ABSTRACT BOOK

June 10th - 12th, 2026
A Coruña (Spain)

SEMA | 2026

30th Spanish Environmental Mutagenesis and Genomics Society (**SEMA**) meeting

https://ojs.diffundit.com/index.php/sema/issue/view/97

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Sociedad Española de Mutagénesis y Genómica Ambiental

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DAY 1 – JUNE 10th WEDNESDAY

14:30 – 15:00 Registration

15:00 – 15:15 Opening and welcome

SESSION 1: HUMAN BIOMONITORING IN CLINICAL, OCCUPATIONAL AND ENVIRONMENTAL SETTINGS

Moderators: *Isabel Gaivão (UTAD) and Irene Barguilla (UAB)*

15:15 – 16:00 Keynote speaker: Dr. Joao Paulo Teixeira (National Institute of Health Dr. Ricardo Jorge, Portugal): ***Inside the fire: advancing human biomonitoring in firefighters***

16:00 – 16:15 Marko Gerić (Institute for Medical Research and Occupational Health, Croatia): ***The assessment of health-related biomarkers among different dietary patterns***

16:15 – 16:30 Ali Hemadeh (University of A Coruña, Spain): ***Lifestyle, environment, and major determinants of frailty: UK Biobank study***

16:30 – 16:45 Carlota Lema-Arranz (University of A Coruña, Spain): ***Endocrine biomarkers for physical and cognitive frailty in older adults***

16:45 – 17:00 Elena Lendoiro (University of Santiago de Compostela, Spain): ***Occupational exposure to cytostatic drugs: combined environmental and biological monitoring in a Spanish hospital***

17:00 – 17:30 Coffee break

SESSION 2: DNA REPAIR AND GENOME INSTABILITY

Session sponsored by Thermo Fisher Scientific

Moderators: *Natalia Mallo (UDC) and Laura Rubio (UAB)*

17:30 – 18:15 Keynote speaker: Dr. María Teresa Roldán Arjona (University of Córdoba, Spain): ***Epigenetic and repair functions of DNA base excision***

18:15 – 18:30 Rocío Santiago-Domínguez (University of Córdoba, Spain): ***Role of an AP Lyase pathway in the repair of AP sites generated by DNA* methylating agents**

18:30 – 18:45 Yolanda Lorenzo (Oslo University Hospital, Norway): ***DNA oxidation damage and expression of the DNA repair enzyme OGG1 in organ-cultured human conjunctival epithelial cells***

18:45 – 19:00 Maite Saavedra-Rodríguez (University of A Coruña, Spain): ***Assessment of cytotoxicity and genotoxicity in human glial cells exposed to platinum nanoparticles***

19:00 – 19:15 Goran Gajski (Institute for Medical Research and Occupational Health, Croatia): ***Living with higher air pollution: Does it affect genomic stability?***

19:15 – 20:00 New Investigators Assembly

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DAY 2 – JUNE 11th THURSDAY

SESSION 3: ENVIRONMENTAL AND HEALTH RISK ASSESSMENT OF EMERGING POLLUTANTS (I)

Moderators: *Amaya Azqueta (UNAV) and Natalia Fernández-Bertólez (UDC)*

9:30 – 10:15 Keynote speaker: Dr. Mario Durán Prado (University of Castilla la Mancha, Spain): ***When wonder materials meet DNA: graphene's toxic and genotoxic footprint***

10:15 – 10:30 Ana Teresa Reis (National Institute of Health Dr. Ricardo Jorge, Portugal): ***Genotoxic responses to nanomaterials and metal(loid)s co-exposure: insights from complementary DNA damage biomarkers***

10:30 – 10:45 Rita Sofia Vilela (National Institute of Health Dr. Ricardo Jorge, Portugal): ***Comparative genotoxicity of Alternaria mycotoxins in HepG2 cells***

10:45 – 11:00 Hannes Van Goethem (Autonomous University of Barcelona, Spain): ***Assessment of genotoxic effects induced by true-to-life micro and nanoplastics derived from orthodontic thermoplastics***

11:00 – 11:15 Kevin Barrios-Garay (Autonomous University of Barcelona, Spain): ***Changes in the oral microbiota after six months of clear aligner orthodontic treatment: Assessment of micro- and nanoplastic exposure using MinION sequencing***

11:15 – 11:30 Julia Catalán (Finnish Institute of Occupational Health, Finland): ***Genotoxic effects in workers of the plastic recycling industry exposed to micro- and nanoplastics***

11:30 – 12:00 Coffee break

SESSION 4: EPIGENOMICS AND EPITRANSCRIPTOMICS, AND EMERGING POLLUTANTS (II)

Moderators: *Susana Pastor (UAB) and Adriana Rodríguez-Garraus (UNAV)*

12:00 – 12:45 Keynote speaker: Dr. Diana Guallar Artal (University of Santiago de Compostela, Spain): ***Time written in RNA: The epitranscriptomic code of ageing***

12:45 – 13:00 Carmen Ayala-Roldán (University of Córdoba, Spain): ***H3K27 acetylation as a key regulator of onco-lncRNA expression in lung cancer***

13:00 – 13:15 Irene Barguilla (Autonomous University of Barcelona, Spain): ***Chronic exposure to nanoplastics alters stem cell type-specific mechanisms, promoting cancer development***

13:15 – 13:30 Alba García-Rodríguez (Autonomous University of Barcelona, Spain): ***Genotoxic and functional endothelial responses to nanoplastics: the role of physicochemical properties***

13:30 – 13:45 Jéssica Arribas Arranz (Autonomous University of Barcelona, Spain): ***An innovative biomarker-based blood assay for monitoring micro- and nanoplastic exposure in occupational and environmental settings***

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13:45 – 14:00 Raquel Egea (Autonomous University of Barcelona, Spain): ***Multi-omic analysis of the biological impacts of exposure to PTFE micro- and nanoplastics in Drosophila melanogaster***

14:00 – 15:30 Lunch

SESSION 5: ANIMAL MODELS IN TOXICITY TESTING AND HUMAN DISEASE RESEARCH
Moderators: *Sara Maisanaba (UPO) and Alba García-Rodríguez (UAB)*

15:30 – 16:15 Keynote speaker: Dr. Laura Sánchez Piñón (University of Santiago de Compostela (campus Lugo), Spain): ***Zebrafish as an animal model for biomedicine research***

16:15 – 16:30 Lucía Ramos-Pan (University of A Coruña, Spain): ***In vivo evaluation of three differently charged gold nanoparticles using the zebrafish embryo model***

16:30 – 16:45 Assia Touzani (University of A Coruña, Spain): ***Toxicity assessment of platinum nanoparticles in Drosophila melanogaster: acute and chronic exposure effects***

16:45 – 17:00 Diego López-Aragón (University of A Coruña, Spain): ***Morphological and behavioural alterations in Drosophila melanogaster offspring and adults after developmental exposure to gold nanoparticles***

17:00 – 17:15 Mohamed Alaraby (Autonomous University of Barcelona, Spain): ***Hazard impacts of polytetrafluoroethylene micro-nanoplastics: in vivo study***

17:15 – 17:30 Mario Cabaleiro (University of A Coruña, Spain): ***Natural flavonoids induce dose-dependent cytotoxicity in marine ciliated protozoa parasites through putative mitochondrial mechanisms***

17:30 – 20:00 Social event

20:00 Conference dinner

DAY 3 – JUNE 12th FRIDAY

SESSION 6: NEW APPROACH METHODOLOGIES IN TOXICITY/SAFETY ASSESSMENT
Moderators: *Alba Hernández (UAB) and Ana Teresa Reis (INSA)*

9:00 – 9:45 Keynote speaker: Dr. Verónica Bolón Canedo (University of A Coruña, Spain): ***AI for nanotoxicology: in-silico tools to reduce experimental testing***

9:45 – 10:00 Myriam Benito-Fuertes (University of Navarra, Spain): ***Evaluation of New Approach Methodologies (NAMs) for the detection of compounds classified as non-genotoxic carcinogens (NGTxCs)***

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10:00 – 10:15 Carolina Ramos (National Institute of Health Doutor Ricardo Jorge, Portugal): ***Model matters: Comparative toxicity of BPA and BPS-MAE in 2D and 3D HepG2 models***

10:15 – 10:30 Sara Maisanaba (University Pablo de Olavide, Spain): ***Use of the nematode Caenorhabditis elegans as a biomodel to investigate the toxic effects of the plasticizer bisphenol A and their chemical analogues***

10:30 – 10:45 Javier Guitiérrez-García (Autonomous University of Barcelona, Spain): ***Micro- and nanoplastic interference in THP-1 immune response in an inflammatory context***

10:45 – 11:00 Laura Rubio (University Pablo de Olavide, Spain): ***Unraveling the effects of real-life micro- and nanoplastics on the lung barrier using an ALI co-culture model***

11:00 – 11:30 Coffee break

11:30 – 12:15 PhD thesis contest

12:15 – 13:00 SEMA Annual Assembly

13:00 – 13:30 Awards and closing ceremony

KEYNOTE LECTURES

Inside the fire: advancing human biomonitoring in firefighters

F. Esteves[1], J. Madureira[1], S. Bonassi[8], S. Costa[1], J. P. Teixeira[1]*

[1] Environmental Health Department, National Institute of Health, Porto, Portugal
[2] Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Roma, Rome, Italy
* joao.teixeira@insa.min-saude.pt

In recent decades, several European countries, including Portugal, have experienced increasingly frequent and severe wildfires, a trend expected to intensify due to climate change. Wildfire smoke contains hazardous pollutants such as polycyclic aromatic hydrocarbons (PAHs), many of which are classified as carcinogenic. The International Agency for Research on Cancer has classified occupational exposure in firefighting as carcinogenic to humans. Nevertheless, firefighting remains insufficiently studied, particularly under real wildfire conditions.

This study aimed to identify and integrate suitable biomarkers for monitoring occupational exposure and early biological effects among Portuguese wildland firefighters.

A longitudinal study design was implemented, assessing firefighters at two time points: before the wildfire season (baseline) and after participation in a real wildfire event. Information on sociodemographic characteristics, health status, lifestyle, and occupational factors was obtained through questionnaires. Biological samples (blood, buccal cells, and urine) were collected in both phases. Biomarkers of genetic instability, primary DNA damage, oxidative DNA damage, and inflammatory response were evaluated. Urinary hydroxylated metabolites of polycyclic aromatic hydrocarbons (OH-PAHs) were quantified as exposure biomarkers.

Significant increases in DNA damage and inflammatory biomarkers were observed following wildfire exposure. Positive associations were identified between biomarkers of effect and urinary OH-PAHs levels, supporting a link between internal exposure and early biological alterations.

These findings highlight measurable adverse biological changes associated with wildfire firefighting. The results reinforce the importance of structured biomonitoring programmes, preventive strategies, and occupational health surveillance to protect firefighters in the context of escalating wildfire events driven by climate change.

Funding: This work was supported by FCT-MCTES and European Social Funds through PCIF/SSO/0017/2018 and FCT fellowships UI/BD/150783/2020 and 2020.07394.BD.

Base excision: evolving complexities of a crucial pathway for epigenetic control and DNA repair

T. Roldán Arjona[1,2,3*]

[1] *Maimónides Biomedical Research Institute of Córdoba (IMIBIC), Córdoba, Spain*
[2] *Department of Genetics, University of Córdoba, Córdoba, Spain*
[3] *Reina Sofía University Hospital, Córdoba, Spain*
* ge2roarm@uco.es

Base Excision Repair (BER) is a key pathway that preserves genome integrity by removing damaged, mispaired, or non-canonical bases. In plants, BER fulfills both canonical DNA repair functions and specialized epigenetic roles, notably the active replacement of 5-methylcytosine (5-meC) with cytosine to shape DNA methylation landscapes. Plant active DNA demethylation is mediated by plant 5-meC DNA glycosylases typified by *Arabidopsis* REPRESSOR OF SILENCING 1 (ROS1). The C-terminal domain of ROS1 mediates interaction with the N-terminal tail of histone H3, and this interaction is specifically disrupted by phosphorylation at H3S28 indicating sensitivity to chromatin state. Conserved residues within the C-terminal domain are required for histone interaction, efficient DNA binding, and catalytic activity. Thus, the ROS1 C-terminal domain functions as a histone reader module that links chromatin marks to targeted DNA demethylation.

Following base excision, downstream BER steps depend on the coordinated action of additional repair enzymes. AP endonucleases incise abasic sites generated by glycosylases, producing strand breaks with 5′-deoxyribose phosphate (5′-dRP) termini that must be further processed to complete repair. In contrast to mammals, which use DNA polymerase β (Pol β) for 5′-dRP removal and gap filling, plants encode a single X-family polymerase, Pol λ. *Arabidopsis* Pol λ exhibits intrinsic dRP lyase activity and is required for efficient BER, establishing it as a functional Pol β analog in plants.

Divergence between plant and animal BER is also evident at the level of AP endonuclease specificity. In contrast to APE1, the major human AP endonuclease, its *Arabidopsis* ortholog ARP displays orphan base-dependent cleavage and limited activity on single-stranded DNA (ssDNA). Differences in two key DNA-intercalating residues determine such substrate divergence. Notably, the residue governing ssDNA activity in APE1 is essential for mammalian antibody diversification, suggesting an evolutionary adaptation in metazoans. Collectively, these observations reveal the evolving molecular complexity of BER at the intersection of genome maintenance, chromatin signaling, and epigenetic regulation.

Funding: Ministerio de Ciencia e Innovación (MICINN), Spain, [PID2019-109967 GB-I00, PID2022-140458NB-I00].

When wonder materials meet DNA; graphene's toxic and genotoxic footprint

M. Durán-Prado[1,2*]

[1] *Medical School, University of Castilla-La Mancha, Ciudad Real, Spain*
[2] *Instituto de Investigación Sanitaria de Castilla-La Mancha, IDISCAM, Toledo, Spain*
* mario.duran@uclm.es

Background: Graphene and related two-dimensional materials are widely promoted as "wonder materials", yet their long-term safety profile at realistic exposure levels, especially regarding DNA integrity in human barrier tissues, remains poorly unknown.

Aims: We aimed to characterize the toxic and genotoxic footprint of graphene-related materials (GRMs) in human skin cells, focusing on HaCaT keratinocytes, and to translate these findings into robust in vitro DNA-damage testing strategies and regulatory-relevant guidance.

Methods: We investigated acute and subchronic exposures to well-characterized graphene oxide (GO), few-layer graphene (FLG) and small FLG at cytotoxic and non-cytotoxic doses using primary human skin cells and HaCaT keratinocytes. Endpoints included viability, ROS production, Ca^{2+} signaling, metabolomics, mitochondrial function, proliferation and genotoxicity assays (comet, γ-H2AX, micronucleus), followed by optimization of GRM-adapted in vitro DNA-damage assays and guidance for regulatory risk assessment.

Results: GRMs induced dose-dependent oxidative stress, Ca^{2+} dysregulation and cell death, but also profound metabolic rewiring at sublethal doses, including a shift towards glycolysis, increased TCA cycle intermediates, glutamine dependence and mitochondrial damage in HaCaT cells. Subchronic, non-cytotoxic exposures to GO and FLG in HaCaT keratinocytes triggered DNA damage, activation of the DNA damage response, and, after prolonged exposure, persistent genotoxic lesions comparable to those induced by arsenite. These findings led to the refinement of micronucleus and comet-based assays, the proposal of GRM-specific testing batteries, and their positioning within emerging European regulatory frameworks for graphene-enabled products.

Conclusions: Our work reveals that wonder graphene materials leave a measurable toxic and genotoxic footprint in human skin cells, even at sublethal doses, driven by oxidative stress, metabolic reprogramming and sustained DNA damage. It also underscores that tailored in vitro genotoxicity batteries are essential to capture these risks and to support experimentally based regulation of graphene and other 2D materials.

Time Written in RNA: The Epitranscriptomic Code of Ageing

D. Guallar[1*]

[1] *Center for Research in Molecular Medicine and Chronic Diseases (CiMUS). University of Santiago de Compostela. Santiago de Compostela, Spain.*
* diana.guallar@usc.es

Cellular ageing is characterized by a conserved set of molecular, cellular, and systemic alterations, known as the “hallmarks” of ageing. Among these, epigenetic alterations (i.e., DNA methylation and histone modifications) have been widely recognized as central players of the cellular decline resulting from the passage of time. In contrast, the role of RNA methylation, a prevalent epitranscriptomic modification involved in regulation of stability, localization and translation of RNA molecules, in ageing has not been studied in detail. Recent evidence indicates that specific RNA modifications change in an age-dependent manner, and influence pathways connected to ageing hallmarks, suggesting a regulatory role for the epitranscriptome in ageing and age-related pathologies.

Our research explores how one specific RNA modification, 5-methylcytosine (m5C), changes during ageing. Using RNA bisulfite sequencing of primary cells isolated from young and old mice, we have uncovered age-dependent remodelling of m5C across coding and non-coding RNAs. Notably, methylation increases with age at sites within motifs associated with the RNA methyltransferases NSUN2. Experimental modulation of this enzyme can partially restore youthful transcriptomic profiles, underscoring the functional relevance of RNA methylation in cellular homeostasis.

These findings establish m5C as an emerging player in the regulatory networks that drive ageing and adaptation to environmental and metabolic challenges. By revealing how reversible RNA modifications shape the ageing transcriptome, this work opens new perspectives for developing strategies aimed at promoting resilience and healthy ageing.

Funding: Research at the D.G. lab was funded by Agencia Estatal de Investigación (PID2022-136608OB-I00/FEDER,UE), Xunta de Galicia (ED431F 2022/011) and Fundación Ramón Areces (CIVP21A7026).

Zebrafish as a Versatile Vertebrate Model for Cancer, Rare Disease, and Toxicology Research

Á. J. Arana[1,2*], L. Sánchez[1,2*]

[1] AQUIGEN Group (GI-1251), Faculty of Veterinary Medicine, Campus Terra, Universidade de Santiago de Compostela, 27002, Lugo, Spain
[2] Preclinical Animal Models (E032), Oncology Area, Health Research Institute of Santiago de Compostela (IDIS), 15706, Santiago de Compostela, Spain
* lauraelena.sánchez@usc.es

The zebrafish (*Danio rerio*) has emerged as a powerful vertebrate model for biomedical research due to its genetic similarity to humans, optical transparency during early development, rapid life cycle, and suitability for high-throughput studies. In recent years, zebrafish has been widely adopted to investigate the molecular basis of cancer, rare diseases, and toxicological responses. In cancer research, transgenic and xenograft zebrafish models enable real-time visualization of tumor initiation, progression, angiogenesis, and metastasis.

In the context of rare diseases, zebrafish provides a versatile platform for functional genomics and disease modelling. Techniques such as CRISPR/Cas9 gene editing allow the rapid generation of mutant lines that mimic human genetic disorders, facilitating the identification of pathogenic mechanisms and potential therapeutic drug targets. Because many human disease genes have zebrafish orthologs, the model is particularly valuable for studying rare developmental and genetic disorders that are difficult to investigate in traditional mammalian models.

Zebrafish is also extensively used in toxicology and environmental health research. Embryonic and larval stages are highly sensitive to chemical exposures, making them suitable for assessing the toxicity, teratogenicity, and pharmacological effects of environmental pollutants, pharmaceuticals, and industrial compounds. High-throughput screening platforms using zebrafish enable rapid evaluation of compound safety, biological impact and potential environmental risk.

AI for toxicology: in silico tools to reduce experimental testing

V. Bolón-Canedo[1*], M. Martínez-Matamoros[1], L. Morán-Fernández[1], N. Fernández-Bertólez [2,3], B. Laffon[3,4] & V. Valdiglesias[2,3]

[1] Universidade da Coruña, Grupo LIDIA, CITIC – Centro de investigación TIC, Departamento de Ciencias de la Computación y Tecnologías de la Información, Facultad de Informática, Campus Elviña s/n, 15071, A Coruña, Spain
[2] Universidade da Coruña, Grupo NanoToxGen, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Biología, Facultad de Ciencias, Campus A Zapateira s/n, 15071, A Coruña, Spain
[3] Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas, As Xubias, 15006, A Coruña, Spain
[4] Universidade da Coruña, Grupo DICOMOSA, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Psicología, Facultad de Ciencias de la Educación, Campus Elviña s/n, 15071, A Coruña, Spain
** veronica.bolon@udc.es*

The rapid development of nanotechnology in areas such as biomedicine, electronics, and advanced materials has significantly increased the need to understand the potential toxic effects of engineered nanomaterials. Assessing nanoparticle toxicity remains a complex challenge because their biological impact depends on a wide range of physicochemical properties, including size, surface chemistry, and experimental exposure conditions. Traditional toxicological evaluation, particularly in vivo testing, is often costly, time-consuming, and raises ethical concerns, highlighting the importance of complementary approaches that can help guide experimental work.

This work explores how data-driven methods can support nanotoxicology by identifying patterns between nanoparticle properties and biological responses. After briefly introducing the role of machine learning in toxicological research, I will present a case study focused on predicting the toxicity of nanoparticles from physicochemical descriptors.

The study uses an experimental dataset derived from in vitro assays evaluating the biological effects of iron oxide nanoparticles and cerium dioxide nanoparticles. Several machine learning classifiers were trained to predict cytotoxicity and genotoxicity outcomes using physicochemical characteristics and experimental variables. Feature selection methods were applied to identify the most informative attributes influencing toxicity.

The results show that predictive models can capture relevant relationships between nanoparticle properties and toxicological outcomes, highlighting key variables such as hydrodynamic size and exposure conditions. Beyond prediction, these models can help prioritize experiments, guide safer material design, and contribute to the development of alternative strategies that reduce the need for extensive experimental testing.

Funding: This research was funded by Ministry of Science and Innovation MCIN/AEI [Grant PID2024-160400OB-I00], the Ministry for Digital Transformation and Civil Service and Next-GenerationEU/PRTR under Grant TSI-100925-2023-1, and Xunta de Galicia (ED431B 2025/26).

SESSIONS

01. Human biomonitoring in clinical, occupational and environmental settings

The assessment of health-related biomarkers among different dietary patterns

M. Gerić[1*] for the UZDRAVLJe team

[1] *Institute for Medical Research and Occupational Health, Zagreb, Croatia*
* mgeric@imi.hr

Vegetarian and vegan diets are increasingly popular for their apparent health and environmental benefits, yet their biological effects are complex and influenced by nutrition, metabolism, and environmental exposures. A recent review of biomarkers related to oxidative stress, inflammation, and genomic stability underscores the variability in findings regarding the effects of different dietary patterns.

Previously, we showed that vegetarians had lower levels of nutrients primarily obtained from animal foods, including calcium, zinc, copper, and vitamins B_{12} and D. These deficiencies coincided with reduced antioxidant defences, higher homocysteine, and increased markers of genomic instability, such as DNA strand breaks, micronuclei formation, and shorter telomeres. In contrast, omnivorous diets increased exposure to toxic metals, notably mercury and arsenic, reflecting consumption of fish and other animal products. Nevertheless, in a pilot study, we managed to show that female pescatarians had a lower baseline DNA damage profile, indicating that dietary choices can influence early biomarkers of DNA damage, thus emphasising the role of diet in long-term disease risk.

Since dietary patterns significantly shape nutritional status, exposure to food-borne contaminants, and molecular markers of health, we recently launched a new project, UZDRAVLJe (/'uzdravʎe/, Croatian expression for "cheers"), that integrates nutrition, toxicology, and molecular epidemiology through a multi-biomarker approach. Future research will focus on biochemical parameters, DNA damage, microbiome, and also the diet quality in order to clarify the long-term health implications of vegetarian, vegan, and omnivorous diets.

Funding: HrZZ (UZDRAVLJe #2823), BioMolTox (304IP).

Lifestyle, environment, and major determinants of frailty: UK Biobank study

A. Hemadeh[1,2*], C. Lema-Arranz[1,2], S. Bonassi[3,4], L. Buscarini[5], F. Infarinato[5], P. Romano[5], A. Finti[6], F. Marinozzi[6], F. Bini[6], N. Fernández-Bertóléz[2,7], L. Lorenzo-López[8,] V. Valdiglesias[2,7], & B. Laffon[1,2]

[1] Universidade da Coruña, Grupo DICOMOSA, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Psicología, A Coruña, Spain
[2] Instituto de Investigación Biomédica de A Coruña (INIBIC), A Coruña, Spain
[3] IRCCS San Raffaele Pisana, Rome, Italy
[4] San Raffaele University, Rome, Italy
[5] Rehabilitation Bioengineering Laboratory, IRCCS San Raffaele Pisana, Rome, Italy
[6] Department of Mechanical and Aerospace Engineering (DIMA), Sapienza University of Rome, Rome, Italy
[7] Universidade da Coruña, Grupo NanoToxGen, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Biología, A Coruña, Spain
[8] Universidade da Coruña, Gerontology and Geriatrics Research Group, INIBIC-CHUAC-SERGAS, A Coruña, Spain
* ali.hemadeh@udc.es

Background: Frailty is a geriatric syndrome that describes an old person's increased vulnerability to stressors due to the loss of physiological reserves. While some functional decline is expected with age, frailty represents an accelerated and multifactorial deterioration that goes beyond normal ageing, potentially driven by genetic, environmental and lifestyle factors.

Aim: The study aimed to identify exposome determinants of frailty using data from the UK Biobank.

Methods: The study analysed cross-sectional data from over 220,000 participants aged ≥60 years, which were classified as non-frail, pre-frail, and frail using the Fried's phenotype. Over 50 variables were analyzed covering lifestyle, diet, environmental exposure parameters, and several biomarkers. Univariate analysis was followed by Principal Component Analysis (PCA), machine learning, and logistic regression to identify the top frailty predictors.

Results: Frailty risk was mainly shown to be driven by chronological age and C-reactive protein, a marker of chronic inflammation. Higher frailty risk was associated with external factors like poor air quality, poor dietary patterns, smoking, heavy alcohol use, and polypharmacy. Frail participants also showed lower levels of vitamin D. Early life factors, like maternal smoking and the lack of breastfeeding, were linked to higher odds of frailty in late life.

Conclusion: This study showed the multifactorial nature of frailty, driven by lifestyle and environmental factors, while achieving high predictive accuracy. Future longitudinal studies are required to validate these findings and implement preventive measures.

Funding: This work was supported by the Spanish Ministry of Science and Innovation: MCIN/AEI [Grant PID2020-113788RB-I00] and Xunta de Galicia (ED431B 2025/26, and ED481A-2025/085 to C. Lema-Arranz).

Endocrine biomarkers for physical and cognitive frailty in older adults

C. Lema-Arranz[1,2*], A. Hemadeh[1,2], N. Cibeira[3], R. López-López[3], J.L. Rodríguez-Villamil[3], J.C. Millán-Calenti[3], A. López-Cortón[1], N. Fernández-Bertólez[2,4], V. Valdiglesias[2,4], L. Lorenzo-López[3], & B. Laffon[1,2]

[1] Universidade da Coruña, Grupo DICOMOSA, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Psicología, A Coruña, Spain
[2] Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas, A Coruña, Spain
[3] Universidade da Coruña, Gerontology and Geriatrics Research Group, Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas, A Coruña, Spain
[4] Universidade da Coruña, Grupo NanoToxGen, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Biología, A Coruña, Spain
** carlota.lema.arranz@udc.es*

Frailty has emerged as a clinical marker of biological ageing, characterised by diminished physiological reserves and heightened vulnerability to stressors. Cognitive frailty is defined by the co-occurrence of physical frailty and mild cognitive impairment, and is associated with accelerated cognitive decline, functional loss, and higher risk of institutionalisation and death. The endocrine system plays a fundamental role in the maintenance of homeostasis. Beyond genetic determinants of frailty, the endocrine system becomes progressively dysregulated with advancing age and the onset of frailty. A range of hormones have been proposed as potential biomarkers of both physical and cognitive frailty; however, evidence regarding their associations remains inconsistent. This cross-sectional study examined the relationship between endocrine biomarkers and physical and cognitive frailty in a cohort of older adults (N=155; aged ≥65 years) classified according to their physical frailty status using two approaches: the frailty index and the frailty phenotype. Cognitive impairment was identified with the Montreal Cognitive Assessment questionnaire. Serum concentrations of endocrine biomarkers [insulin-like growth factor 1 (IGF-1), cortisol, brain-derived neurotrophic factor (BNDF), α-klotho, and 25-hydroxyvitamin D (25(OH)D)] were determined. Our results showed IGF-1 concentrations declining progressively with increasing frailty severity across all classifications, exhibiting a male-specific association. Adjustment for cognitive performance indicated that physical frailty exerted a greater influence on IGF-1 than cognitive status, highlighting its potential as a biomarker of physical frailty. Lower cortisol levels were detected in individuals with MCI, particularly among those without depression or with less than two chronic conditions. No significant associations were observed for serum BDNF, α-klotho, or 25(OH)D in relation to either physical or cognitive frailty. These findings underscore the potential of IGF-1 as a biomarker of frailty and cortisol as a biomarker of MCI, while highlighting the need for further studies to confirm these associations and elucidate the underlying mechanisms in larger and more diverse cohorts.

Funding: This work was supported by the Spanish Ministry of Science and Innovation: MCIN/AEI [Grant PID2020-113788RB-I00] and Xunta de Galicia (ED431B 2025/26, and ED481A-2025/085 to C. Lema-Arranz).

Occupational exposure to cytostatic drugs: combined environmental and biological monitoring in a Spanish hospital

J. A. Lema-Atán[1], E. Lendoiro[1]*, A. Cruz[1], B. Bernardez[2,3], M. Cobo-Golpe[1], N. Mayo[4], M. Blanco-Ces[1], M. Tourís[2], Á. López-Rabuñal[1], I. Zarra[2,3], M. López-Rivadulla[1], & A. de-Castro-Ríos[1]

[1] Servicio de Toxicología, Instituto de Ciencias Forenses "Luis Concheiro", Universidade de Santiago de Compostela, Santiago de Compostela, Spain
[2] Unidad de Farmacia Oncológica, Servicio de Farmacia, Hospital Clínico Universitario de Santiago de Compostela, Santiago de Compostela, Spain
[3] Grupo de Farmacología, Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Santiago de Compostela, Spain.
[4] Servicio de Oncohematología, Hospital Clínico Universitario de Santiago de Compostela, Santiago de Compostela, Spain
* elena.lendoiro@usc.es

Occupational exposure to cytostatic drugs in healthcare workers has been associated with acute and chronic adverse effects. The increasing use of these agents in clinical practice may increase the risk, particularly due to repeated exposure to multiple compounds over time.

This study aimed to evaluate occupational exposure to multiple cytostatic drugs in a Spanish hospital using a combined environmental and biological biomonitoring approach.

Surface samples were collected across three monitoring campaigns in Pharmacy and Onco-Haematology services of the Clinical University Hospital of Santiago de Compostela (Spain), including preparation areas, equipment, materials and patient-care settings. Additionally, during the third campaign, forearm bracelets worn by workers throughout the work shift and urine samples were collected. All samples were analysed with a validated LC-MS/MS method for detection of 12 commonly used cytostatic drugs (gemcitabine, dacarbazine, methotrexate, cyclophosphamide, doxorubicinol, doxorubicin, epirubicin, irinotecan, vinorelbine, etoposide, docetaxel and paclitaxel) with limits of detection of 5-100 pg/cm^2 for surfaces and 5-250 pg/mL for urine, depending on the analyte.

A total of 153 surface samples were collected. Contamination was detected in 57.6% of samples from the Pharmacy service and 32.3% from the Onco-Haematology service. All monitored compounds were identified at least once, with cyclophosphamide and gemcitabine being the most frequently detected. Most contaminated samples (≥76%) showed low levels (<100 pg/cm²), except in patient-area bathrooms, where less than 40% were below this threshold and all samples were positive. Contamination was also observed in 60.7% of vials, blisters and infusion bags (0.6-1,631.5 ng), with cross-contamination detected in 7 out of 17 cases. Only 2 out of 82 bracelets showed contamination, both with cyclophosphamide (7.4 pg/cm^2 and 31.4 pg/cm^2). Finally, all urine samples (n=165, from 29 workers) were negative.

Overall, cytostatic drugs were widely distributed across monitored hospital settings, although generally low contamination levels were found. These findings highlight the need for continued biomonitoring as a preventive strategy to assess occupational exposure.

SESSIONS

02. DNA repair and genome instability

Role of an AP Lyase pathway in the repair of AP sites generated by DNA methylating agents

R. Santiago-Domínguez[1,2,3*], A. Muñoz-Fernández[1,2,3], S. Conde-Maestre [1,2,3], T. Morales-Ruiz[1,2,3], R. R. Ariza [1,2,3], T. Roldán-Arjona [1,2,3], & M. I. Martínez-Macías[1,2,3]

[1] Department of Genetics, University of Córdoba, Córdoba, Spain
[2] Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Córdoba, Spain
[3] Reina Sofía University Hospital, Córdoba, Spain
* b92sador@uco.es

Abasic (AP) sites are major DNA lesions generated during temozolomide (TMZ)–induced genotoxic stress in human cells. It is generally accepted that they are primarily repaired through the canonical base excision repair (BER) pathway initiated by AP endonucleases. In plants, AP sites derived from spontaneous loss of N7-methylguanine (N7-meG), the predominant lesion induced by TMZ and others DNA methylating agents, are processed through an AP lyase/DNA phosphatase pathway involving FPG and ZDP. The human homologs of these enzymes are the bifunctional glycosylases/AP lyases NEIL1 and NEIL2, and the polynucleotide kinase 3′-phosphatase (PNKP). Our aim was to investigate the role of the NEIL1/2-PNKP axis in the repair of TMZ-induced AP sites and its impact on cellular sensitivity to DNA methylating agents. We used human glioblastoma (GBM) cells to study AP site accumulation and repair upon TMZ treatment. To inhibit AP site repair, we used the BER inhibitor methoxyamine. PNKP activity was inhibited with A12B4C3 a selective small-molecule inhibitor, and NEIL1/2 were depleted by siRNA. TMZ sensitivity was then evaluated using cell viability assays in both TMZ-sensitive and TMZ-resistant GBM cell lines. Finally, DNA single-strand break (SSB) formation and repair kinetics were analysed using comet assay and immunofluorescence detection of poly-(ADP-ribosylation) (PARylation). We found that TMZ treatment induced AP site accumulation in GBM cells. Inhibition or depletion of PNKP significantly enhanced TMZ sensitivity and delayed SSB repair. Importantly, combined downregulation of NEIL1 and NEIL2 partially rescued TMZ resistance and reduced PARylated SSB levels in PNKP-inhibited cells. Collectively, our findings support a model in which NEIL1 and NEIL2 process TMZ-induced AP sites and generate SSB intermediates that require PNKP for efficient resolution of methylation-induced DNA damage.

DNA oxidation damage and expression of the DNA repair enzyme OGG1 in organ-cultured human conjunctival epithelial cells

Y. Lorenzo[1*], B.O. Nicolaissen[1,3], G. Nguyen[2], K. Beraki[1], M.C. Moe[1,2], G. Petrovski[1,2], D. Krohn-Hansen[1], & B. Nicolaissen[1,2]

[1] Center for Eye Research and Innovative Diagnostics, Department of Ophthalmology, Oslo University Hospital, Oslo, Norway
[2] Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway
[3] Department of Ophthalmology, Vestre Viken Hospital Trust, Drammen, Norway
* y.l.corrales@ous-research.no

Background: Loss of corneal transparency, often resulting from overwhelming cellular and molecular stress, is a leading cause of blindness worldwide. Restoration of vision commonly relies on transplantation of full-thickness or lamellar donor corneal tissue. Prior to transplantation, donor tissue is preserved either under hypothermic conditions or in organ culture in eye banks. However, organ culture represents a non-physiological environment that may promote oxidative stress and associated DNA damage in ocular surface cells.

Aim: To investigate oxidative DNA damage and the expression of the DNA repair enzyme 8-oxoguanine DNA glycosylase-1 (OGG1) in organ-cultured human conjunctival epithelium.

Methods: Human corneoscleral tissue was obtained from residual rings following penetrating keratoplasty or automated Descemet's stripping endothelial keratoplasty and maintained in organ culture. Conjunctival samples were fixed, embedded, and analyzed by immunohistochemistry for 8-oxo-7,8-dihydroguanine (8-oxoG) and OGG1. *OGG1* gene expression was further assessed using RNA *in situ* hybridization (ISH). All procedures adhered to the Declaration of Helsinki and were approved by local ethics committees.

Results: Immunohistochemical analysis demonstrated detectable levels of 8-oxoG in conjunctival epithelial cells following organ culture, indicating the presence of oxidative DNA damage. OGG1 protein expression was observed throughout the epithelial layers.
Consistently, RNA ISH demonstrated *OGG1* gene expression throughout the conjunctival epithelium, confirming active transcription.

Conclusions: Organ culture conditions are associated with DNA oxidation damage in human conjunctival epithelial cells. Importantly, these cells retain the capacity to express the DNA repair enzyme OGG1 at both gene and protein levels. This suggests that conjunctival epithelium maintains an active defense mechanism against oxidative stress during storage, which may be relevant for tissue preservation and *ex vivo* epithelial cell expansion.

Funding: Supported in part by Arthur and Clauson's Legacy, Inger Holms Memorial Fund, Blindemissionen IL, The Norwegian Association of the Blind and Partially Sighted, Center for Eye Research, Oslo University Hospital, and the University of Oslo.

Assessment of cytotoxicity and genotoxicity in human glial cells exposed to platinum nanoparticles

M. Saavedra-Rodríguez[1,2*], A. Touzani[1,2], L. Ramos-Pan[1,2], A. Insua[1], M. A. Maestro[1], N. Fernández-Bertólez[1,2], B. Laffon[2,3], & V. Valdiglesias[1,2]

[1] Universidade da Coruña, Grupo NanoToxGen, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Biología, Facultad de Ciencias, Campus A Zapateira s/n, 15071, A Coruña, Spain
[2] Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas, As Xubias, 15006, A Coruña, Spain
[3] Universdade da Coruña, Grupo DICOMOSA, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Psicología, Facultad de Ciencias de la Educación, Campus Elviña s/n, 15071, A Coruña, Spain
* maite.saavedra@udc.es

Platinum (Pt) is a noble metal, biologically inert and non-allergenic, which makes it exceptionally valuable for industrial, medical, and jewellery applications. Nowadays, platinum nanoparticles (PtNP) are playing an important role not only in the field of nanotechnology but also in nanomedicine, being promising tools for therapeutic and diagnostic applications, or as drug delivery systems, among others. This is due to their physicochemical properties (i.e. catalytic properties, high stability, etc.) and their ability to interact with biological systems. Hence, it is crucial to understand their toxicity profile, as well as their potential effects on genetic material and cellular repair capacity, to ensure a safe use for both patients and consumers. The aim of this work was to assess the potential cytotoxicity and genotoxicity induced by PtNP exposure *in vitro* in A172 glioblastoma cells. Cytotoxic effects were addressed by evaluating cellular membrane disruption and viability decreases in the presence of PtNP, whereas primary DNA damage and effects on DNA repair capacity were assessed by employing the Comet assay and the Challenge-comet assay, respectively. A range of concentrations and different exposure periods were tested in all cases. Also, cellular uptake of PtNP was assessed before the evaluation of toxic effects to verify their ability to enter the cells. Results obtained showed no significant cytotoxicity or genotoxicity under any of the conditions evaluated, confirming the biocompatibility of PtNP previously reported in the literature for other cell types, and supporting their use in biomedical applications. Still, further analysis must be undertaken to completely rule out any other potential harmful effects and to fully understand the biological behaviour of these nanoparticles.

Funding: Ministry of Science and Innovation MCIN/AEI [Grant PID2024-160400OB-I00] and Xunta de Galicia (ED431B 2025/26), L.R.-P. was supported by a Ministry of Science and Innovation predoctoral fellowship (grant number FPU2023/03379).

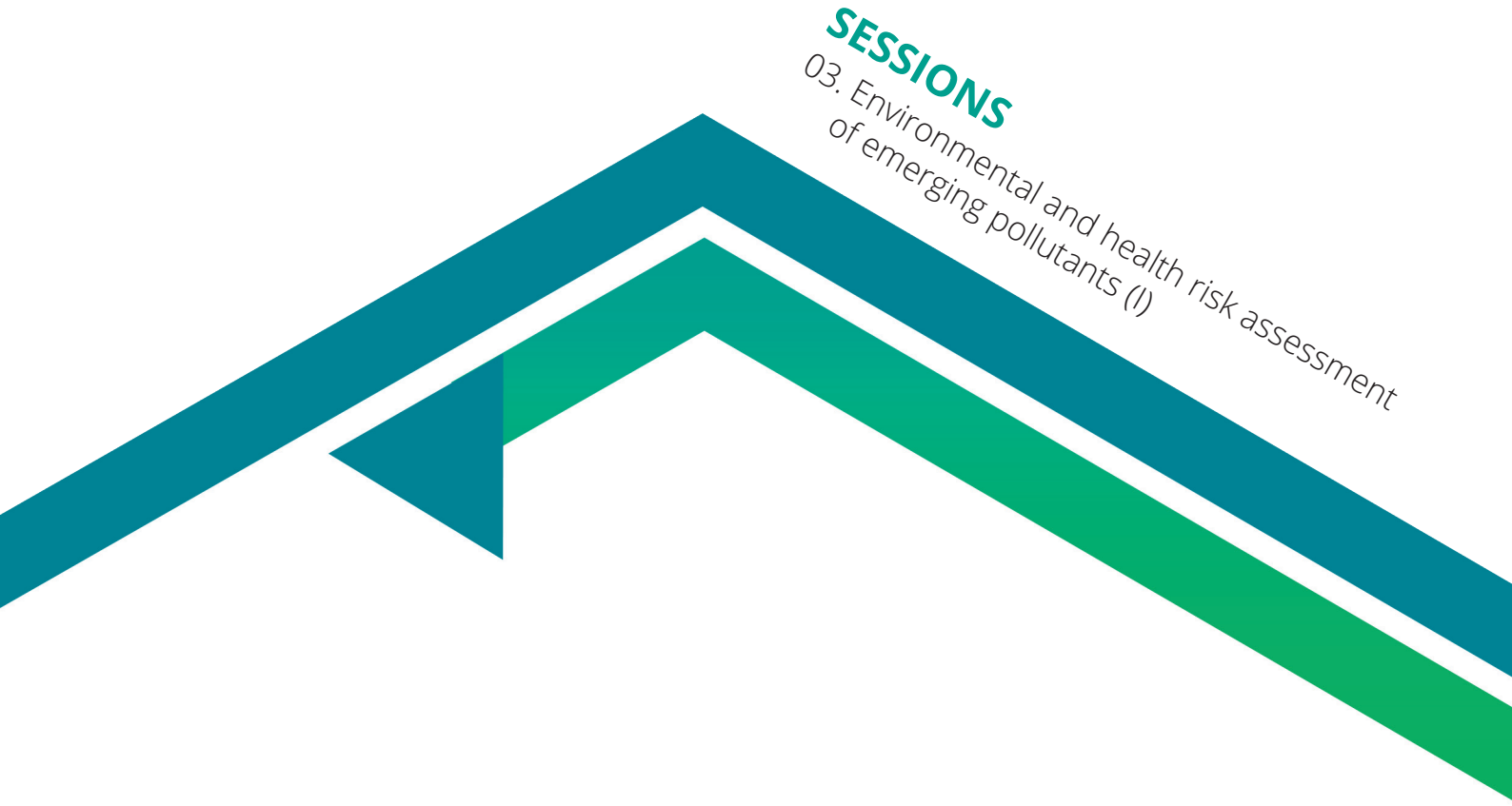
Living with higher air pollution: Does it affect genomic stability?

G. Gajski[1*], K. Matković[1], M. Gerić[1], A. Cvitković[2,3,4], M. Sanković[5], V. Kašuba[1], M. Milić[1], L. Kazensky[1], A. Jurič[1], I. Brčić Karačonji[1], I. Jakovljević[1], S. Davila[1], G. Pehnec[1], A. M. Domijan[6], A. Šumanovac[3,7], P. Wild[8,9], I. Guseva Canu[8], & N. B. Hopf[8]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] Teaching Institute of Public Health Brod-Posavina County, Slavonski Brod, Croatia
[3] Faculty of Medicine, J. J. Strossmayer University of Osijek, Osijek, Croatia
[4] Faculty of Dental Medicine and Health, J. J. Strossmayer University of Osijek, Osijek, Croatia
[5] City of Vinkovci, Vinkovci, Croatia
[6] Faculty of Pharmacy, University of Zagreb, Zagreb, Croatia
[7] County General Hospital Vinkovci, Vinkovci, Croatia
[8] Center for Primary Care and Public Health, University of Lausanne, Lausanne, Switzerland
[9] PW Statistical Consulting, 54520, Laxou, France
* ggajski@imi.hr

According to the latest report from the European Environment Agency, the city of Slavonski Brod has one of the highest average annual $PM_{2.5}$ concentrations in the European Union (26 µg/m³), approximately five times higher than the guideline value recommended by the World Health Organization. As air pollution is widely recognised as a major environmental health risk, such exposure may adversely affect the health of local populations. To investigate potential biological effects, we conducted a human biomonitoring study during the colder season, when air pollution levels are typically elevated. The study aimed to evaluate genotoxicity biomarkers by comparing residents of Slavonski Brod, characterised by higher air pollution levels, with residents of Vinkovci, a less polluted city in the Slavonia region. The study included two comparable groups of non-smoking participants: 55 individuals from Slavonski Brod (51% female; average outdoor exposure 162 min/day; age 39±11 years; BMI 24.2±2.6 kg/m²; residence duration 19±13 years) and 54 individuals from Vinkovci (57% female; outdoor exposure 126 min/day; age 38±10 years; BMI 24.3±3.1 kg/m²; residence duration 16±12 years). Air quality monitoring confirmed higher pollutant levels in Slavonski Brod, including PM_{10} (40.9±22.4 vs. 33.6±16.1 µg/m³), NO_2 (18.3±8.3 vs. 13.1±6.1 µg/m³), and benzo[a]pyrene (5.8±5.1 vs. 2.9±2.5 ng/m³), compared with Vinkovci. Despite these differences in environmental exposure, no significant differences were observed in the analysed biomarkers of genomic instability. Frequencies of micronuclei (4.9±4.1 vs. 3.8±2.9), nuclear buds (5.3±6.9 vs. 4.8±4.7), and nucleoplasmic bridges (1.0±1.9 vs. 1.1±1.8) were comparable between the groups. Similarly, baseline DNA damage assessed by the comet assay showed identical tail intensity values (1.0±0.4%). Measurement of exhaled nitric oxide (FeNO) as an indicator of respiratory inflammation also revealed no significant differences between the two populations (21.3±13.9 vs. 18.6±18.3 ppm). Overall, the results indicate that the observed differences in ambient air pollution between the two cities did not translate into detectable changes in the investigated biomarkers, consistent with findings from our previous studies. Future research will include additional biomarkers and employ advanced statistical and modelling approaches to further elucidate potential relationships between air quality and health outcomes.

Funding: Funded by the Croatian Science Foundation (HUMNap) and the NextGen EU (BioMolTox and EnvironPollutHealth).



SESSIONS

03. Environmental and health risk assessment of emerging pollutants (I)

Genotoxic responses to nanomaterials and metal(loid)s co-exposure: insights from complementary DNA damage biomarkers

A. T. Reis[1,2*], F. Rosário[3], C. Costa[1,2], C. Vilaça[1,2], C. Lopes[4], A. C. Estrada[4], V. Valdiglesias[5,6], & J. P. Teixeira[1,2]

[1] Environmental Health Department, National Institute of Health, Porto, Portugal
[2] EPIUnit ITR, Institute of Public Health of the University of Porto, University of Porto, Rua das Taipas, n.º 135, 4050-600 Porto, Portugal
[3] CESAM—Centre for Environmental and Marine Studies & Department of Biology, University of Aveiro, Aveiro, Portugal
[4] Department of Chemistry and Aveiro Institute of Materials (CICECO), University of Aveiro, Aveiro, Portugal
[5] Universidade da Coruña, Grupo NanoToxGen, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Biología, Facultad de Ciencias, Campus A Zapateira s/n, 15071, A Coruña, Spain
[6] Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas, As Xubias, 15006, A Coruña, Spain
* ana.reis@insa.min-saude.pt

Background: Human exposure to nanomaterials (NMs) and metal(loid)s rarely occurs in isolation, yet toxicological studies still predominantly assess single substances. Co-exposure scenarios may alter both the magnitude and the type of DNA damage induced. The Comet and the γ-H2AX assays represent complementary approaches for detecting DNA damage and DNA double-strand breaks, respectively, providing insight into genotoxic responses.

Aim: This study investigated the genotoxic effects of NMs and metal(oid)s under single and binary co-exposure conditions, evaluating whether the latter alters the extent and nature of DNA damage.

Methods: Two human cell models (A549 and HepG2) representing distinct target tissues were exposed to individual contaminants and binary mixtures. Primary DNA damage and oxidative lesions were assessed using a medium-throughput Comet assay and its FPG-modified version. DNA double-strand breaks were evaluated through quantification of γ-H2AX formation by flow cytometry. Responses following single and combined exposures were compared to identify potential interaction patterns.

Results: Single exposures to metal(loid)s resulted in marked increases in primary DNA damage, while NMs alone induced modest and cell-type-dependent effects (more pronounced in HepG2 cells). Co-exposure responses differed between cell models but frequently resulted in enhanced primary DNA damage relative to NMs exposures alone. In contrast, γ-H2AX analysis revealed that co-exposure generally reduced double-strand break formation compared with the corresponding single metal(loid) exposures, particularly in A549 cells, whereas HepG2 showed intermediate responses. These findings suggest that co-exposure may modify the severity and mechanisms of DNA damage, potentially shifting responses toward less severe lesion types. Some co-exposure conditions also indicated reduced oxidative DNA damage compared with exposure to single compounds.

Conclusions: The use of complementary genotoxicity biomarkers demonstrates that co-exposure can modulate both the extent and the nature of DNA damage. These results highlight the importance of incorporating co-exposure scenarios and multiple mechanistic endpoints in environmental mutagenesis and risk assessment studies.

Funding: This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., through project MixIng-Tox with reference 2022.03491.PTDC, and also projects with references UID/4750/2025, LA/P/0064/2020, UIDB/50011/2020, and LA/P/0006/2020, Ministry of Science and Innovation MCIN/AEI Grant PID2024-160400OB-I00, and Xunta de Galicia (ED431B 2025/26). A.T. Reis thanks the support of Horizon Europe Framework Programme under Grant Agreements No. 101057014 and No. 101157269. C.B. Lopes thanks the support of FCT - Fundação para a Ciência e Tecnologia, I.P. for researcher contract with DOI identifier 10.54499/2021.03739.CEECIND/CP1659/CT002.

Comparative genotoxicity of *Alternaria* mycotoxins in HepG2 cells

R. S. Vilela[1], C. Ventura[1,2], B. Guerreiro[1], H. Louro[1,2], & M. J. Silva[1,2*]

[1] Department of Human Genetics, National Institute of Health Doutor Ricardo Jorge, 1649-016 Lisbon, Portugal
[2] Comprehensive Health Research Centre (CHRC), NOVA Medical School, Universidade NOVA de Lisboa, Lisboa, Portugal
* m.joao.silva@insa.min-saude.pt

Background: *Alternaria* mycotoxins are widespread emerging food contaminants to which humans are frequently exposed through diet. However, toxicological data on several relevant *Alternaria* toxins remain limited, particularly regarding their potential hepatic genotoxicity.

Aims: This study aimed to evaluate the cytotoxic and genotoxic effects of six selected *Alternaria* mycotoxins — alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA), altertoxin-I (ATX-I), altenuene (ALT), and tentoxin (TEN) — in human hepatocellular carcinoma (HepG2) cells.

Methods: Exponentially growing cells were exposed to mycotoxins for 1.5-2 cell cycles lenght (48 h – 51 h). Cytotoxicity was firstly assessed by the MTT assay and then using the cytokinesis-block proliferation index (CBPI) to define the concentration-range for genotoxicity testing. The cytokinesis-block micronucleus (CBMN) assay (OECD Test Guideline 487) was selected for genotoxicity assessment.

Results: A differential toxicity profile was observed among the tested mycotoxins (MTT assay), with AME showing the highest cytotoxicity (IC_{50} = 20.06 µM), followed by ATX-I (IC_{50} = 56.84 µM), AOH (IC_{50} = 116.78 µM) and TeA (IC_{50} = 446.31 µM). In contrast, both ALT and TEN showed limited cytotoxicity (< 20%) under the tested conditions. All six mycotoxins induced a significant increase in the frequency of micronucleated cells at least at one tested concentration, compared with the vehicle control, showing their ability to cause chromosomal damage in HepG2 cells. ATX-I and AOH were the most potent genotoxic compounds, inducing significant micronucleus formation from 0.25 µM and 6.25 µM onward, respectively. AME, ALT, TEN, and TeA induced significant genotoxic effects from 20, 25, and 50 µM onward, respectively.

Conclusion: Overall, these findings indicate that *Alternaria* mycotoxins can exert genotoxic effects in hepatic cells, suggesting a potential ability to induce carcinogenic processes. These results provide relevant evidence for the hazard and risk assessment of these mycotoxins, contributing to their future regulation.

Acknowledgements: Doris Marko (University of Vienna, Austria) and Ann-Cathrin Behr (Bundesinstitut für Risikobewertung, BfR, Berlin, Germany).

Funding: Work funded by the European Union's Horizon Europe research and innovation programme under Grant Agreement No.101057014 (PARC) and co-funded by Instituto Nacional de Saúde Dr. Ricardo Jorge.

Assessment of genotoxic effects induced by real-life micro- and nanoplastics derived from orthodontic thermoplastics

H. R. Van Goethem[1*], A. Vergara[1,2], I. H. Valido[1], & S. Pastor[1]

[1] Universitat Autònoma de Barcelona, Barcelona, Spain
[2] Pontificia Universidad Javeriana, Bogotá, Colombia
* Hannes.vangoethem@uab.cat

The widespread use of clear orthodontic aligners and retainers has raised concerns regarding the potential release of polymeric nanoplastics during routine wear. In this study, we explored the potential cellular interactions and genotoxic effects of polyurethane nanoplastics derived from Invisalign retainer material in human oral cells. Polyurethane nanoplastic particles were generated manually in the lab from retainer material and characterized prior to experimental exposure.

Following incubation, cellular internalization of the particles was assessed, indicating that polyurethane nanoplastics could be taken up by oral cells under the experimental conditions tested. To investigate potential genetic effects, two commonly used genotoxicity assays were employed: the comet assay to evaluate DNA strand breaks and the micronucleus assay to assess chromosomal damage and genome instability.

Preliminary observations suggest an increase in markers associated with DNA damage in exposed cells compared with untreated controls. While these findings are still under investigation, they point toward the possibility that polyurethane nanoplastics released from orthodontic retainer materials may interact with oral cells and influence genomic stability.

Overall, this work provides early evidence that nanoplastic particles originating from polyurethane-based orthodontic retainers can enter oral cells and may contribute to genotoxic stress under experimental conditions. Further development of the study is required to confirm these observations and to better understand the potential long-term biological implications and safety of the polyurethane-based materials used in orthodontic appliances.

Funding: This research was funded by the Spanish State Research Agency (AEI) under project PID2023-146489OB-I00, in accordance with Order CIN/1025/2022, within the State Plan for Scientific and Technical Research and Innovation 2021–2023.

Changes in the oral microbiota after six months of clear aligner orthodontic treatment: Assessment of micro- and nanoplastic exposure using MinION sequencing

K. Barrios-Garay[1,2*], A. Rocabert[1], O. Camps-Font[2], E. Valmaseda-Castellón[2], & S. Pastor-Benito[1]

[1] Group of Mutagenesis, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain
[2] Faculty of Medicine and Health Sciences, Department of Dentistry, Universitat de Barcelona, Barcelona, Spain
* kevinbarriosgaray@outlook.es

Malocclusion is a common oral health issue that affects the normal functioning of the orofacial system. Orthodontic therapy aims to correct malocclusion and craniofacial skeletal discrepancies whilst also improving mastication and appearance. Clear aligners (CA) have transformed the field of orthodontics providing a more discreet and convenient alternative to fixed appliances, as they offer a more comfortable and aesthetically pleasing orthodontic treatment. CA are manufactured from different thermoformed materials that are susceptible to degradation into micro- and nanoplastics (MNPLs), which is worsened by various oral cavity factors such as pH fluctuations, mechanical attrition, among others. The use of CA and their associated MNPLs release may influence oral bacterial communities and impact oral health.

The objective of our study is to assess the changes in the oral microbiota of individuals undergoing CA orthodontic treatment. Bacterial DNA was extracted from buccal swabs using the PureLink Microbiome DNA Purification Kit (Invitrogen, Thermo Fisher Scientific), followed by sequencing with the MinION system and subsequent bioinformatic analysis.

At present, a total of thirty buccal samples from fifteen patients undergoing orthodontic treatment with CA were analyzed. Two different samples were taken from the same individual, one obtained before starting treatment with CA and one after six months of CA use. Preliminary data suggest that metrics related to alpha and beta diversity might shift between samples. Exposure to MNPLs may contribute to oral microbiota changes and therefore influence oral health.

Funding: This work is supported by the Ministry of Science, Innovation and Universities and National Research Agency (PID2023-146489OB-I00). AR is funded by the Generalitat de Catalunya (FI-SDUR 2023).

Genotoxic effects in workers of the plastic recycling industry exposed to micro- and nanoplastics

H. Pulli[1], T. Venus[2], M. Venäläinen[1], D. Abass[3,4], J. Domenech[1], D. Angosto[5], M. Morataya-Reyes[3], C. Moreno[6], K. Siivola[1], L. Martínez-Alarcón[5,7], K. Portillo[8], P. Millán-Billi[8], E. Cervera[8], I. Estrela-Lopis[2], P. Pelegrín[5,9], A. Hernández[3], S. Pastor[3], & J. Catalán[1,6*]

[1]Finnish Institute of Occupational Health, Helsinki, Finland
[2] Institute of Medical Physics and Biophysics, University of Leipzig, Leipzig, Germany
[3] Universitat Autònoma de Barcelona, Cerdanyola Del Vallès, Spain
[4] Sohag University, Sohag, Egypt
[5] Molecular Inflammation Group, Biomedical Research Institute of Murcia (IMIB), Murcia, Spain
[6] University of Zaragoza, Zaragoza, Spain
[7] University Clinical Hospital Virgen Arrixaca, Murcia, Spain
[8] Hospital Universitari Germans Trias I Pujol, Badalona, Spain
[9] Department of Biochemistry and Molecular Biology B and Immunology, Faculty of Medicine, University of Murcia, Spain.
** jcatalan@unizar.es*

Micro- and nanoplastics (MNPLs) pollution constitutes one of the greatest current threats for the environment and human health. In the present study, we investigated the internal exposure and early health effects induced by MNPLs exposure. Blood and buccal samples were taken from 17 male employees of a plastic recycling company (mean age, 44.3±3.0 y) and 21 male volunteers (45.6±2.7 y) non-occupationally exposed to MNPLs. Workers also provided an exhaled breath condensate (EBC) sample. The study was approved by the local ethics committees and all participants provided written informed consent. MNPLs were characterised by Confocal Raman Microscopy (CRM). Local chromosome damage was assessed by the micronucleus assay in buccal cells, whereas systemic DNA and chromosome damage was assessed from blood cells by means of the comet and micronucleus assays. Furthermore, the levels in plasma of ten pro-inflammatory cytokines and chemokines were also investigated. The number of particles in blood increased by 114% in recycling workers compared to the control group. Workers also showed a higher percentage of particles below 1 µm (40% vs 38%) and 1-10 µm (58% vs 54%) diameter, but a lower percentage of particles above 10 µm diameter (2% vs 6%). Particle composition was similar in both groups, except for polydimethylsiloxane, an additive used in the manufacture of thermoplastics, which was only detected in the group of workers. Furthermore, workers showed a statistically significantly higher rate of DNA damage than the corresponding control group, although the damage was not connected to oxidative stress, as well as a significantly higher level of interleukin (IL)-1α, IL-1 receptor antagonist (IL-1Ra) and C-X-C motif chemokine ligand 5 (CXCL5). No significant differences were detected for the other genotoxic or inflammatory biomarkers. In conclusion, exposure to MNPLs during recycling-related processes seems to induce alterations of some pro-inflammatory cytokines and DNA damage.

Acknowledgements: The authors would like to thank the company, workers and volunteers who participated in the study.

Funding: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 965196 (PlasticHeal).

SESSIONS

04. Epigenomics and epitranscriptomics, and emerging pollutants (II)

H3K27 acetylation as a key regulator of onco-lncRNA expression in lung cancer

C. Ayala-Roldán[1,2,3]*, L. Gisella Bermúdez Liscano[1,4], J. Romero Simmonds[4], R. R. Ariza[1,2,3], T. Roldán-Arjona[1,2,3], T. Morales-Ruiz[1,2,3], & A. P. Rojas Moreno[1,2,3]

[1] Department of Genetics. University of Cordoba, Córdoba, Spain.
[2] Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Córdoba, Spain
[3] Reina Sofia University Hospital, Córdoba, Spain
[4] Institute of Human Genetics, School of Medicine, Pontificia Universidad Javeriana, Bogotá, Colombia
* b82ayroc@uco.es

Background: Lung cancer is the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of cases. Beyond genetic alterations, epigenetic mechanisms play a central role in tumor progression by modulating gene expression programs. Long non-coding RNAs (lncRNAs), which are transcripts longer than 200 nucleotides that do not encode proteins, are emerging as key regulators of cancer-associated cellular processes, acting as either oncogenes or tumor suppressors. However, the epigenetic mechanisms that govern their transcription process remain poorly understood.

Aim: To investigate whether histone H3 lysine 27 acetylation (H3K27ac), a chromatin mark associated with transcriptional activation, regulates the expression of a deregulated onco-lncRNA identified in lung cancer models.

Methods: H3K27ac enrichment at putative regulatory regions of the lncRNA locus was first assessed. To determine the functional relevance of this modification, targeted acetylation was induced using a CRISPR/deactivated Cas9 (dCas9)-p300 epigenome editing system in HEK293 cells, which lack endogenous expression of the lncRNA. Guide RNAs were designed to direct the dCas9-p300 complex to specific regulatory regions. Following transfection, lncRNA expression levels were quantified by reverse transcription quantitative polymerase chain reaction (RT-qPCR). A catalytically inactive p300 construct was used as a control.

Results: Regulatory regions of the lncRNA locus displayed enrichment of H3K27ac. Targeted acetylation of individual regulatory regions significantly increased lncRNA expression compared with control conditions.

Conclusions: These findings demonstrate that H3K27 acetylation directly regulates the transcriptional activation of this lncRNA and highlight the utility of epigenome editing tools to dissect the regulatory mechanisms governing lncRNA expression in lung cancer.

Chronic exposure to nanoplastics alters stem cell type-specific mechanisms, promoting cancer development

I. Barguilla[1*], L. Billon[2], K. Geistlich[2], J Gutiérrez-García[1], R. Egea[1], L. Rubio[1], S Jeanpierre[2], A. Hernández[1], B. Guyot[2], & V. Maguer-Satta[2]

[1] Group of Mutagenesis, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona; Cerdanyola del Vallès, Barcelona, Spain
[2] CNRS UMR5286, Inserm U1052, Universite Claude Bernard Lyon 1, Centre Léon Bérard, Centre de Recherche en Cancérologie de Lyon, Lyon, France
* irene.barguilla@uab.cat

Micro- and nanoplastics (MNPLs) are increasingly recognized as a potential threat to human health. These small plastic particles can be internalized in primary organs, translocate through physiological barriers, reach the bloodstream and potentially bioaccumulate in secondary organs. Increasing evidence demonstrates potential long-term impacts of MNPLs, including carcinogenicity. However, the molecular mechanisms leading to these effects are largely unexplored.

In this work, we compared the pre-neoplastic effects of polystyrene (PS) and polyethylene terephthalate (PET) nanoplastics on mammary stem cells, which may be especially sensitive to the cumulative impacts of environmental pollutants due to the long lifespans in the human body. Both plastic types induced similar oncogenic outcomes, particularly when combined with physiologically relevant microenvironmental signalling such as BMP2, a key regulator of stem cell niche. Notably, a comprehensive omic analysis revealed that the molecular mechanisms leading to these effects are different for PS and PET. While PS mainly activates kinase networks, PET induced large transcriptional deregulations. Besides, our approach allowed us to identify a molecular signature especially affected by MNPL exposure and comprised of genes associated with poorer overall survival in patients with breast cancer.

Our work underscores the urgent need to refine current approaches and to incorporate a mechanistic perspective for risk assessment. Further, our findings support the integration of stem-cell based assays, microenvironmental factors and realistic test materials in testing frameworks to inform regulation and support decision-making to mitigate the impact of MNPLs.

Funding: This project received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant agreement No. 965196. IB (Academic record 2023-BP-00212) holds a Beatriu de Pinós Postdoctoral Program from the Secretariat of Universities and Research of the Department of Business and Knowledge of the Government of Catalunya.

Genotoxic and functional endothelial responses to nanoplastics: the role of physicochemical properties

A. García-Rodríguez[1*], J. Martín-Pérez, J. Gutiérrez-García, R. Egea, R. Marcos, & A. Hernández

[1]Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193, Spain.
* alba.garcia.rodriguez@uab.cat

The vascular endothelium represents a critical biological interface for circulating micro- and nanoplastics (MNPLs) following systemic translocation. This thesis investigates MNPL-induced endothelial dysfunction using a primary human umbilical vein endothelial cell (HUVEC) model, integrating mechanistic and functional endpoints while systematically addressing the role of physicochemical properties. A progressive material strategy was implemented, beginning with well-defined polystyrene (PS) nanoplastics to dissect the effects of surface functionalization and nanoscale size (30–100 nm), and extending to environmentally relevant materials, including irregular polytetrafluoroethylene (PTFE) and real-life polyethylene terephthalate (PET) fragments derived from post-consumer bottles.

All tested nanoplastics were rapidly internalized; however, internalization efficiency did not predict biological impact. MNPL exposure induced a predominantly sub-lethal endothelial dysfunction phenotype characterized by intracellular cholesterol accumulation, impaired migration and angiogenesis, oxidative stress, DNA strand breaks, transcriptomic alterations, and IL-6 modulation. Surface functionalization emerged as a key determinant, with aminated PS exhibiting the highest toxicity despite lower uptake. Particle size modulated the magnitude and profile of responses, with 30 nm particles showing the most distinct transcriptomic signature. Environmentally realistic PET induced the strongest functional impairment.

Overall, MNPL-induced endothelial dysfunction occurs under sub-cytotoxic conditions and is strongly governed by surface chemistry, size, and environmental realism, underscoring the need to integrate physicochemical characterization into hazard assessment.

Funding: This work was partially supported by the EU Horizon 2020 programme (965196, PlasticHeal), the Spanish Ministry of Science and Innovation (PID2020-116789, RB-C43), the Generalitat de Catalunya (2021-SGR-00731), and the ICREA-Academia programme to AH.

An innovative biomarker-based blood assay for monitoring micro- and nanoplastic exposure in occupational and environmental settings

J. Arribas Arranz[1*], I. Barguilla[1], C. Pommerenke[2], R. Egea[1], M. Morataya-Reyes[1], A. Villacorta[3], P. Pelegrín[4], JF. Ferrer[5], J. Catalán[6], R. Marcos[1], & A. Hernández[1]

[1] Group of Mutagenesis, Department of Genetics and Microbiology, Faculty of Biosciences, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain
[2] German Collection of Microorganisms and Cell Cultures GmbH (DSMZ). Braunschweig, Germany
[3] Facultad de Recursos Naturales Renovables, Universidad Arturo Prat, Iquique, Chile
[4] BioMedical Research Institute of Murcia, Murcia, Spain
[5] AIMPLAS, Plastics Technology Center, Valencia Parc Tecnològic, 46980 Paterna, Spain
[6] Department of Anatomy, Embryology and Animal Genetics, Faculty of Veterinary Medicine, University of Zaragoza, Zaragoza, Spain
** jessica.arribas@uab.cat*

Background: Human exposure to micro- and nanoplastics (MNPLs) is increasingly recognized due to their ubiquitous presence; however, their assessment is still based on physicochemical particle detection. These approaches are analytically complex, poorly standardized, and largely unsuitable for routine application, resulting in a major gap in the ability to quantify exposure-related biological effects and to identify at-risk individuals. This limitation is particularly relevant in occupational settings but also affects vulnerable population groups and limits translation to broader environmental contexts.

Aim: To present an innovative biomarker-based strategy that overcomes current limitations in MNPL exposure assessment by enabling detection of early biological responses using a simple blood-based molecular approach.

Methods: A MNPL-specific gene expression signature was identified using an *ex vivo* human whole-blood exposure model combined with integrative omics analyses. Candidate biomarkers were selected based on their consistent response across white blood cell subtypes and different MNPL types and tested by a quantitative polymerase chain reaction. The approach has been validated in occupationally exposed workers, and performance benchmarked against conventional genotoxicity and inflammatory biomarkers.

Results: The biomarker panel reliably discriminated MNPL-exposed individuals from controls, being able to identify early, potentially reversible molecular alterations not captured by current particle-based methods. The assay is compatible with standard blood sampling workflows, requires minimal sample volumes, and significantly reduces analysis time and cost. The biomarkers provide specific biological information associated with MNPL exposure, supporting their applicability in occupational health surveillance and environmental human biomonitoring.

Conclusions: This innovative blood-based biomonitoring approach addresses a critical methodological gap in MNPL exposure assessment by moving beyond particle quantification and detection towards biologically meaningful indicators. It offers a scalable tool for occupational health programs and has potential to be extended to vulnerable human populations and to other species relevant for environmental and food-chain monitoring, supporting integrated and preventive risk assessment strategies.

Funding: Part of this work was carried out within the PlasticHeal project, funded by the European Union's Horizon 2020 programme (Grant Agreement No. 965196), and supported by the Generalitat de Catalunya (2021-SGR-00731) and the ICREA-Academia programme (A. Hernández, Ac2232418).

Multi-omic analysis of the biological impacts of exposure to PTFE micro- and nanoplastics in *Drosophila melanogaster*

R. Egea*, A. Rocabert, M. Gámez, M. Alaraby, A. García-Rodríguez, R. Marcos, & A. Hernández

Group of Mutagenesis, Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain
* raquel.egea@uab.cat

The increasing environmental presence of micro and nanoplastics (MNPLs) raises growing concerns regarding their biological effects and underlying molecular mechanisms. Polytetrafluoroethylene (PTFE, Teflon®) is of particular relevance due to its extensive industrial and consumer applications, yet its impact on living organisms remains poorly understood. Its widespread use in household and cooking wares facilitates particle transfer into food, representing a potential route of direct exposure and a possible risk to human health.

In this study, we employed an integrated omic approach combining 16S rRNA gene amplicon sequencing and transcriptomics to evaluate the biological effects of PTFE micro- and nanoparticles using *Drosophila melanogaster* as an *in vivo* model. *D. melanogaster* is well suited for investigating the dynamics of gastrointestinal microbiota due to its advantages such as short life cycle, well-characterised genetic background, and intestinal structure and function similar to humans.

Drosophila was exposed to PTFE particles of micro- (MPLs) and nanoscale (NPLs) dimensions to identify size-dependent biological responses. Whole-organism RNA sequencing was conducted to characterise host transcriptional responses, while 16S rRNA gene sequencing was used to assess changes in gut microbial community composition. The analyses revealed pronounced size- and concentration-dependent alterations affecting both host gene expression and microbiota structure. Integrated multi-omic analyses evaluated potential interactions between host transcriptional regulation and microbiota dynamics, highlighting the role of the gut ecosystem in mediating nanoparticle-induced biological effects.

Funding: This project has received funding from the Generalitat de Catalunya (2021-SGR-00731) and the ICREA-Academia programme to A. Hernández (Ac2232418).

SESSIONS

05. Animal models in toxicity testing and human disease research

In vivo evaluation of three differently charged gold nanoparticles using the zebrafish embryo model

L. Ramos-Pan[1,2*], M. Novak[3], A. Touzani[1,2], M. Saavedra-Rodríguez[1,2], M. Paz[4], J. Mosquera[4], A. Criado[4], M.L. Díaz-Prado[5], N. Fernández-Bertólez[1,2], B. Laffon[2,6], B. Zegura[3], & V. Valdiglesias[1,2]

[1] Universidade da Coruña, Grupo NanoToxGen, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Biología, Facultad de Ciencias, Campus A Zapateira s/n, 15071, A Coruña, Spain
[2] Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas, As Xubias, 15006, A Coruña, Spain
[3] National Institute of Biology (NIB), Department of Genetic Toxicology and Cancer Biology, Večna pot 121, Ljubljana, Slovenia
[4] Universidade da Coruña, Grupo Nanoself, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Química, Facultad de Ciencias, Campus A Zapateira s/n, 15071, A Coruña, Spain
[5] Universidade da Coruña, Grupo NEUROVER, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Biología, Facultade de Ciencias, Campus A Zapateira s/n, 15071 A Coruña, Spain
[6] Universidade da Coruña, Grupo DICOMOSA, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Psicología, Facultad de Ciencias de la Educación, Campus Elviña s/n, 15071, A Coruña, Spain
** lucia.ramosp@udc.es*

Due to their unique physicochemical properties, gold nanoparticles (AuNP) are widely used for numerous applications in different fields, and in recent years they have gained considerable interest in biomedical research. Their small size enables them to cross biological barriers such as the blood-brain barrier, which makes them promising candidates for diagnosis and treatment of neurological disorders. In this context, evaluating their potential toxicity is essential to ensure their biocompatibility and safe use in biomedical applications. On this basis, the main objective of the present study was to assess the biological effects of three differently charged AuNP (i.e. anionic, cationic and neutral) over a wide range of concentrations (6.25–100 µg/mL) using zebrafish (*Danio rerio*) embryos as an *in vivo* model. Embryos were exposed for 96 hours, and toxicity was assessed by the Fish Embryo Acute Toxicity (FET) test according to the established OECD Test Guideline No. 236. Several developmental endpoints were analyzed, including embryonic viability, hatching rate and morphological alterations. Additionally, heart rate and blood flow were measured to detect sublethal effects. Under the experimental conditions tested, no significant alterations were observed for any of the AuNP tested. To gain deeper insight into their mechanism of action, targeted gene expression analysis using microfluidic qPCR on chips (Fluidigm) was performed, focusing on genes associated with oxidative stress, DNA damage response, and neuronal development. Findings from this study provide a better understanding of AuNP biological behaviour and support their possible use in nervous system-targeted applications.

Funding: Ministry of Science and Innovation MCIN/AEI [Grant PID2024-160400OB-I00] and Xunta de Galicia (ED431B 2025/26), L.R.-P. was supported by a Ministry of Science and Innovation predoctoral fellowship (grant number FPU2023/03379). The research stay was funded by the INDITEX-UDC Predoctoral Mobility Program 2025 (to L.R.-P.), ARIS P1-0245.

Toxicity assessment of platinum nanoparticles in *Drosophila melanogaster:* acute and chronic exposure effects

A. Touzani[1,2*], M. Saavedra-Rodríguez[1,2], L. Ramos-Pan[1,2*], A. Insua[1], J. Moreda-Piñeiro[3], N. Fernández-Bertólez[1,2], B. Laffon[2,4] & V. Valdiglesias[1,2]

[1] Universidade da Coruña, Grupo NanoToxGen, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Biología, Facultad de Ciencias, Campus A Zapateira s/n, 15071, A Coruña, Spain
[2] Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas, As Xubias, 15006, A Coruña, Spain
[3] Universidade da Coruña, Grupo Química Analítica Aplicada (QANAP), Instituto Universitario Medio Ambiente (IUMA), Departamento de Química, Facultad de Ciencias, Campus A Zapateira s/n, 15071 A Coruña, Spain.
[4] Universidade da Coruña, Grupo DICOMOSA, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Psicología, Facultad de Ciencias de la Educación, Campus Elviña s/n, 15071, A Coruña, Spain
* assia.touzani@udc.es

The increasing use and potential applications of platinum nanoparticles (PtNP) in various fields raise important concerns regarding their safety and long-term biological effects. Despite their promising catalytic and antioxidant properties, data on their toxicity and impact on living organisms remain limited and insufficiently documented.

In this context, the present study aims to evaluate the *in vivo* effects of PtNP using *Drosophila melanogaster* as a reference biological model. Oral exposures to different concentrations of PtNP were conducted in adult flies as well as in third instar larvae, under both acute and chronic exposure conditions. Analysis of survival, morphological, and behavioural parameters were assessed.

Results indicate that PtNP induced dose- and exposure duration-dependent effects. At the morphological level, no modifications were observed in adult individuals, either males or females, following acute exposure. However, size alterations were detected in adult males after chronic exposure. In addition, no morphological changes were observed in third-instar larvae, regardless of the exposure conditions. Regarding behavioural assessment, the crawling assay revealed no alteration in larval locomotion after acute exposure but a decrease in locomotor activity after long-term exposure. In adults, the climbing assay demonstrated a significant and marked dose-dependent decline in flight and climbing behaviour after both acute and chronic exposures.

Overall, these findings suggest that PtNP may induce functional impairments, particularly affecting locomotor activity, which could reflect potential neurotoxicity or physiological stress. They also highlight the importance of exposure duration and developmental stage in assessing the toxic effects of nanoparticles.

Funding: Ministry of Science and Innovation MCIN/AEI [Grant PID2024-160400OB-I00] and Xunta de Galicia (ED431B 2025/26), L.R.-P. was supported by a Ministry of Science and Innovation predoctoral fellowship (grant number FPU2023/03379).

Morphological and behavioural alterations in *Drosophila melanogaster* offspring and adults after developmental exposure to gold nanoparticles

D. López-Aragón[1,2*], L. Ramos-Pan[1,2], L. Martínez[1], J. Moreda-Piñeiro[3], B. Laffon[2,4], & V. Valdiglesias[1,2]

[1] Universidade da Coruña, Grupo NanoToxGen, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Biología, Facultad de Ciencias, Campus A Zapateira s/n, 15071, A Coruña, Spain
[2] Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas, As Xubias, 15006, A Coruña, Spain
[3] Universidade da Coruña, Grupo Química Analítica Aplicada (QANAP), Instituto Universitario Medio Ambiente (IUMA), Departamento de Química, Facultad de Ciencias, Campus A Zapateira s/n, 15071 A Coruña, Spain
[4] Universdade da Coruña, Grupo DICOMOSA, CICA - Centro Interdisciplinar de Química e Bioloxía, Departamento de Psicología, Facultad de Ciencias de la Educación, Campus Elviña s/n, 15071, A Coruña, Spain
* d.aragon@udc.es

Nanoparticles (NP) are the particles of matters with the diameter usually ranging from 1 to 100 nanometres. Their composition is highly variable, and their applications differ according to their characteristics. Gold nanoparticles (AuNP) are highly promising nanomaterials in the therapeutic field due to their surface plasmon resonance property and high biocompatibility. Furthermore, due to their small size, they are able to cross the blood-brain barrier and penetrate the central nervous system, something that very few agents are able to achieve. However, this advantage may also be associated with a potential risk to the nervous system due to the accumulation of NP within this tissue cells. The objective of this study was to investigate the potential toxicological effects that oral exposure to AuNP during larvae development phase may cause in the adulthood or even afterwards in the offspring, using the model organism *Drosophila melanogaster*. With this aim, three concentrations ranging from 0.02 to 2 µg/mL of anionic and neutral AuNP were administered to the parental generation during their larvae development, i.e. individuals were exposed to AuNP only during their larval stage, after which the development of the adults was monitored until their death. Together with adult survival, the effects of the AuNP on morphology and behaviour (climbing and crawling) were assessed in adults and offspring. The data obtained yielded relevant findings in both cases: for anionic AuNP, adverse effects on the locomotor capacity of larvae were detected at high concentrations, along with size changes depending on the concentration and the characteristics of the subject (larva, adult male, or adult female). For neutral AuNP, variations in larval locomotor capacity cannot be confirmed; however, a trend toward size reduction in exposed adult females and an increase in larval size at high concentrations was observed. This study shows that anionic AuNP have a greater effect at the neurological level, impairing the motor capacity of larvae, while neutral AuNP affect development by primarily reducing the size of adult females.

Funding: Ministry of Science and Innovation MCIN/AEI [Grant PID2024-160400OB-I00] and Xunta de Galicia (ED431B 2025/26), L.R.-P. was supported by a Ministry of Science and Innovation predoctoral fellowship (grant number FPU2023/03379).

Hazard impacts of polytetrafluoroethylene micro-nanoplastics: *in vivo* study

M. Alaraby[1,2*], D. Abass[1,2], R. Egea[1], A. Velázquez[1], A. Hernández[1], & R. Marcos[1]

[1]Group of Mutagenesis, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain
[2]Zoology Department, Sohag University (82524), Sohag, Egypt
* mohamed.alaraby@uab.cat

Background: Polytetrafluoroethylene (PTFE) widely used in household and cookware, has recently been identified as a potential source of micro- and nanoplastics (MNPLs). Despite its widespread use, the contribution of PTFE-derived particles to MNPL exposure and their associated biological risks remain poorly understood, representing a significant gap in toxicological research.

Aim: This study aimed to systematically evaluate the biological effects of PTFE-MNPLs of different sizes, categorized into microscale (MPLs) and nanoscale (NPLs) fractions.

Methods: A comprehensive experimental approach was applied using *Drosophila melanogaster* as an *in vivo* model. Well-characterized PTFE particles were used to assess uptake, internalization, and tissue distribution. Physiological endpoints included oxidative stress (ROS generation), DNA damage, and mitochondrial membrane potential disruption. Molecular responses were investigated through genome-wide transcriptomic (omics) analyses, complemented by targeted gene expression profiling using RT-PCR.

Results: Both PTFE-MNPLs, irrespective of size, were localized within the gut lumen, near the peritrophic membrane and symbiotic microbiota. Evidence of bioerosion and the formation of secondary nanoscale particles was observed. Exposure induced significant oxidative stress, DNA damage, and mitochondrial dysfunction, with more pronounced effects for NPLs. Transcriptomic analyses, supported by RT-PCR, revealed widespread alterations in gene expression associated with stress response, metabolic processes, and cellular transport.

Conclusions: PTFE-MNPLs, regardless of size, induce significant physiological and molecular alterations, highlighting their capacity to penetrate biological barriers and disrupt cellular function.

Natural flavonoids induce dose-dependent cytotoxicity in marine ciliated protozoa parasites through putative mitochondrial mechanisms

J. L. R. Rama[1*], M. Cabaleiro[1], N. Bocija-Villaverde[1], S. Cabaleiro[2], & N. Mallo[1]

[1] Grupo GIBE, CICA, UDC, A Coruña, Spain
[2] CETGA, Ribeira, Spain
* cabaleiro.diaz@gmail.com

Marine ciliated protozoa are metabolically versatile microorganisms that frequently rely on flexible mitochondrial pathways to adapt to fluctuating environmental conditions, including variations in oxygen availability. In this study, we evaluated the cytotoxic effects of several naturally occurring phenolic compounds on *Philasterides dicentrarchi*, a marine ciliate parasite of turbot, causing important economical losses, developing a Trypan Blue based exclusion assay (Vi-CELL BLU, Beckman Coulter). Flavonoids such as quercetin and kaempferol are known to interact with mitochondrial metabolism, including modulation of reactive oxygen species production and interference with electron transport. *P. dicentrarchi* possess an Alternative Oxidase (AOX), which allows metabolic flexibility under stress conditions.

Cells were exposed to increasing concentrations of Quercetin, Kaempferol, Rutin, Gallic acid, and the reference inhibitors Cycloheximide and Chloramphenicol. Among the tested compounds, quercetin displayed the strongest cytotoxic effect, reducing viable cell counts by more than 95% at 100 µM and showing an estimated IC50 of approximately 48.9 µM. Kaempferol and rutin also exhibited significant dose-dependent cytotoxicity, with IC50 values of approximately 31.0 µM and 35.7 µM, respectively. Gallic acid produced moderate effects, while chloramphenicol showed limited but statistically significant cytotoxicity. In contrast, cycloheximide did not produce significant reductions in cell viability under the tested conditions.

The observed cytotoxic effects may involve disruption of mitochondrial respiration. The moderate effect observed with chloramphenicol, which targets mitochondrial-like ribosomes, further supports mitochondrial involvement in the cytotoxic response.

Overall, our results indicate that naturally occurring flavonoids exert significant cytotoxic effects on *P. dicentrarchi*, likely through mitochondrial-related mechanisms. These findings highlight the potential ecological relevance of environmental phenolic compounds in regulating marine protozoan populations of aquaculture importance and suggest mitochondrial metabolism, including AOX-related pathways, as potential targets for further investigation.

Funding: CNS2022-135616. PHILANAUTA: Viaje hacia el entendimiento: explorando la biología molecular del parasito *Philasterides dicentrarchi*.

SESSIONS

06. New Approach Methodologies in toxicity/safety assessment

Evaluation of New Approach Methodologies (NAMs) for the detection of compounds classified as non-genotoxic carcinogens (NGTxCs)

M. Benito-Fuertes[1*], A. Rodriguez-Garraus[1], & A. Azqueta[1]

[1] Department of Pharmaceutical Sciences, School of Pharmacy and Nutrition, University of Navarra, Pamplona, Spain
* mbenitof@unav.es

NGTxCs represent a major challenge for chemical risk assessment because they do not induce DNA damage through genotoxic mechanisms and therefore escape detection by standard genotoxicity testing strategies. They act through diverse and often complex mechanisms and are mainly identified through long-term *in vivo* carcinogenicity studies, which are resource-intensive and raise scientific and ethical concerns. Within the European Partnership for the Assessment of Risks from Chemicals (PARC), our group contributes to the WP 5.2.1.a working team, which aims to develop a battery of *in vitro* methods for NGTxC identification.

In a first phase, eight compounds classified as non-carcinogens or NGTxCs were evaluated using the standard and enzyme-modified comet assays in 2D and 3D HepG2 models after short- and long-term exposures. Potassium bromate and methyl methanesulfonate were used as positive controls and produced the expected responses, whereas all tested compounds were negative. In a second phase, eleven additional compounds representing diverse mechanisms of action related to carcinogenesis were assessed using the Fpg-modified comet assay in a 2D HepG2 model after short exposure. All compounds yielded negative results, including the oxidizing agents, for which additional experiments are currently ongoing.

In parallel, the Cell Transformation Assay (CTA) was implemented following the OECD guidance to assess chemically induced cell transformation associated with carcinogenic activity. The Growth in Low Attachment (GILA) assay was also established to detect anchorage-independent growth. Six compounds have been analysed to date, and the results are being compared with their expected activity profiles.

Ongoing analyses focusing on oxidative stress-related endpoints and cell transformation-based assays aim to support the development of *in vitro* approaches for NGTxC identification.

Funding: European project Partnership for the Assessment of Risks from Chemicals (PARC; HORIZON-HLTH-2021-ENVHLTH-03; 101057014; https://www.eu-parc.eu).

Model matters: Comparative toxicity of BPA and BPS-MAE in 2D and 3D HepG2 models

C. Ramos[1*], C. Ventura[1,2], A. Tavares[1,2], H. Louro[1,2], & M. J. Silva[1,2]

[1] Human Genetics Department, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal
[2] Comprehensive Health Research Centre (CHRC), NOVA Medical School, Universidade NOVA de Lisboa, Lisbon, Portugal
* carolina.ramos@insa.min-saude.pt

Background: The phase-out of Bisphenol A (BPA) led to the introduction of analogues such as Bisphenol S 4-allyl ether (BPS-MAE), yet concerns remain about their safety. Evaluating their genotoxicity is paramount to hazard assessment and regulation. New Approach Methodologies (NAMs), including three-dimensional (3D) cell culture models, are increasingly adopted in toxicology and enhance human relevance and reduce reliance on animal testing, gaining trust and acceptability in regulatory frameworks like the European Partnership for Assessment of Risk from Chemicals (PARC).

Aim: To comparatively assess the cytotoxicity and genotoxicity potential of BPA and BPS-MAE across 2D and 3D HepG2 cell culture systems, following the characterization of the HepG2 3D spheroid model established via liquid overlay technique.

Methods: HepG2 spheroids (2,500 cells) were maintained for 13 days and characterized by total cell counting, metabolic activity (Alamar Blue assay), and morphological analysis. Cytotoxicity and genotoxicity of BPA and BPS-MAE were assessed in 2D and 3D HepG2 cell cultures via Cytokinesis-Block Micronucleus (CBMN) assay.

Results: Preliminary characterization showed stable spheroid morphology over 13 days, and an observed increase in metabolic activity in 3D versus 2D cultures. Preliminary cytotoxicity data revealed model-dependent responses. Both compounds exhibited higher cytotoxicity in 2D than in 3D cultures: BPA: ≃ 55% at 160 µM in 2D vs. 35% in 3D cultures; BPS-MAE: ≃ 55% at 60 µM in 2D and at 80 µM ≃ 28% in 3D cultures. Additionally, BPS-MAE exhibited higher cytotoxicity than BPA in both models. Genotoxicity assessment is underway.

Conclusions: Spheroids showed higher tolerance to both compounds, likely attributable to diffusion barriers, structural complexity, or differential metabolic activity, possibly related to cells organization in a tissue-like structure. The fact that BPS-MAE was more toxic to liver cells than BPA suggest that a regrettable substitution may have occurred. These findings underscore the critical importance of NAMs development in genotoxicity.

Acknowledgements: Naouale El Yamani (NILU, Norway) and Yvonne Kohl (IBMT, Germany) for their support with the establishment of 3D cultures.

Funding: Work funded by the European Union's Horizon Europe research and innovation programme under Grant Agreement No. 101057014 (PARC) and co-funded by Instituto Nacional de Saúde Dr. Ricardo Jorge.

Use of the nematode *Caenorhabditis elegans* as a biomodel to investigate the toxic effects of the plasticizer bisphenol A and their chemical analogues

S. Maisanaba[1*], M. Cano-Caballero[1], H. Flores- Mayal [1], C. Álvarez-Herrera[1], R. Rojas[1], O. Herrero[2], R. Planelló[2], A.P. Zaderenko[3], R. García-Repetto[1], M. Llana-Ruiz-Cabello[1], & G. Repetto[1]

[1] Area of Toxicology, University Pablo de Olavide, Seville, Spain
[2] Faculty of Science UNED, Madrid, Spain
[3] Area of Nanotechnology, University Pablo de Olavide, Spain
* smaiher@upo.es

The nematode *Caenorhabditis elegans* is a widely used and promising model organism in various areas of research, including developmental biology, genetics, medical sciences, and toxicology.

Interest in *C. elegans* as a biomodel is based on several key advantages, such as: (i) ease of use and maintenance, together with its low cost; (ii) a short life cycle (approximately 3 days from egg to adult) and high reproductive capacity; (iii) high genetic homology (60–80%) with humans; (iv) a transparent body that allows direct observation of complex biological processes such as embryogenesis and morphogenesis; and (v) a strong capacity for genetic manipulation, with the availability of mutants and fluorescent reporters for mechanistic studies.

The main objective of this study was to investigate the effects on growth, reproductive rate, longevity and obesity following exposure to the plasticizer bisphenol A and its analogues in different strains of *C. elegans*.

Depending on the evaluated parameter, L1 or L4 larvae were selected, and different exposure times were considered in each case. In general, the assays were carried out at 20°C in liquid medium, or, at 25°C for longevity assays, using increasing concentrations of the tested chemicals. Body size, larval number and survival time were quantified using a digital fluorescence microscopy system.

The preliminary results showed some significant effects in the reproductive, longevity and obesity tests compared to the growth assays; however, further investigation is required to expand the current findings.

In conclusion, the methodologies applied indicate that *C. elegans* is a useful biomodel for environmental toxicology research.

Funding: This work was partially funded within the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC), Horizon Europe, Grant Agreement No. 101057014 and the project Estrategia de una-salud para evaluar la interacción del cambio climatico en la toxicidad ambiental de plásticos biodegradables y compostables. PID2024-160709OB-I00.

Micro- and nanoplastic interference in THP-1 immune response in an inflammatory context

J. Gutiérrez-García[1*], S. Trischitta[2], G. M. Ferraz [3], L. Pareras[1], C. Anguita-Soler[1], R. Marcos[1], A. Hernández[1], & L. Rubio[1]

[1] Group of Mutagenesis, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona; Cerdanyola del Vallès, Barcelona, Spain
[2] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale F. Stagno D'Alcontres, Messina, Italy
[3] Environmental Biogeochemistry Laboratory, Department of Chemistry, Federal University of São Carlos, São Carlos, São Paulo, Brazil
* Javier.Gutierrez.Garcia@uab.cat

Micro- and nanoplastics (MNPLs) are increasingly recognized as threatening environmental pollutants. Their low density and small mass facilitate airborne transport, creating a potential risk to human health as these particles can be inhaled and subsequently accumulate in lung tissue. There, MNPLs harmful effects disclose special threat in inflammatory processes and long-term exposure scenarios.

In vitro human-relevant models, such as the bronchial Calu-3 air–liquid interface (ALI) system, are widely used to evaluate the harmful potential of MNPLs at primary biological barriers. Under ALI conditions, epithelial cells undergo differentiation, produce mucus and form tight junctions, creating a structure that closely mimics the human airway epithelium while also supporting long-term exposure studies. In this context, macrophages play a major role in directing the immune-response and particle clearance.

Understanding MNPLs impact on macrophages is crucial to advance on the development of new strategies to evaluate toxicological risks using the co-culture system. Thereby, in this work we assessed inflammatory-response interference of poly lactic acid (PLA), polyethylene terephthalate (PET) and polytetrafluoroethylene (PTFE, Teflon) MNPLs in THP-1 that have already undergone inflammatory differentiation, as happens in bronchial macrophage recruitment. Ongoing work is focused on evaluation of inflammatory endpoints including proinflammatory cytokine secretion, mitochondrial membrane potential disruption and inflammatory gene expression at short- and long-term timepoints.

Preliminary and ongoing data suggest an increased activation of inflammatory pathways due to the exposure. The in-depth characterization of such impact induced by the diverse real-life polymers tested will contribute to the knowledge base of MNPLs health risk.

Funding: This project has received funding from the Generalitat de Catalunya (2021-SGR-00731 and FI-SDUR 2025 scholarship), the ICREA-Academia program to A. Hernández (Ac2232418); and by the São Paulo Research Foundation (FAPESP, grant no. 2025/11879-0).

Unraveling the effects of real-life micro- and nanoplastics on the lung barrier using an ALI co-culture model

J. Gutiérrez-García[1], L. Pareras[1], G. M. Ferraz [2], S. Trischitta[3], R. Marcos[1], A. Hernández[1], & L. Rubio[1*]

[1] Group of Mutagenesis, Department of Genetics and Microbiology, Faculty of Biosciences, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona, Spain
[2] Environmental Biogeochemistry Laboratory, Department of Chemistry, Federal University of São Carlos, São Carlos, São Paulo, Brazil
[3] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale F. Stagno D'Alcontres, Messina, Italy
** Laura.Rubio@uab.cat*

Plastic materials undergo environmental weathering, leading to the formation of micro- and nanoplastics (MNPLs) that are widely dispersed and can enter living organisms through air, water, and food. Inhalation represents a major route of human exposure, yet interactions between MNPLs and the lung epithelium remain poorly understood. This underscores the need for advanced new approach methodologies (NAMs) that better recapitulate the complexity of the respiratory barrier.

In this study, a human-relevant *in vitro* lung model based on Calu-3 epithelial cells cultured under air-liquid interface (ALI) conditions and co-cultured with THP-1-derived macrophages is used to investigate the effects of nanoplastics on epithelial barrier function. ALI conditions promote epithelial differentiation, mucus production, and tight junction formation, closely resembling the human airway epithelium while enabling long-term exposure. In addition, the inclusion of THP-1-derived macrophages incorporates a key immune component, allowing evaluation of epithelial-immune interactions and inflammatory responses.

The established model is exposed to real-life MNPLs, including polyethylene terephthalate (PET), polylactic acid (PLA), and polytetrafluoroethylene (PTFE, Teflon), at two time points (24 hours and 1 week). Multiple endpoints are assessed, including barrier integrity (TEER), cellular internalization (confocal and TEM microscopy), mitochondrial function (Mitoprobe assay), oxidative stress (DCF assay), and genotoxicity (Comet assay). In addition, gene expression analysis by qPCR evaluates molecular responses related to inflammation, oxidative stress, and epithelial function.

Preliminary and ongoing results indicate that all tested real-life MNPLs are internalized, while no significant changes in barrier integrity are observed under the conditions tested. Early data suggest material-specific effects on mitochondrial function, with PET nanoplastics showing a greater impact at 24 hours compared to PLA and PTFE.

Overall, this approach enables comparative assessment of MNPLs with different physicochemical properties and supports the use of advanced co-culture systems as robust NAMs for studying inhalation toxicology.

Funding: This project has received funding from the Generalitat de Catalunya (2021-SGR-00731 and FI-SDUR 2025 scholarship), the ICREA-Academia program to A. Hernández (Ac2232418); and by the São Paulo Research Foundation (FAPESP, grant no. 2025/11879-0).

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June 10th - 12th, 2026
A Coruña (Spain)

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