amazonicum* (96h-LC_{50} 0.13 µg/L) > *D. similis* (48h-LC_{50} 7.87 µg/L) > *D. rerio* (144h-LC_{50} 1.68 mg/L). Major effects of the cypermethrin formulation included reduced length of shrimps and zebrafish, as well as early hatching and increased incidence of developmental deformities in zebrafish embryos. The very high toxicity of the cypermethrin formulation to both shrimps, particularly the endemic *M. pantanalense*, highlights the sensitivity of Pantanal species to anthropogenic chemicals and thus, the importance of using local species for risk evaluation. Moreover, this study raises concern about the effects of pesticides on this biome.

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P47.

OPTIMIZATION OF MICROBIAL DETOXIFICATION FOR AN AQUATIC MERCURY-CONTAMINATED ENVIRONMENT

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Mercury reduction performed by microorganisms is well recognized as a biological way for the remediation of contaminated environment. Recently, we found that mercury resistant microorganisms of Tagus estuary are involved in mercury reduction processes. In the present study, aerobic microbial community isolated from a highly mercury-contaminated area of Tagus was used to study the optimization of the reduction process in conditions similar to the contaminated ecosystem. Factorial design methodology was used to study the effect of glucose, sulphate, iron and chloride on mercury reduction. In the presence of several concentrations of these elements, microbial community reduced mercury in a range of 37-61% of the initial 0.1 mg/mL of Hg^{2+}. The response prediction through central composite design showed that the increase of sulphate concentration leads to an optimal response in mercury reduction by microbial community, while the increase of chloride decreases mercury reduction sharply. Iron can have antagonistic effects depending on the media composition. These results are important for bioremediation strategies planning.

P48.

NEW FINDINGS ON MERCURY NEUROTOXICITY DISCLOSED BY OXIDATIVE STRESS PROFILES IN FISH BRAIN

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Fish brain has shown to be a target organ for Hg, mainly methylmercury (MeHg). However, the information about the neurotoxicity of inorganic mercury (iHg) and its ability to accumulate in fish brain is still scarce. The prevalent information on MeHg is likely based in the perception of its higher toxicity associated with rapid uptake and distribution. Nevertheless, it has been also stated that the different forms of Hg share the same toxic chemical entity, thus neurotoxicity may depend mainly on the environmental bioavailability.

In order to contribute to the clarification of this controversy, two experiments comprising exposure and post-exposure periods were performed with juveniles of white seabream (*Diplodus sargus*), namely: a waterborne exposure to iHg (as 2 μg L^{-1} of Hg^{2+}) and a dietary exposure to MeHg (8.7±0.5 μg g^{-1}). A similar experimental design was followed for both experiments, consisting in 4 exposure periods (E) (days 1, 3, 7 and 14) and 2 post-exposure periods (PE) (days 14 and 28). For both experiments, a control group was kept in clean seawater or fed with uncontaminated food. At each time, brain was collected for determination of total Hg (tHg) levels and oxidative stress endpoints.

In both experiments, tHg accumulation showed maximum values in the brain after 14 days of exposure, reaching the highest levels upon exposure to MeHg (mean values of 7.0 μg g^{-1} for MeHg exposure vs. 1.4 μg g^{-1} for iHg). Interestingly, fish brain exposed to iHg did not show the ability to eliminate Hg, while tHg levels in brain decreased significantly in the post-exposure to MeHg (to a mean of 3.5 μg g^{-1}). Moreover, there was a poor activation of antioxidant defences in fish brain exposed to iHg, mainly characterized by an increase of superoxide dismutase (SOD) and glutathione reductase (GR) activities. The low protection afforded by antioxidants (confirmed by glutathione peroxidase (GPx) activity decrease) was probably on the basis of oxidative damage, as revealed by the enhanced protein carbonyl groups levels in exposure and post-exposure periods. MeHg exposure led to a different scenario, mostly characterized by an activation of antioxidant defences (SOD, catalase (CAT), GPx, glutathione S-transferase (GST) that were able to prevent oxidative damage on proteins and lipids. Despite the higher accumulation of tHg in fish brain after MeHg exposure, there was a higher vulnerability of this tissue to iHg depicted in the occurrence of oxidative damage and less responsiveness of the antioxidant system. Thus, iHg revealed a high neurotoxicity potential, pointing out the relevance of considering this Hg form, together with MeHg, in further studies concerning wildlife and human health.

P49.

MERCURY NEUROTOXICITY IN WILD FISH (*Liza aurata*) AND THE INTERFERENCE OF ESTUARINE ENVIRONMENTAL VARIABLES

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Brain is a target organ for mercury (Hg), and oxidative processes have been described as a key pathway for Hg neurotoxicity. Thus, alterations of oxidative stress endpoints have been proposed as biomarkers of Hg neurotoxicity. It has also been referred that Hg may interfere with neurotransmission. However, the evaluation of oxidative stress and neurotransmission-related endpoints in fish upon environmental exposure to Hg remains elusive. Moreover, there is a lack of studies on the interference of water physicochemical conditions with these biochemical endpoints in estuarine fish, which is a very important topic in ecotoxicology, keeping in view that in estuaries such conditions can vary greatly within small time and spatial scales. Hence, the current study was designed to mitigate such lack of knowledge by evaluating the Hg neurotoxicity in brain of an estuarine fish species (*Liza aurata*), as well as the interference of water physicochemical parameters with accumulation levels and biochemical endpoints. A field study was carried out in winter and summer 2013, in a Portuguese coastal lagoon (Ria de Aveiro), namely in: (i) Largo do Laranjo (LAR) - Hg contaminated area, and (ii) São Jacinto (SJ) - reference site. The study covered the characterisation of tHg and MeHg levels in water and sediments, as well as water physicochemical parameters (temperature, dissolved oxygen and salinity). Furthermore, the brain of *L. aurata* was analysed for tHg and MeHg bioaccumulation (inorganic Hg- iHg - was estimated by the difference between tHg and MeHg) and for alterations on oxidative stress and neurotransmission-related endpoints. A canonical correspondence analysis (CCorA) was performed in order to search for associations between environmental parameters (in water and sediment), bioaccumulated levels and biochemical endpoints in *L. aurata* brain. tHg, MeHg and iHg in brain were higher at LAR than SJ, both in winter and summer, reflecting environmental spatial differences. Moreover, fish caught at LAR in winter showed an increased superoxide dismutase (SOD) activity and total glutathione (GSHt) content, an occurrence of peroxidative damage, and a decreased glutamine synthetase (GS) activity. In summer, solely an increment of GSHt at LAR was recorded in comparison with SJ. CCorA showed a clear separation of the sampling sites and respective seasons, emphasising also a relationship between accumulated tHg and MeHg in brain with, environmental availability of tHg and MeHg, particularly at LAR in winter. Nevertheless, CCorA did not establish a strictly cause-effect relationship between accumulated Hg levels and biochemical effects. Contrarily, some of the biochemical endpoints varied significantly with water physicochemical parameters, namely the increased GSHt levels, that seems to be associated with higher temperatures, while changes of dissolved oxygen in water can be on the basis of the GS variation. Current data demonstrated the susceptibility of the fish's brain to Hg neurotoxicity, mainly in scenarios of higher Hg environmental availability. It was also pointed out the relevance of evaluating the potential influence of environmental parameters in the variation of biochemical endpoints in estuarine studies.

P50.

ENDOCRINE DISRUPTORS MIXTURES: THE REAL SCENARIO OF HUMAN EXPOSURE

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Endocrine disrupting chemicals (EDCs) are exogenous agents that have the ability to interfere with/or mimic estrogenic hormones and, therefore can simultaneously and differentially trigger specific signaling pathways responsible for the nature and magnitude of biological responses in diverse cell types. EDCs may occur naturally (e.g. phytoestrogens), whereas others are industrial chemicals and plasticizers commonly utilized. Human exposure to these compounds, particularly at low-doses, is ubiquitous, persistent and occurs in complex mixtures. Additionally, EDCs can bioaccumulate in lipid compartments of tissues forming a mixed "body burden" of contaminants of different origins. Although the independent action of chemicals has been considered the main principle in EDCs mixture toxicity, recent studies have demonstrated that several effects cannot be predicted when analysing single compounds independently.

Here we performed a revision of the literature existent in Pubmed focused in studies that evaluated EDCs mixture effects. Based on published data we describe a potential real scenario of human exposure, namely the scenario of a pregnant woman exposed to BPA trough oral intake (canned food), Nonylphenol (cosmetics) and PBDE-99 flame-retardant (house). These compounds have been reported to be transplacental transferred, which indicates that during embryonic development, the fetus is chronically exposed to mixtures of these compounds, potentially resulting in an early "body burden", which may have severe adverse effects considering that the proper development of tissues and organs growth requires accurate timing of hormone action. Prenatal exposure to mixtures of xenoestrogens have been reported to alter epigenetic marks (DNA methylation) in placenta tissues and particular genes and epidemiological studies demonstrated that parental exposure to EDCs during pregnancy is correlated with decreased birth weight of offspring and with shortened anogenital distance in male offspring.

Although the assessment of potential risks to human health as a result of exposure to mixtures of EDCs is a major topic for consumer safety, information regarding EDCs mixtures effects in the context of real exposure scenarios is still inexistent.

P51.

IMPACT OF SURFACE CHARGE AND FUNCTIONALIZATION ON UPTAKE AND TOXICITY CELLS OF SILVER NANOPARTICLES IN MAMMALIAN CELLS

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The increasing interest and exponentially growing applications of metallic nanoparticles necessitate research on their bioavailability and effects on living tissues. Understanding of effect of the characteristics of nanoparticles on their distribution, cellular uptake and response is still imperative.

This study aimed to explore the toxicity and uptake mechanisms of silver nanoparticles (AgNPs) with different surface functionalization on human hepatoblastoma (HepG2) cells. Comparison of cellular response was made including AgNPs coated with cetyltrimethylammonium bromide (CTAB), polyvinylpyrrolidone (PVP), bis(2-ethylhexyl) sulfosuccinate (AOT), poly-*L*-lysine (PLL), and bovine serum albumin (BSA).

Comprehensive evaluation on characteristics and stability of different AgNPs showed particles organized in nanometric, but also in micrometric agglomerates upon suspension in cell culture medium. It was observed that the cellular uptake of AgNPs depends on the time of incubation and the concentration of AgNPs in the medium. Screening of the AgNPs uptake was performed by flow cytometry and showed that HepG2 internalized NPs by the mechanism of macropinocytosis. Transmission electron microscopy images revealed that AgNPs were localized preferentially in endosomes. The cell viability test showed that all AgNPs reduced cell viability in a dose-dependent manner, while alkaline Comet assay revealed that AgNPs caused DNA damage in HepG2 cells.

Finally, our study provides evidence that the type of surface group of AgNPs has a substantial influence over toxicity in mammalian cells *in vitro*. Further steps will include detailed analysis of the mechanisms in which toxicity is induced within the cells and different organelles with the cell.

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P52.

IN VITRO BIOCOMPATIBILITY STUDIES OF POLYESTER FABRICS COATED WITH PHOTOCATALYTIC TITANIUM DIOXIDE NANOPARTICLES

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Nowadays, many products containing manufactured nanomaterials (MNMs) have entered the market in very diverse fields of applications; among them is a new concept called “self cleaning textiles”. However, before being used in the development of novel textiles with improved antibacterial properties and self-cleaning capacity, these modified fabrics must be strictly characterized and analyzed.

This research aims to assess the biocompatibility and safety of polyester knitted fabrics coated with titanium dioxide nanoparticles (TiO_2 NPs) after a direct contact with the skin or when the nanoparticles could be released from fabric under the influence of various mechanical or chemical factors. Therefore, the cell viability, the cell membrane integrity and the inflammatory potential were evaluated *in vitro* on normal human dermal fibroblasts, after 6 and 12 hours of exposure to PES fabrics previously treated with TiO_2 NPs doped with 1% Fe atoms and nitrogen, which were used as photocatalysts. Also, fluorescent microscopy have been used to assess the cellular morphology and the actin cytoskeleton organization following exposure to TiO_2 NPs-coated polyester fabrics.

At 6 hours of incubation with NPs treated polyester samples, there were no significant changes in any of the analyzed parameters, suggesting that the proliferative capacity of the cells has not been disrupted in the presence of textiles. Only after 12 hours of exposure the cell viability decreased by 30% and the amount of nitric oxide (NO) released in medium registered a slightly increased by 10%, while released lactate dehydrogenase level remained unchanged compared to control. So, NPs coated polyester did not affect cell membrane integrity nor induced inflammatory processes.

These results demonstrate that the treatment of PES materials with photocatalytic TiO_2 NPs is harmless for skin cells and could be used for the development of innovative self-cleaning antimicrobial textiles with great potential biomedical applications in preventing patients’ accidental contamination with microorganisms from the hospital environment.

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P53.

CONTRIBUTION OF MACROPHAGE ACTIVATION TO THE GENOTOXIC EFFECT OF NANOFIBERS IN LUNG EPHITELIUM

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Nanofibers are nano-objects with two similar dimensions in the nanoscale (size range from approximately 1nm to 100nm) and the third dimension significantly larger. The most widespread group of nanofibers is carbon nanotubes (CNTs) that consist of graphite sheets with a cylindrical arrangement of various lengths, and diameter at the nanoscale. CNTs may have a single, double or multiple walls arranged in concentric layers, and embedded metals. In recent years, several studies performed in rodents exposed to liquid suspensions of CNTs by intratracheal instillation or pharyngeal aspiration showed the development of acute or chronic pulmonary inflammation and persistent interstitial fibrosis with granuloma formation, and bronchiolar or bronchioloalveolar hyperplasia. High thin and crystalline CNTs may also have a carcinogenic effect similar to asbestos. Macrophages play a key role in the response to poorly soluble nanomaterials as nanofibers. Activated macrophages degrade SWCNTs via NADPH oxidase pathway facilitating lung clearance, and if oxidative species formation is exaggerated, injury to the neighbour cells can occur. After macrophage activation, nanofibers phagocytosis also leads to the release of cytokines (TNF-α, IL-6, etc.) and transcription factors associated to inflammation (NF-κB and AP-1). In addition, when nanofiber length exceeds the pleural macrophages length, it triggers an inflammatory response in the pleural cavity due to "frustrated phagocytosis", which in turn stimulates a cytokine proinflammatory response by adjacent mesothelial cells. Thin and highly crystalline CNTs may also have a piercing effect in the mesothelial cell membrane causing *in vitro* cytotoxicity, and *in vivo* inflammatory and carcinogenic effects. The aim of this work is to elucidate the role of macrophage activation in the genotoxic effects of a thick and high aspect ratio multiwalled CNT in human lung epithelium. For that purpose, a co-culture of the human alveolar epithelial cell line A549 with the human monocytic leukemia cell line THP-1 differentiated into macrophages by 48 h incubation with 100ng/ml 12-O-tetradecanoylphorbol-13-acetate (TPA) was established. The co-culture as well as the A549 cell culture alone was exposed to a dose-range of the multiwalled CNT NRCWE-006 for 24 hours. Genotoxicity was evaluated using the comet assay. The results obtained in the co-culture and in the epithelial cells will be compared, and the same methodology will be performed using asbestos, a well known cause of mesothelioma. The comparative analysis of data from NRCWE-006 and asbestos fibers is expected to contribute to the hazard assessment of CNTs.

P54.

CYTO- AND GENOTOXICITY ASSESSMENT OF CERUIM DIOXIDE NANOMATERIALS CERIUM DIOXIDE IN THE A549 CELL LINE

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In the past decades the growing application of nanomaterials (NMs) in diverse consumer products has raised various concerns in the field of toxicology. They have been extensively used in a broad range of applications and cover most of the industrial sectors as well as the medicine and the environmental areas. The most common scenarios for human exposure to NMs are occupational, environmental and as consumers and inhalation is the most frequent route of exposure, especially in occupational settings. Cerium dioxide NMs (nano-CeO_2) are widely used in a number of applications such as in cosmetics, outdoor paints, wood care products as well as fuel catalysts. For such reason, nano-CeO_2 is one of the selected NMs for priority testing within the sponsorship program of the Working Party of Manufactured Nanomaterials of the OECD. In this context, the aim of this study is to assess the safety of nano-CeO_2 (NM-212, Joint Research Center Repository) through the characterization of its cytotoxicity and genotoxicity in a human alveolar epithelial cell line.

The nano-CeO_2 particles are spherical, displaying a diameter of 33 nm and 28 m^2/g of surface area. A dispersion of the NM in water plus 0.05% BSA was prepared and sonicated during 16 minutes, according to a standardized protocol. DLS analysis was used to characterize the quality of the NM dispersion in the culture medium. To evaluate the cytotoxicity of nano-CeO_2 in the A549 cell line, the colorimetric MTT assay was performed; the capacity of cells to proliferate when exposed to CeO_2 was also assessed with the Clonogenic assay. The genotoxicity of this NM was evaluated by the Comet Assay (3 and 24h of exposure) to quantify DNA breaks and the FPG-modified comet assay to assess oxidative DNA damage. The Cytokinesis-Block Micronucleus (CBMN) assay was used to further detect chromosome breaks or loss.

The DLS data demonstrated that the dispersion of the NM was achieved and was stable at least for 48h in culture medium. The results of the MTT assay did not show any decrease in cells viability following treatment with a dose-range of nano-CeO_2 during 24h. Nevertheless, the highest concentrations of this NM were able to significantly reduce the colony forming ability of A549 cells, suggesting that a prolonged exposure may be cytotoxic to these cells. Data from both genotoxicity assays revealed that nano-CeO_2 was neither able to induce DNA breaks nor oxidative DNA damage. Likewise, no significant micronucleus induction was observed. Taken together, the present results indicate that this nano-CeO_2 is not genotoxic in this alveolar cell line under the tested conditions, although further studies should be performed, e.g., gene mutation in somatic cells and *in vivo* chromosome damage (rodent micronucleus assay) to ensure its safety to human health.

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P55.

EXPOSURE TO ULTRAFINE PARTICLES IN MAG STEEL WELDING: INFLUENCE OF METAL TRANSFER MODES AND SHIELDING GAS COMPOSITION

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MAG welding tests were performed in laboratory for mild steel plates of AISI 1020, and also for austenitic stainless steel plates in AISI 304. Different welding parameters were tested, varying the gas shielding mixture, the current intensity and the voltage in order to produce short-circuit, globular and spray metal transfer modes. Gas protection was selected amongst the most frequently used gas mixtures. For measuring ultrafine particle exposure a Nanoparticle Surface Area Monitor (NSAM) was used for estimating Alveolar Deposition Surface Area (ADSA), and particles were also collected using a Nanometer Aerosol Sampler (NAS). The main conclusions from this work are: the emission of ultrafine particles, seems to be related to element volatilization and not so much to spatter formation and it increases with the increase of welding parameters such as current intensity and voltage. Regarding the studied metal transfer modes, the spray mode originated higher emissions of nanoparticles, while it is known for usually not showing spatter. The short-circuit mode resulted in lower average ADSA values for all gas mixtures tested. This exhibits the “coldest” electric arc thus volatilizing lower quantities of elements from the base material and the wire. The globular transfer mode, for the majority of tested conditions, resulted in ADSA values between short-circuit and spray modes. This transfer mode is known for arc instability and, thus, it seems that electric arc instability is not the main cause for ultrafine particles emission but, instead, the electric arc temperature. This information is quite useful in order to select operating parameters and protection gas mixtures leading to achieve low welders exposure and, thus, cleaner welding processes.

P56.

ASBESTOS – IDENTIFICATION ON BULK MATERIALS

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Asbestos is the generic term for various types of natural silicates of magnesium and / or iron, which have fibrous forms. Due to its properties, asbestos has been widely used in industry, it is estimated that is present in approximately three thousand different products. It is currently known that asbestos causes, various types of diseases in exposed human beings, such as asbestosis, lung cancer and mesothelioma (cancer of the pleura or peritoneum). Although the use of asbestos is already prohibited, its extended use in the past, requires the adequate surveillance in places where it is applied in order to reduce as much as possible the risk of exposure to this agent.

The Air and Occupational Health Unit of the National Institute of Health Doutor. Ricardo Jorge (INSA) identifies since 1985, the presence of asbestos fibers in materials, using the Polarised Light Microscopy, method 9002 of NIOSH, Manual of analytical methods, fourth edition. Since its ban in 2005, by the Community Directive 2003/18/EC, requests for such assessments have risen considerably (about 300%) mainly in the assessment of air surveillance in schools. Requests for asbestos identification in materials, had a very significant increase since 2014, when the government undertook to carry out a survey of “materials suspected of containing asbestos” (MCA) in buildings, facilities and public facilities provided for in Law No. 2/2011 but, to date, it had not yet been made.

This study aims to make the evaluation of the results for all material samples analyzed in INSA since 2012, with regard to asbestos detection.

Conclusions from that study demonstrate that in 75% of the analyzed materials was not detected the presence of asbestos. The majority (84%) of materials where the presence of asbestos fibers was detected corresponds to asbestos cement sheets containing asbestos chrysotile type and in older cement sheets asbestos chrysotile and crocidolite type in accordance with the expectable.

P57.

SAFE PRODUCTION AND USE OF NANOMATERIALS IN THE CERAMIC INDUSTRY: THE CERASAFE PROJECT

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The ceramic industry is a growing industrial sector, which is benefitting from advances made available through nanotechnology and a number of innovative industrial processes. However, production of nanomaterials, including the manufacture and use of nanoceramics, cannot be considered safe without a thorough investigation regarding exposure and toxicity of nanoceramic materials, which is a current research gap. This requires better knowledge of workers' exposure in the ceramic sector and during nanoceramics manufacturing, handling and processing, which will firstly require the understanding of exposure scenarios.

In this framework, the ERANET-SIINN project CERASAFE aims to assess and improve environmental health and safety (EHS) in the ceramic industry. The objective of this project is to study industrial processes and activities which may generate nanoparticle emissions into workplace air, and to assess worker exposure by evaluating the particle release processes, characterizing the emitted particles, and understanding their toxicity. Two main types of nanoparticles will be characterised during the nanoceramic value chain: (1) engineered nanoceramics (e.g., Al-doped zinc oxide, La-based, $BaSO_4$, TiO_2 and silica nanoparticles, and ceramic pigments), and (2) process-generated nanoparticles from e.g., laser ablation, plasma thermal projection, laser sintering of ceramic tiles, physical vapour deposition, and inkjet printing. Toxicity assessments will be carried out with the aim to address biological interactions of nanoceramics, by performing in vitro and in vivo studies to provide insights on toxicity profiles namely on those related with oxidative stress, inflammatory and genotoxic responses. Finally, we will develop a tool to discriminate engineered nanoceramic particles from background aerosols based on hygroscopicity measurements, thus innovating in the field of characterization methods relevant for EHS. Mitigation measures will also be proposed.

P58.

THE IMPACT OF SIZE ON TISSUE DISTRIBUTION AND ELIMINATION OF SILVER NANOPARTICLES IN MICE

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Silver nanoparticles (AgNPs) have been extensively used for its antimicrobial and anti-inflammatory properties, being incorporated in a variety of consumer products. Their large dissemination raises concerns about the potential risks of the inadvertent exposure to human health. Therefore, their toxicity towards mammalian cells and animal models has been extensively investigated in the last years. Several studies have demonstrated that AgNPs can exert toxicity to different organs and especially to the lung, however, the fate and behavior of AgNPs in the human body is still not completely understood. A Physiologically based pharmacokinetic (PBPK) modelling could provide an insight into the relationships between an external dose and internal organ, blood or excretion dose. Therefore, in this study we aimed to construct a PBPK model for two different sizes of AgNP (5nm and 50nm, AgNP5 and AgNP50, respectively) and ionic silver ($AgNO_3$) in order to understand the ADME behaviour of the AgNPs in biological systems after the entry into the lungs. We assessed the distribution and excretion of AgNP and ionic silver, after single and repeated intratracheal instillations on mice. The organ distribution pattern for 1day after single instillation and 1, 7 and 28 days after repeated instillations of AgNPs or ionic silver was evaluated by inductively coupled plasma-mass spectrometry (ICP-MS). Moreover, urine and feces were collected for silver quantification after the same period of time. After a single instillation, the AgNP5 distributed in high concentrations to the main organs. After repeated instillations, AgNP50 concentration decrease from each organs, until 28 days. On contrary, for the AgNP5, the repeated administration resulted in high silver accumulation until 28 days. Overall, we found a size-dependent tissue distribution and accumulation. Also, we found that the feces seems to be the major route for elimination of this particles.

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P59.

TOXICITY OF GOLD NANORODS ON ZEBRAFISH (*DANIO RERIO*) EMBRYOS

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Gold nanoparticles (NPs) are being incorporated into various consumer products and are very promising in biomedicine as diagnostic and therapeutic delivery platforms. However, concerns about their safety, environmental and health impact have risen, as they might establish harmful interactions with biological systems. In this context, it is of major importance to understand the implications of exposure to these NPs on early life stages of biota, which have been considered, in general, as the most sensitive to chemical contamination.

The present study aimed to evaluate the lethal and sublethal effects that gold nanorods (GNRs) may provoke on early-life stages of the fish species *Danio rerio*. Therefore, zebrafish eggs at 6 h post-fertilization (hpf) were exposed to different concentrations of 10x35 nm GNRs suspensions, following the OECD TG 236. The embryos were observed at 24, 48, 72 and 96 hpf for evaluation of survival, somite formation, incidence of pericardial edema, heartbeat, malformations (general, spinal, tail and head), hatching and body length. The median lethal concentration ($LC_{50,96h}$) was calculated by fitting concentration-response curves with cumulative mortality obtained after 96 h of exposure.

The results revealed no mortality in embryos exposed to GNRs concentrations lower than 0.2 µg/mL. However, 66.7% of embryo mortality was observed at 0.3 µg/mL. At 96 hpf, the LC_{50} value obtained was 0.288 µg/mL with a 95% confidence interval ranging from 0.278 to 0.303 µg/mL. The tested GNRs concentrations did not elicit any significant malformation on zebrafish embryos. In addition, no alteration in the heartbeat of zebrafish embryos was observed at 48 hpf. However, a significant reduction of 8% in body length was detected in embryos exposed to the highest sublethal concentration tested (0.243 µg/mL) compared with controls.

In conclusion, under our experimental conditions, GNRs caused significant lethal and sublethal (body length) effects at low concentrations of the NPs, highlighting the need to perform predictive risk assessment of these NP in order to establish environmental safety values.

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P60.

HOW IMPORTANT IS THE STUDY OF ASSAY INTERFERENCE PRIOR TO NANOTOXICITY ASSESSMENT? CASE STUDY

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Most of the toxicity assays were developed for the evaluation of conventional drug compounds *in vitro*. In fact, researchers have realized that not all cytotoxic and genotoxic assays are appropriate for the evaluation of nanoparticle toxicity [1, 2] as several nanoparticles are capable to interfere with these methods [3]. For instance, the binding and possible inactivation of assay components involved and/or the interference with colorimetric detection are examples of nanoparticle interference on the cytotoxic assays [2]. For a correct assessment of the toxicity of the nanoparticles under evaluation, possible nanoparticle-assay interactions need to be identified.

In the present work was evaluated the possible interferences between 3 nanomaterials (TiO_2 nanoparticles, nanokaolin clay and TiO_2 nanoparticles immobilized in nanokaolin substrates) and the cytotoxicity (MTT, neutral red uptake (NRU), alamar blue (AB) and LDH) and genotoxicity (alkaline comet assay) assays under evaluation. This stage was essential to identify which of them were suitable for toxicity assessment of the studied materials, and to obviate possible interference between materials and assay reading by introducing alterations to assay protocols.

To reveal possible interferences between nanomaterials and cytotoxicity assays experimental procedures, two main sets of experiments were conducted: (1) light-absorption interference and (2) catalytic interference. For the LDH assay, an additional experiment was carried out to understand the possible nanomaterials interference on the enzymatic activity of LDH. For the alkaline comet assay it was performed a lysis test to in order to estimate the nanomaterials capacity to damage DNA.

Results obtained in these interference studies suggest that nanokaolin and TiO_2 nanoparticles immobilized in nanokaolin substrates were able to adsorb NRU and LDH assay components, decreasing the signal in both assays with the increase of particles concentrations. As these interferences could not be eliminated by protocol alterations, only MTT and AB assays were found to be suitable for further *in vitro* cytotoxicity studies. Regarding the alkaline comet assay, after a slight alterations to the protocol, this assay was appropriate for the genotoxicity evaluation of all tested nanomaterials.

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P61.

TIO_2-NP EFFECTS IN PLANTS ARE FORMULATION AND SPECIES DEPENDENT

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In the past years nanotechnology applications and related industries are increasing, with consequent release of nanoparticles (NPs) into the environment. The implications of NPs presence in the environment on plant growth/yield and agro-food quality and safety are under great concern [1]. Therefore, NPs impact in crops (considering physiological, biochemical and molecular levels) need to be more studied. At this moment, it seems that plant response to NPs exposure depends on several factors such as plant species, NPs characteristics and growth conditions. One of the metallic NPs widely spread in industrial and consumer products are Titanium dioxide (TiO_2) NPs [2]. TiO_2 NPs can be found in many daily products, such as paints, papers, cosmetics, food, and as photocatalyst in environmental technology [3, 4]. TiO_2 NPs exist in three allotropic forms: anatase (ana), rutile (rut) and brookite [5]. Rut is used mostly as a pigment, whereas ana is used for its superior photocatalytic properties [5]. The available studies on TiO_2 NP phytotoxicity indicate that these NPs may become toxic to plants.

The present work investigates the impact of two different TiO_2 NPs formulation on germination and growth as crucial parameters to agro-industry. Related with impaired growth, we also assess the impact of these NPs on cell cycle/mitosis disturbances, by the formation of micronucleus (MNs). These endpoints were also used to compare different degrees of tolerance using three agro-food relevant species: *Triticum aestivum* (wheat), *Lactuca sativa* (lettuce) and *Ocimum basilicum* (basil). Seeds were exposed to 5-150 mg L^{-1} of ana or rutile+anatase (rut+ana) TiO_2 NPs for 5 d and after exposure the different parameters were analyzed. Data showed that TiO_2-NPs may affect germination and growth. However, the effects are formulation and species dependent. Also, both formulations induced MN formation, supporting that both NPs may become genotoxic to plants species in a dose dependent manner. Rut+ana seems to be more genotoxic than ana at lower concentrations. By showing higher sensitivity to these formulations, MN test seems to be a reliable endpoint to assess TiO_2 nanoparticles toxicity in plant species.

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P62.

CHILDREN'S EXPOSURE TO VOLATILE ORGANIC COMPOUNDS OF HEALTH RELEVANCE IN KINDERGARTENS AND PRIMARY SCHOOLS

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Various volatile organic compounds (VOC) are classified as carcinogens, irritants, and toxicants, as well as promoters of the onset and exacerbations of asthma. This study, part of the EME (More Efficient Schools) project, aimed to assess children's exposure to indoor air pollutants in schools in Porto Metropolitan Area by: *(i)* quantifying the most abundant VOC in 54 naturally ventilated school buildings and *(ii)* comparing the results associated to the type of classrooms (kindergarten or primary rooms). VOC were collected by active air sampling using stainless steel tubes over 60 min in 108 classrooms at 54 schools. In addition, carbon dioxide (CO_2), temperature and relative humidity levels were measured concurrently using an IAQ-CALC monitor.

The mean concentrations of every target VOC were always higher indoors compared to outdoors. Among the aromatic hydrocarbons, benzene, toluene, m,p-xylenes and styrene were detected and quantified in all classrooms. High concentrations of terpenes were found in all monitored schools: α-pinene and limonene concentrations ranged from 1.7-13.8 μg/m^3 and 2.1-649.0 μg/m^3, respectively. These results pointed for the presence of specific indoor sources, namely, of products emitting VOC associated to cleaning and children's specific activities (like paints and glues). No significant differences were found for VOC concentrations between the kindergarten and primary classrooms, except for benzene (higher in primary classrooms, p=0.035). The mean CO_2 concentration for the 108 studied classrooms during the occupied period was 1430 ppm, ranging from 399 to 3810 ppm, with a median value of 1271 ppm. Overall, the studied classrooms presented mean CO_2 concentration above 1000, 1250, and 1500 ppm in 74%, 54%, and 39% of the cases, respectively. Indoor mean temperature was 20.5±3.6 ºC being 21.4±4.0 ºC for kindergartens and 20.2±3.0 ºC for primary classrooms; while the relative humidity indoors fluctuated between 50 and 54%, respectively.

Few studies assessed simultaneously the air concentrations of different groups of chemical compounds in schools. This study highlights a ubiquitous presence of VOC with known or suspected respiratory toxicity in kindergartens and primary classrooms. The identification and control of VOC sources are relevant, especially for vulnerable individuals. Moreover, ventilation conditions may not be negligible.

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P63.

A PRELIMINAR STUDY OF CARBON DIOXIDE CONCENTRATIONS AND STUDENTS' ACTIVITY PERFORMANCE IN PORTUGUESE PRIMARY SCHOOLS

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Poor indoor air quality (IAQ) has been associated with a number of health effects in children as well as with a decrease in their academic performance. However, limited data exist on how IAQ affects children' scholar performance. The aim of this study was to investigate the effect of classroom's carbon dioxide (CO_2) concentrations on schoolchildren performance.

Concentrations of CO_2 were monitored over a 5-day period in 59 classrooms from 13 naturally ventilated public primary schools located in the urban area of Porto, using infrared-based equipment. Standardized performance tests were applied in 841 children from the 3rd and 4thgrades (8 to 10 years old). The test included 24 math skills (mathematic test) and 119 numeric strings (Chipher test) that should be solved in 5 and 2 minutes, respectively. The performance assessment was conducted on a scholar day in the first hour of the classroom and repeated in the last hour of the day. The proportion of variation in CO_2 concentrations between the first hour and the last hour of the day was calculated. The proportion of variation in results of performance tests was also estimated. Regression coefficients (β) and respective 95% confidence intervals were computed to evaluatethe effect of CO_2 concentration on students' performance.

In the 1st hour of the class, children resolve correctly 17±6 mathematic operations and 40±9 of the numeric strings. Concerning to the second assessment, better results were observed for both tests (19±6 for mathematic test and 47±11 for Chipher test). No significant association was found between increase on CO_2 concentrations and schoolchildren performance [β=-0.090 (-0.201; 0.022) for mathematic test and β=0.021 (-0.020; 0.062) for Chipher test].

Although we observed lower improvement in the mathematic test with the increasing CO_2 concentrations, this effect did not reach statistical significance.

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P64.

FUNGAL COMMUNITIES IN HOUSE DUST SAMPLES FROM PATIENTS WITH ASTHMA – PRELIMINARY RESULTS

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People spend about 90% of their time indoors, being exposed to a large number of indoor contaminants, including fungi. Some fungi are associated with a wide range of adverse health effects, including the risk of asthma onset or exacerbation. Many studies support this fact, showing associations between the exposure to indoor damp and mould and the risk of asthma. A previous study by this team demonstrated that house dust is a suitable matrix to evaluate indoor fungal communities. Therefore in this survey fungal communities from houses of asthma patients and controls (non-asthmatics) were characterized in dust samples collected from the participants' vacuum cleaner bags. Volunteers were recruited by a medical doctor (Allergist) during their routine consultation and those willing to participate signed an informed consent. For the fungal identification, the samples were treated by three different culture methods (direct plating, suspension and dilution). Fungal quantification was performed by naked eye and the identification was performed based on the phenotypic characteristics of each fungus, identified through an optical microscope. The results hereby presented correspond to the first group of samples analyzed: 6 samples from asthma patients' houses and 7 samples from controls. The number of Colony Forming Units of fungi per gram (CFU/g) ranged from 833 to 1583 CFU/g for asthmatics and from 933 to 2767 CFU/g for the controls. No significant differences (Man Whitney U test, $p>0.05$) were found between the CFUs in the house dust from the two groups. *Aspergillus niger*, *Penicillium* sp., *Mucor* sp. *Alternaria alternata* and yeasts were the most common fungi, being detected in both groups of samples. Considering the limited number of samples analyzed so far it is difficult to draw any conclusions. More samples are being analyzed which will provide opportunity for further discussion.

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P65.

UNEXPECTED EFFECT OF DRY OLIVE LEAF EXTRACT (DOLE) BEFORE AND AFTER $CaNa_2EDTA$ CHELATION THERAPY IN COMET ASSAY IN LEAD INTOXICATED WORKERS

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The $CaNa_2EDTA$ chelation is a standard therapy for lead (Pb) intoxication in occupationally exposed workers. Application of antioxidant nutrients through exogenous supplementation is often practiced with the chelation therapy, although their synergistic effect in reduction of Pb-induced oxidative damage has not been investigated conclusively. Dry olive Leaf extract (DOLE) is polyphenol rich natural antioxidant. The effects of DOLE on the levels of DNA damage were investigated *ex vivo* in peripheral blood lymphocytes (PBLs) of 19 male workers occupationally exposed to Pb, before and after application of five day $CaNa_2EDTA$ chelation therapy. Comet assay was used to assess levels of DNA damage.

While the level of DNA damage in PBLs of workers before chelation were moderately increased (24.21±14.26) compared to controls (6.0±3.37), the incubation of the same samples with 1mg/mL DOLE for 30 min at 37^0C lead to a severe increase in DNA damage levels (64.03±20.96). After the exposure of workers to a five day $CaNa_2EDTA$ chelation regimen, the experiment was repeated. Following chelation, the level of DNA damage in PBLs of workers was decreased (8.26±4.62) significantly compared to the baseline value and was then similar to the control level. When the PBLs after chelation were treated with 1mg/mL DOLE for 30 min, high level of damage was obtained (41.82±23.17). The antigenotoxic effects of five day $CaNa_2EDTA$ chelation were demonstrated in PBLs of Pb exposed workers. On the contrary, the applications of DOLE lead to an increase of oxidative DNA damage after 30 min incubation, exhibiting prooxidant rather than antioxidant effect. After $CaNa_2EDTA$ treatment, the acute prooxidant effects of DOLE remained following the incubation, but, the oxidative DNA damage was less severe compared to the same experiment with DOLE before the chelation, probably as a result of partial removal of Pb from cells by chelation therapy. Prooxidant nature of DOLE could be a result of Pb-mediated hydroxyl radical formation, where heavy metals serve as catalysts for the reactions which oxidize DOLE and reduce oxygen. Removal of Pb by complexation with CaNa2EDTA seems to significantly depress these oxidative events. However, this mechanism remains to be explored on molecular level. It could be concluded that the DOLE exhibits prooxidant effects in presence of Pb in lymphocytes of exposed workers, and its effect is less pronounced following the removal of Pb after standard chelation therapy.

P66.

ASSESSMENT OF DNA DAMAGE ON A GROUP OF PROFESSIONAL DANCERS

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Despite the numerous health benefits of physical activity, it is known that the induction of oxidative stress, on the cells and tissues metabolism, is a direct consequence of this practice and may contribute for various diseases [1]. Cellular damage induced by oxidative stress can be represented through the modifications of several macromolecules such proteins, lipids and nucleic acids. Genotoxicity evaluation is a crucial tool for studying important occupational hazards. The aim of the present study was to evaluate both DNA damage and oxidative stress in blood of a group of professional dancers before and after the season, comparing the first moment with general population.

Study population consisted of a total of 28 healthy subjects, 14 professional dancers and 14 controls. In case of professional dancers, blood samples were taken immediately before and after the season. For each subject, relevant information was assessed by questionnaire. It was used the classical comet assay version to measure the basal DNA damage through the percentage of DNA in the comet tail. It was also used the comet assay enzyme version to calculate the oxidative damage. Analyses were conducted using the IBM SPSS for Windows statistical package 23.0. Different statistical tests were used and the level of significance considered was 0.05.

Both oxidative damage and basal damage showed an increase after the season among professional dancers. Oxidative damage was significantly higher (p-value: 0.001) after the season (mean ± sd: 4.67 ± 2.54) than before (mean ± sd: 1.06±0.59) the season in this group of study.

Data obtained in this work indicate that intensive physical exercise exposure is associated to an increase level of oxidative DNA damage.

These results may offer the support needed to develop new studies or implement effective measures in order to protect professional dancers health and another occupations with same physical requirements, including regular monitoring and surveillance activities.

[1] V. Rani, et al., *Life Sci*, doi:10.1016/j.lfs.2016.02.002 (2016)

P67.

WILDLAND FIREFIGHTERS: DNA DAMAGE AND OXIDATIVE STRESS ASSESSMENT

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Portugal is a high risk country for forest fires. In 2013 alone, Portugal forests accounted for more than 50% of the total burned area of Southern Europe. Portuguese firefighting is supported by a human force of 30.000 firefighters. Firefighters are often exposed to many toxic combustion products, including known carcinogens (benzene, vinyl chloride, formaldehyde, polychlorinated biphenyls, particulate matter). IARC classifies firefighting (or occupational exposure as firefighters) as possible carcinogenic to humans (Group 2b). Epidemiological studies suggest that firefighters have an elevated risk of developing cardiovascular and respiratory diseases and various types of cancer. Biomonitoring data are limited, inconsistent and inconclusive. Genotoxicity evaluation is a valuable tool for studying the most important occupational hazards allowing a reasonable epidemiological evaluation of cancer prediction. The aim of this study was to evaluate DNA damage, total and oxidative, in Portuguese wildland firefighters when compared with the general population. A total of 123 individuals were engaged in the study, 60 volunteer firefighters and 63 non-exposed control subjects. Total and oxidative DNA damage were evaluated by comet assay in whole blood samples; oxidative damage was measured by formamidopyrimidine glycosylase (FPG). Both total and oxidative damage were increased in firefighters compared to controls. However, only total DNA damage was significantly higher (11.23±0.36 vs 6.38±0.42). The influence of life style factors and work-related variables (duration and recent exposure) was also studied, but no significant effect was found. Firefighters with more than 7 years of activity had a higher basal DNA damage and showed an increasing trend in oxidative DNA damage with increasing years; however statistical significance was not reached. To our knowledge, this is the first Portuguese study to investigate the potential genotoxic effects of wildland firefighting exposure. Data obtained in this work indicate that wildland firefighting exposure is associated to an increase level of DNA damage; firefighters also showed higher oxidative damage compared to controls namely oxidised purines. Results provide new data regarding potential mechanisms underlying the health effects of wildland firefighting exposure. However, further studies with a larger population and different biomarkers are needed to confirm these results. Nevertheless, data from this study may offer the support needed to implement effective measures in order to protect firefighter's health, including regular monitoring and surveillance activities, such as medical surveillance, good practice campaigns, training programs and implementation of written policies and procedures.

P68.

IMPORTANCE OF SIZE-SELECTIVE PARTICLE MEASURING FOR ASSESSING OCCUPATIONAL EXPOSURES – A CASE STUDY "FROM FIELD TO FORK"

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Sampling the total air concentration of particulate matter (PM) only provides a basic estimate of exposure that normally not allows correlating with the observed health effects. Therefore is of great importance to recognize the particles size distribution and, particularly, the exposure to fine particles (≤ 2.5 μm). This particles dimension corresponds to the respirable fraction, the one that can implicate local and systemic effects due to particle deposition and clearance from the lungs and transport within the organism.

This study intended to describe occupational exposure to PM2.5 in three units related with swine production and consumption, namely: feed production, swine production and swine slaughterhouse. A size-selective particle measuring in five to six workplaces of each unit was performed. Measurements of PM were done using a portable direct-reading hand-held equipment (Lighthouse, model 3016 IAQ).

Data showed slaughterhouse unit with higher values, with values ranging from 0.030 to 0.142 mg/m^3 (0.073$\pm$ 0.043), being the cutting room the workplace with higher values. In feed production unit, values were between 0.026 and 0.033 mg/m^3 (0.028 $\pm$ 0.003) with the warehouse of pharmacy products as the workplace with higher values. Finally, in swine unit values ranged from 0.006 to 0.048 mg/m^3 (0.023$\pm$ 0.017) with the batteries area presenting the higher values.

PM can be rich in fungi and bacteria and their metabolites, such as endotoxins and mycotoxins. Previous publications already showed high contamination in these occupational settings and particles can have an important role in exposure since can easily act as carrier of these agents.

Data acquired allow not only a better prediction of particle penetration into respiratory regions of the respiratory tract, but also a better estimation of PM health effects. Moreover, data permit to identify the workplaces where investment should be made to prevent and reduce exposure.

P69.

OCCUPATIONAL TOXICOLOGY IN THE AGRICULTURE OF MOLDOVA

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In the Republic of Moldova, more than 40% of the total number of farm workers are activating in unfavorable working conditions, including toxic substances exposure. There are used more than 2.400 tons of plant fertilizer and pesticides each year and the intensity of their use is 1.52 to 5.62 kg/ha [1]. Occupational - environmental contamination with pesticides may contribute to the development of professional diseases among farm workers. The objective of this study was to analyze the structure and the dynamics of professional diseases among farm workers.In this work were applied statistical and descriptive-analytical methods. As research materials were used 215 medical cards of machine operators with diagnosed occupational disease (1998-2015 years).

Occupational disease was diagnosed more among machine operators in agriculture (73.5% of total) with 26.1±0.71 years (SD=7.6) work experience. Their working conditions are influenced by negative microclimate, high levels of noise and vibration; during labor on farmland the air in the occupational environment is also contaminated with dust, particles of fertilizer and pesticides. So, professional diseases among machine operators were caused by the following agents: ergonomic/ overuse of certain human /machine operator organs in the work (89.2% cases), physical (19.2%) and chemical determinants (33.9%).

Agricultural toxicology is one of the key branches of occupational health. It's purpose is monitoring the machine operators' working conditions, especially the use of fertilizer and pesticides, and the threat they represent for the health of workers and the environment.

[1] National Bureau of Statistics. StatBank. Available at: http://www.statistica.md/index.php?l=en (accessed 16 February 2016).

P70.

BPA OCCUPATIONAL EXPOSURE ASSESSMENT IN EUROPE: A SCIENTIFIC GAP

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Bisphenol A (BPA), 2,2-bis(4-hydroxyphenyl) propane is one of the greatest volume industrial chemicals utilized in the world, employed in the manufacture of a variety of indoor applications and consumer products with increased production every year.

Human environmental exposure to this xenoestrogen is considered a generalized phenomenon and several studies have focused in estimate human daily intake and potential associated health effects. Recently, the European Food Safety Authority (EFSA) temporarily reduced BPA Tolerable Daily Intake (TDI) from 50 µg/kg body weight/day to 4 µg/kg body weight/day. However, occupational exposure to BPA have been overlooked and considered safe by the European authorities.

Here we performed a revision of the literature published in Pubmed that assessed BPA occupational exposure and associated effects. Despite of the massive BPA production and consumption in European countries, with policarbonate and epoxy resins as the major applications, we have only found 13 studies from 2009 to 2015 performed in the context of occupational exposure in plastic and/or epoxy resin factories in China. These studies demonstrate that in occupationally exposed individuals detected BPA levels are significantly higher (2.22 - 685.9 µg/gCr (urine); 18.75 – 101.94 ng/ml (serum)) considering the levels associated with environmental exposures (10 µg/gCr (urine); 0.5-3 ng/ml (serum)) and the detection rate of serum BPA increase is correlated with time of exposure (27.18 ng/ml 5 years or less; 9.73 ng/ml more than 5 years). BPA-exposed male workers had consistently higher risk of male sexual dysfunction across all domains of male sexual function, endocrine disruption, alterations on epigenetic marks (DNA methylation) and epidemiological evidences demonstrate that parental exposure to BPA in the workplace during pregnancy is associated with decreased birth weight of offspring and shortened anogenital distance in male offspring.

European authorities underestimate the potential adverse of BPA and reliable data on the number of workers at risk at a European level, or the number of occupational diseases arising from BPA exposure are unknown. There is an urgent need to assess the actual exposure of workers to BPA, to create occupational standards and take effective preventive measures to protect workers from potential health adverse effects.

P71.

FIREFIGHTERS' OCCUPATIONAL EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS AT PORTUGUESE FIRE STATIONS

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Polycyclic aromatic hydrocarbons (PAHs) are among the most relevant pollutants due to their toxic, mutagenic, and carcinogenic properties. Although scarce information exists regarding firefighters' exposure to PAHs, there is a great gap of knowledge regarding this topic. Thus the aim of this study was to assess firefighters' personal exposure to PAHs during regular work shifts at fire stations, with emphasis on carcinogenic compounds and potential heath risks.

Eighteen PAHs (16 USEPA priority PAHs, dibenzo[a,l]pyrene, and benzo[j]fluoranthene recommended by EU Directive 2008/50/EC) were collected during a period of 4 consecutive hours in the breathing air zone of healthy and non-smoking firefighters, that were not directly involved in firefighting activities. The exposure of individuals from five different fire stations located in district of Bragança (north of Portugal) was characterized.

Median total PAH (ΣPAHs) concentrations ranged from 46.8 to 154 ng/m^3, with acenaphthylene, acenaphthene, naphthalene, and phenanthrene being the most predominant compounds (73.3 – 95.7% of ΣPAHs). Total carcinogenic PAH levels (sum of benzo[a]pyrene, naphthalene, benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, chrysene, dibenzo[a,l]pyrene, dizenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene) ranged from 12.7 to 20.2 ng/m^3 and represented 8.2 to 27.2% of ΣPAHs. Benzo[a]pyrene, the marker of exposure to carcinogenic PAHs, was detected in the breathing air zone of several individuals from two fire stations (median values of 0.449 and 0.977 ng/m^3). Firefighter's personal exposure to PAHs at Portuguese fire stations was well below the existent occupational exposure limits.

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P72.

EXPLORATORY STUDY: BACTERIAL CONTAMINATION IN HOTEL ROOMS DURING THE CLEANING ACTIVITY

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Cleaning staff can be also exposed to different types of biological agents such as microorganisms present in dust as well as in aerosols created during the cleaning process, however this exposure is not deeply characterized. This study aims to assess and characterize the occupational exposure to bacterial contamination in hotel rooms, one with carpet floor and another without carpet.

Selected points for air samples were pre-defined in terms of normal cleaning activities of a room. Outdoor sample were also performed to be used as reference. Air samples of 250 L were collected, six in a room with a carpet and other six non-carpeted room, through an impaction method with a flow rate of 140 L/min onto Tryptic Soy Agar (TSA) supplemented with nystatin (0.2%) for analysis of mesophilic bacterial population using the Millipore air Tester (Millipore). Samples surface and air swabs were collected at the same time, and were performed by surface swab technique according Staib and Gross (1983), Panagopoulou *et al*. (2002), and also in accordance with the procedures specified in ISO 18593 (2004). All the collected samples were incubated at 30º for 7 days. After laboratory processing and incubation of the collected samples, quantitative results were obtained through the determination of colony-forming units (CFU/m3 and CFU/m2) and qualitative characterization of bacterial population was performed by morphological typing.

The prevalent morphotype (90%) isolated from indoor airborne microbiota in both room types was gram-positive, catalase-positive cocci.. Comparing the two types of rooms, the total number and type of bacteria was not significantly different ($U = 1358.000$, $p = 0.393$). The results obtained suggested that there is no differential bacterial exposure during cleaning activities in hotel rooms with and without carpet.

P73.

ASSESSMENT OF TRIBUTYLTIN EFFECTS AT THE VASCULAR LEVEL

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Tributyltin (TBT) is one of the most studied chemical within organotins, an important class of organometallic compounds with anthropogenic origin. TBT acts as a potent endocrine disruptor being also considered as a model obesogen, immunotoxic and neurotoxic compound. The human exposure to this and other organotins occurred as a consequence of their widespread commercial applications such as plastic stabilizers, catalytic agents for the production of silicones and polyurethane foams and industrial biocides[1]. Biomedical applications may constitute a potential source of organotins exposure due their presence in silicone based products used as medical devices including breast implants and cardiac valves. According to the World Health Organization the prevalence of cardiovascular diseases is sharply increasing and constitutes the prime cause of death at a global scale. Taking this into account, regulatory agencies recommend the study of organotins' toxicity. Considering the limited number of studies on the cardiovascular effects induced by these compounds, the aim of the present study is to elucidate the effects of TBT at the vascular level. The TBT effect on the contractility of rat artery (aorta) was performed using the organ bath technique in rat aorta without endothelium with two different contractile agents: noradrenaline (1µM) and potassium chloride (60mmol/L). The L-type calcium channels activity in A7r5 (cell line derived from the smooth muscle of embryonic rat aorta) was quantified by whole-cell configuration of the patch clamp technique. The preliminary data suggest that TBT relaxes the rat aorta contracted by noradrenaline or by potassium chloride. The electrophysiological experiments suggest that TBT inhibits the L-type calcium current in A7r5 cell line (rat aorta vascular smooth muscle). These results suggest an effect of TBT on the vascular system, although further studies are necessary to analyze the effects on other pathways. .

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[1] A.C.A. Sousa et al., *Environmental Chemistry Letters* **12**, 117-137 (2014)

P74.

EARLY BIOLOGICAL EFFECTS (CYTOME ASSAY) IN CHILDREN EXPOSED TO DIFFERENT LEVELS OF $PM_{0.5}$ IN FIVE ITALIAN CITIES DURING WINTER 2014-2015: MAPEC (MONITORING AIR POLLUTION EFFECTS ON CHILDREN FOR SUPPORTING PUBLIC HEALTH POLICY) STUDY.

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Epidemiological studies have shown that air pollution can cause harmful health effects. In particular, it was found a consistent association between exposure to particulate matter (PM) and incidence and mortality for several chronic diseases. The International Agency for Research on Cancer of WHO has ranked air pollution among the human carcinogens, and genotoxic damage was indicated as the main mechanism responsible for the adverse effects. The children are more vulnerable than adults to the effects of airborne agents for several reasons. The MAPEC (Monitoring Air Pollution Effects on Children for supporting public health policy) study aims to identify the biological damage markers in buccal cells, such as the presence of micronuclei, which are predictive of the onset of chronic diseases in adulthood. The micronucleus cytome assay was performed in exfoliated buccal mucosa (BM) cells of about 1,000 children (6-8 years-old) from five Italian towns (Brescia, Torino, Pisa, Perugia and Lecce) characterized by different concentrations of air pollutants. The BM cells were collected using a small-headed toothbrush, were fixed on microscope slides and stained with Feulgen/LightGreen for both bright field and fluorescence microscopic analysis. The biomarkers of genome damage (i.e. micronuclei and nuclear buds) were evaluated only in normal differentiated cells. The results from microscope analysis of cells sampled on winter 2014-2015 suggest a significant reduction of micronuclei frequency from Northern to Southern Italy, except for Torino. The frequency of micronuclei is proportional to the annual average concentration of PM in the air.

P75.

FUNGI DISTRIBUTION IN POULTRY FEED

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Feed can easily be contaminated and colonized by fungi that use up the nutrients for their own metabolism and growth, producing secondary metabolites such as mycotoxins that are not eliminated throughout the feed processing. The major problems associated with mycotoxin contaminated animal feed are metabolic disturbances resulting in poor animal productivity. In addition, handling contaminated animal feed can also raise health issues regarding workers exposure to fungi and mycotoxins.

The scope of this work was to characterize fungal distribution in 11 poultry feed samples. Twenty grams of feed were suspended in 180 mL of distilled water and homogenized during 20 minutes at 200 rpm. The washed supernatant was plated in malt extract agar (MEA) and dichloran glycerol agar base (DG18) media for morphological identification of the mycobiota present.

Using macro- and microscopic analysis of the colonies, fungal contamination was evident in 72.7% of the analyzed poultry feed samples. Fungal load ranged from 0 to 13140 CFU/g, and the most prevalent species/genera were *F. graminearum* complex (71.1%), *Penicillium* sp. (11.6%), *Cladosporium* sp. (8.8%), and *Fusarium poae* (3.6%). In addition to these species, we also isolated *Aspergillus* sections *Circumdati*, *Nigri* and *Aspergilli*, and *Mucor* and *Rhizopus* genus albeit at a lower abundance.

The data obtained showed that, besides high fungal contamination, mycotoxins contamination is probably a reality, particularly in the final product since mycotoxins resist to all the processing operations including thermal treatment. Additionally, data claimed attention for the probable co-exposure to fungi and mycotoxins of the workers in feed industries.

P76.

AIR MICROBIOLOGY – EXTERNAL QUALITY ASSURANCE PROGRAM

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The Regulation on Energy Heating and Cooling Systems in Buildings (Decreto-Lei 79/2006, April 4th) imposed efficiency rules for Heating Ventilation and Air Conditioning Systems (HVAC) in buildings, established reference concentrations for chemical and microbiological pollutants and compelled the execution of regular audits to indoor air quality in order to guarantee healthy indoor environments in existing commercial buildings with large HVAC systems.

Given the significant increase in indoor air quality audits it became necessary to harmonize sampling and analysis procedures for microbiological pollutants in order to obtain comparable results.

The Air and Occupational Health Unit (UASO) implemented the required procedures for accreditation of the assay "Determination of airborne culturable microorganisms" according to the International Standard ISO/IEC 17025 [1], unequivocal indicator of technical proficiency.

In the absence of external quality assessment programs (EQA), a requirement of the standard, the UASO Unit in collaboration with the National Program for External Quality Assessment, organized the EQA program - Air Microbiology that consists on the bacteriological and fungal enumeration in air samples collected following the EN 13998 Standard methodology [2]. The EQA program allowed the comparability of results between participant Laboratories leading also to the harmonization of procedures, improving the traceability of measurements, result validation and improving the whole process leading to accreditation.

At the end of each year the organizers of the EQA Program present the results and organize training courses in the areas identified as sensitive. The most debated topics have been the equipment calibration, estimation of uncertainty, the acceptance criteria of duplicate samples and control laboratory test conditions.

The aim of this study is the assessment of the results of the EQA program - Air Microbiology (2010-2015).

[1] International Standard ISO/IEC 17025:2005 - General requirements for the competence of testing and calibration laboratories.
[2] European Standard EN 13098 - Workplace atmosphere – Guidelines for measurement of airborne micro-organisms and endotoxin.

P77.

THE RELATIONSHIP BETWEEN THE IMBALANCE OF ECOSYSTEMS AND POVERTY, A CURRENT PUBLIC HEALTH SITUATION

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Considering that we as human beings are by nature, and in a figuratively sense, blind enough to glimpse a global picture of some situations, especially if those are related to environmental issues and its current relationship to poverty alleviation, commonly treated as non-related topics. Nevertheless, if we take as principal reference pollution and poverty rates, it can be inferred a directly and proportional link, as a result of the necessity consume (indiscriminately) of the surround ecosystem with the ultimate aim of satisfy our own necessities. In the 21st United Nations Climate Change Conference (COP21) edition, 187 Parties marked a decisive turning point towards comprehensive and collective global action, which will accelerate the transition to a climate resilient development, climate neutral global economy. With regard to poverty, an available database on "Population below poverty line" (PBPL) showed the effects of climate change hits the hardest on this population group, posing risks over agriculture, food and water supplies highlighting the relationship with local economy. The aim of this paper is an invitation to rethink the interconnection between the human being, worldwide aid and the recognition that a sustainable development could not be explore away from poverty and its inherent consequences. This document is a preliminary product of a four years' exploratory investigation of worldwide poverty rates, natural resources consumption and the impact over Human Scale Development (HSD).

Poverty cannot be treated separately from the well-being and socio environmental impact and more specifically, imbalance of ecosystem. According to Max Neef et al [1], HSD is as important as the life itself, showing that no matter the way, it is imperative to meet or satisfy the needs of a society, this might give the basis for a possible explanation of many of the current problems arising from excessive consumerism economy, leading to critical awareness at the economic level. With regard to PBPL which are Asia (East and South) 7.2 and 18.8% respectively, Latin America and Caribbean 5.6%, among others, that the effects of climate change such as higher temperatures, changes in precipitation patterns, rising sea levels, and more frequent weather-related disasters hits the hardest affecting the local economy and taking too many lives each year.

Human health depends directly from ecosystem, is the most antique life-support system, not only for human species but also for any other form on life in planet Earth. From a holistic point of view, the most likely way for vulnerable countries to deal with climate changes consequences and contamination derived from a mechanistic economy, which clearly harms the human being, is to adopt the agreement of COP21st, where developed countries from abroad at the "High Ambition Coalition" will engage Parties towards the fulfilment of agreed goals. Maintaining climate change advocacy, diminishing contaminating gas emission and therefor better air quality, increasing efforts to address the nexus of climate change, natural resources including water & air, prosperity, stability and migration are the most important international goals for 2016. To act from now on is a compulsory duty to save our ecosystem and so the human beings.

[1] Max Neef et al., World Development **30**, 181-205 (2002)

P78.

AIR QUALITY BIOINDICATORS IN HEALTH RESEARCH: WHAT WE HAVE LEARNED FROM AN INDUSTRIAL AREA

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Bioindicators are used for characterizing air quality in environmental assessment studies. Among those, lichens are frequently used, and provide information with high spatial resolution. These long-lived organisms incorporate the effect of all pollutants in way that reflect the air quality over the medium-long term. Although lichens have been extensively used to monitor air quality, there is still an unaddressed potential for their use in environmental health research.

Throughout the Gestão Integrada da Saúde e Ambiente project (GISA project) several environmental health research studies were conducted in coastal Alentejo (Portugal) to measure the association between air quality measured with lichen bioindicators and health outcomes. The studies conducted illustrate some concerns of using lichen bioindicators for exposure assessment in health studies. Here we highlight the lessons learned with those studies and point some ways forward to use such bioindicators for exposure assessment in health research.

An important key to take steps forward in these fields is an interdisciplinary work with close collaboration between experts from bioindicators specialists, epidemiologists, and spatial statisticians. On the basis of experience gained by the researchers participating in these studies, some recommendations for utilizing bioindicators in environmental health research are delivered: a) an analysis on seasonal variations and sudden pollution episodes is required to gain insight on the type of air pollution reflected in bioindicators, b) selection of sufficient observations with distinct personal exposure profiles are needed to maximize exposure contrasts, c) specially in urban settings, where concentrations change at very short distances due to sudden changes in land-use and in intensity of pollution, use a spatially stratified sampling design is important to reduce exposure bias, d) spatial statistical models should incorporate spatial uncertainty of exposures, a critical measure for successful use of spatial statistical methods in environmental health research.

P79.

MICROBIOTA ASSESSMENT IN OPTICAL SHOPS: AN IGNORED CONCERN TO PUBLIC HEALTH

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The presence of microorganisms in ophthalmic instruments and surfaces can lead to the exposure of patients to several infections. However, there is no information regarding fungal and bacteria contamination in optical shops. This study aims to characterize fungi and bacteria contamination in air and surfaces from 10 optical shops covering also ophthalmic instruments.

Air samples were collected through an impaction method onto malt extract agar (MEA) supplemented with chloramphenicol (0.05%) used for fungi and Tryptic Soy Agar (TSA) supplemented with nystatin (0.2%) used for bacteria. Outdoor samples were also performed to be used as reference. Surface and equipment's swab samples were also collected side-by-side. All the collected samples were incubated at 27ºC for 5 to 7 days (fungi) or at 30º for 7 days (bacteria).

Regarding fungal distribution, thirteen different species/genera were found in the air, being the most common *Alternaria* sp. (62.0%). Eight different species/genera were identified in the surfaces, ranging from 2 to $5x10^4$ CFU/m^2, being the most common *A. versicolor* complex and *Penicillium* sp. (40.0%). The trial frames were the most contaminated equipment, since 50.0% of the collected samples were with countless colonies. The airborne bacterial population indicated higher concentrations in the contactology office (average: 133 CFU/m^3) than in the client's waiting rooms (average: 126 CFU/m^3). The surface samples indicated bacterial concentrations ranging from $2x10^4$ to $1x10^6$ CFU/m^2, pointing out the automatic refractometer as the surface with higher bacterial load.

P80.

AIR POLLUTION IN LISBON: A LOCAL OR A GLOBAL CONCERN?

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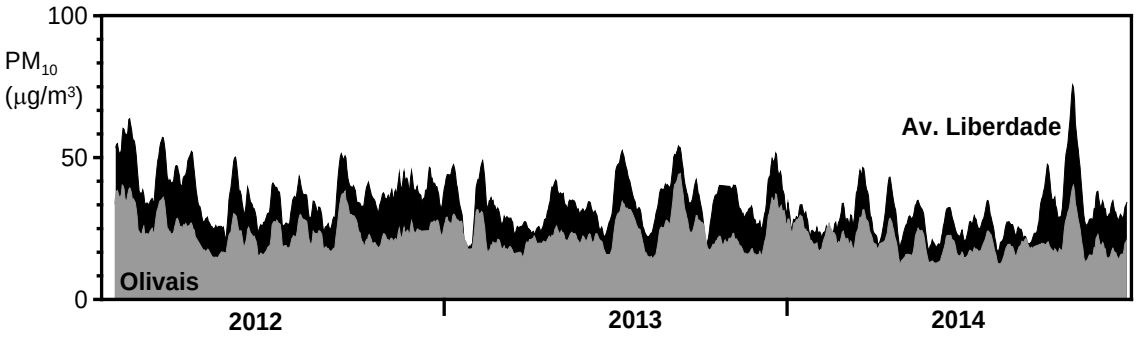
The latest data of WHO (World Health Organization) show that in 2012 ambient air pollution was responsible for 3.7 million deaths, representing 6.7 % of the total deaths in the world. Ambient air pollution is estimated to cause about 16 % of lung cancer deaths, 11% of chronic obstructive pulmonary disease (COPD) deaths, more than 20% of ischaemic heart disease and stroke, and about 13% of respiratory infection deaths. The major causes of these deaths are the pollution by particulate matter and this problem has a great significance in Asia and Africa, namely in countries with low and middle-income economy. Children under five years old are greatly affected by this type of pollution and represent a significant part of the total deaths.

In this work we have investigated the data of air quality in Lisbon, form 2012 to 2014, collected by the monitoring air quality stations that are part of the national and European monitoring air quality network. He have compared the data of the four stations in the city, namely the data of the Av. Liberdade station (Portugal's most polluted site, in terms of particulate matter) with the data of the others tree stations. We found that no significant differences are registered in the pattern of the pollution in the whole city. In the figure bellow we can see, as an example, the data for two stations in Lisbon: Av. Liberdade (black), a high traffic area in the center, and Olivais (grey), a residential area 10 km away from the city center.



The overall behavior in the two above presented sites is similar. It can clearly be seen that the values for Olivais are always bellow the values for Av. Liberdade but presenting an identical pattern all over the studied years.

The similitude of this results has been demonstrated by statistical analysis of the data, namely by correlation analysis and by PCA (Principal Component Analysis). This confirmation reveals a similar and also global pattern of pollution in Lisbon, mostly due to the intense automotive traffic in the city. These results show that the pollution in Lisbon must be lowered, not only in the critical sites, but in the entire city, to provide a most healthy atmosphere to the people that live and work there.

P81.

HEALTHS CONCERN ABOUT HEAVY METALS CONTENTS IN LISBON SOILS

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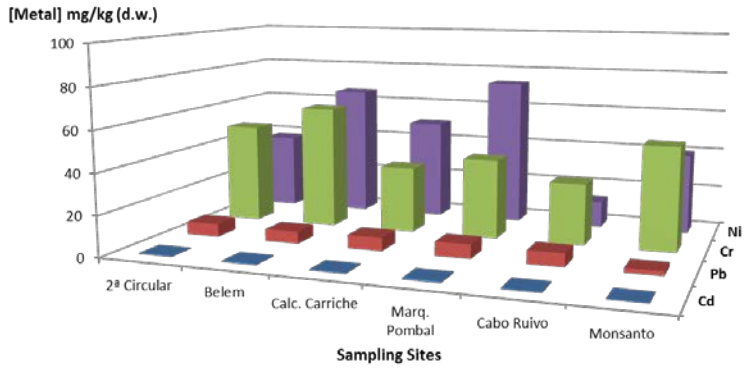
The aerosols that exist as pollutants in the air of Lisbon have two main origins: automobile traffic and suspensions from soils. In Lisbon we may consider that there are no significant industries in terms of production of this kind of pollutants, and so its contribution can be neglected. In this context, the content of metals in soils may be considered as a matter of concern due to population EXPOSURE, by inhalation, oral ingestion and dermal contact.

The evolution of some metal contents, namely cadmium (Cd), chromium (Cr), nickel (Ni) and lead (Pb) in Lisbon soils has been monitored during nine years in six city sites. Exposure to these metals can lead to various types of damage to human health and so they are included in the list of pollutants under legal and mandatory observation in Portugal, Europe and most of the developed countries.

A total of 648 soil samples were collected, always in November, at a deep of 20 cm. These samples gave rise to 54 composite samples, one composite sample by local and year. Total metal contents, in each composite sample, have been determined by graphite furnace atomic absorption spectrometry (GFAAS), after acid digestion. Mean values of soil metal contents, obtained for the six city sites, are shown in the following figure.



The mean contents obtained for Cd (0.46 mg/kg of dry weight, d.w.), Pb (5.7 mg/kg d.w.), Cr (43.9 mg/kg d.w) and Ni (46.6 mg/kg d.w) are below the maximums recommended by the Canadian Soil Quality Guidelines (1.4, 70, 64 and 50 mg/kg d.w for Cd, Pb, Cr and Ni, respectively), though the mean values for Cr and Ni are quite close to the minimum allowed values. However, despite these mean values, it should be noted that, for some samples, the maximum values found were in the order of 1.1 mg/kg d.w for Cd, 12.2 mg/kg d.w for Pb, 88.5 mg/kg d.w for Cr and 120.4 mg/kg d.w for Ni, which raises some concerns in terms of public health in Lisbon city, notably with regard to Cr and Ni, whose values are significantly high.

P82.

BIOMONITORING OF ARSENIC, CADMIUM AND LEAD IN URINE OF CHILDREN FROM THE PORTUGUESE REGION OF TÂMEGA

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Exposure to environmental pollutants occurs through different routes, such as inhalation, ingestion, and dermal absorption. Thus, the body burden of a specific pollutant is determined by several factors, such as the pollutants concentration, their physical and chemical properties and time of exposure, as well as individual factors, such as uptake, metabolism and excretion rates. Human biomonitoring takes into account these factors by measuring the concentrations of a pollutant in human tissues or body fluids. This biomonitoring study was conducted to assess children´s exposure to arsenic (As), cadmium (Cd) and lead (Pb) through urine analysis from a representative sample (n=535). This is a cross-sectional study of a random representative sample of children (6-12 years) from one NUTS III region of northern Portugal (Tâmega). We have used a multi-stage complex sampling method, with clusters in three levels (county, group of schools and classes). The urine levels of As, Cd and Pb was measured by inductively coupled plasma-mass spectrometry (ICP-MS) according to the CDC method 3018.3. This is the first study simultaneously analyzing those three elements in urine from Portuguese children. The median levels (µg/L) were 0.14 for Cd, 0.47 for Pb and 29.11 for As. In general, the urinary concentrations of these toxic metals were below international reference limits (<2 µg/L for Cd, <4 µg/L for Pb and <35 µg/L for As), however concentrations of As were higher than 35 µg/L in 35% of children. These preliminary results reinforce the relevance for more detailed toxicological studies in school-aged children.

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P83.

EVALUATION OF HUMAN EXPOSURE TO ENVIRONMENTAL CHEMICALS: THE INTEGRATIVE APPROACH OF BIOPORTO GROUP

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Environmental cues can have a direct biological effect, so it is not surprising that the profound environmental changes triggered by man in the last century and its subsequent exposure to ubiquitous chemical pollutants, have been correlated with adverse human health outcomes. With its concern, biOporto group presents a multidisciplinary research approach to this problematic. Resorting to specific and complementary skills and accessibility to relevant infrastructures and necessary equipment it aims to measure human exposure to environmental chemicals, as well as to assess their health effects.

Hence, there is a focus on three main thematic lines (TLs) i.e. TL1 - environmental chemicals analysis and development of new detection methods, TL2 - evaluation of their biological effects (*in vitro*/*in vivo* models and humans) and, TL3 - data management and statistical analysis. The TLs are not only strongly aligned with the major priorities defined for Human Biomonitoring research in Europe, but are designed to maximize the research interactions and complementarities of expertise among the member institutions.

Together, the ICETA/REQUIMTE and CINTESIS teams have extended expertize in: 1) identification and quantification of biomarkers of exposure and/or effect and/or susceptibility (e.g. GC-MS/MS; GC-ECD; LC-MS/MS; LC-FL; HR-CS-AAS); 2) development of new analytical methodologies for the detection of biomarkers in several matrices (blood, plasma, serum, urine and adipose tissue); 3) assessment of environmental exposure impact on human health in specific target cohorts (e.g. obese patients, diabetic, autistic, controls); 4) understand the human health impact resorting to experimental and mechanistic approaches (animal and *in vitro* models); 5) study design and sampling in human population; and 6) statistical analysis. Overall, the above described methodology provides a highly transversal research approach that enables the evaluation of the human external/internal exposure to chemical pollutants and its

P84.

THE FOGO VOLCANO (CAPE VERDE) 2014 ERUPTION – IMPACTS ON HUMAN HEALTH

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Volcanic eruptions disturb directly and indirectly the ecosystems and the health of the exposed populations. Hazards include, among others, toxic volcanic ashes with diameters <10 µm (PM_{10}). During volcanic eruptions and their immediate aftermath, increased respiratory system morbidity has been observed as well as mortality among those affected by volcanic eruptions. Unfavorable health effects could partly be prevented by timely application of safety measures.

Fogo island (476 km^2) is an active volcano, which belongs to the archipelago of Cape Verde. The last Fogo eruption occurred on November 23, 2014, on a subsidiary vent of the main cone, after 19 years of inactivity. The lava expelled destroyed two villages, previously evacuated, and covered vast areas of agricultural land, causing very large economic losses. Although the eruption caused no casualties large amounts of gases and dusts were expelled and spread all over the island.

During this mission it was possible to monitoring the PM_1, $PM_{2.5}$, PM_4 and PM_{10}, CO, CO_2 and TVOC's concentrations. Also roof dusts were collected during this mission. Previous results revealed that, regardless the sampling location, both the PM_{10} and $PM_{2.5}$ concentrations largely exceeded the 24-h daily means stipulated by the by the World Health Organization of 25 µg/m^3. The data collected and related to the eruption, associated to the previous knowledge of the island and volcano, will allow the modelling of the dynamic and transfer pathways of the potential toxic elements expelled. The volcano-dusts-human system will be accessed.

The monitoring of the 2014 Fogo eruption was supported by the Collaboratory for Geosciences (C4G - Portugal) in collaboration with the Instituto Nacional de Meteorologia e Geofísica (INMG - Cape Verde), and also by Fundação para a Ciência e Tecnologia (FCT - Portugal) who provided also financial support for the participation of the first authors in the sampling campaign.

P85.

PARTITION OF MERCURY LEVELS ALONG THE MATERNAL-FETAL-PLACENTAL UNIT

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The placenta was early considered one of the most powerful barriers avoiding the transfer of harmful substances to the offspring. Nowadays, it is known that several environmental chemicals are capable to cross this organ leading to teratogenic effects. One of those chemicals is mercury (Hg) that has been linked to the inhibition of fetal brain development leading to a delay in growth, neural tube defects and craniofacial malformations.

Concerning Hg exposure during pregnancy, most authors have been focused in the quantification of this metal simultaneously in scalp hair, maternal and cord blood, cord tissue, breast milk and total placenta. However, the mature human placenta consists of a fetal and a maternal component which turns the placenta in a dual purpose specimen for evaluating the pollutant burden exerted on the mother as well as on the fetus. Besides, placental system is also constituted by extraembryonic membranes (chorion and amnion) which are poorly documented about metal retention capabilities.

In this context the main objective of this study was to improve the knowledge about the partition of Hg levels along the maternal-fetal-placental unit. This work was performed in 50 paired mother-child samples. Total Hg quantification was made in maternal hair, maternal and cord red blood cells and plasma, cord tissue and placenta (maternal and fetal surface and amniotic membrane) by Atomic Absorption (direct combustion). Data analysis and discussion of results were performed in order to understand how different placental tissues may predict both maternal and fetal exposure to Hg enhancing the use of noninvasive biological material as an alternative in biomonitoring studies.