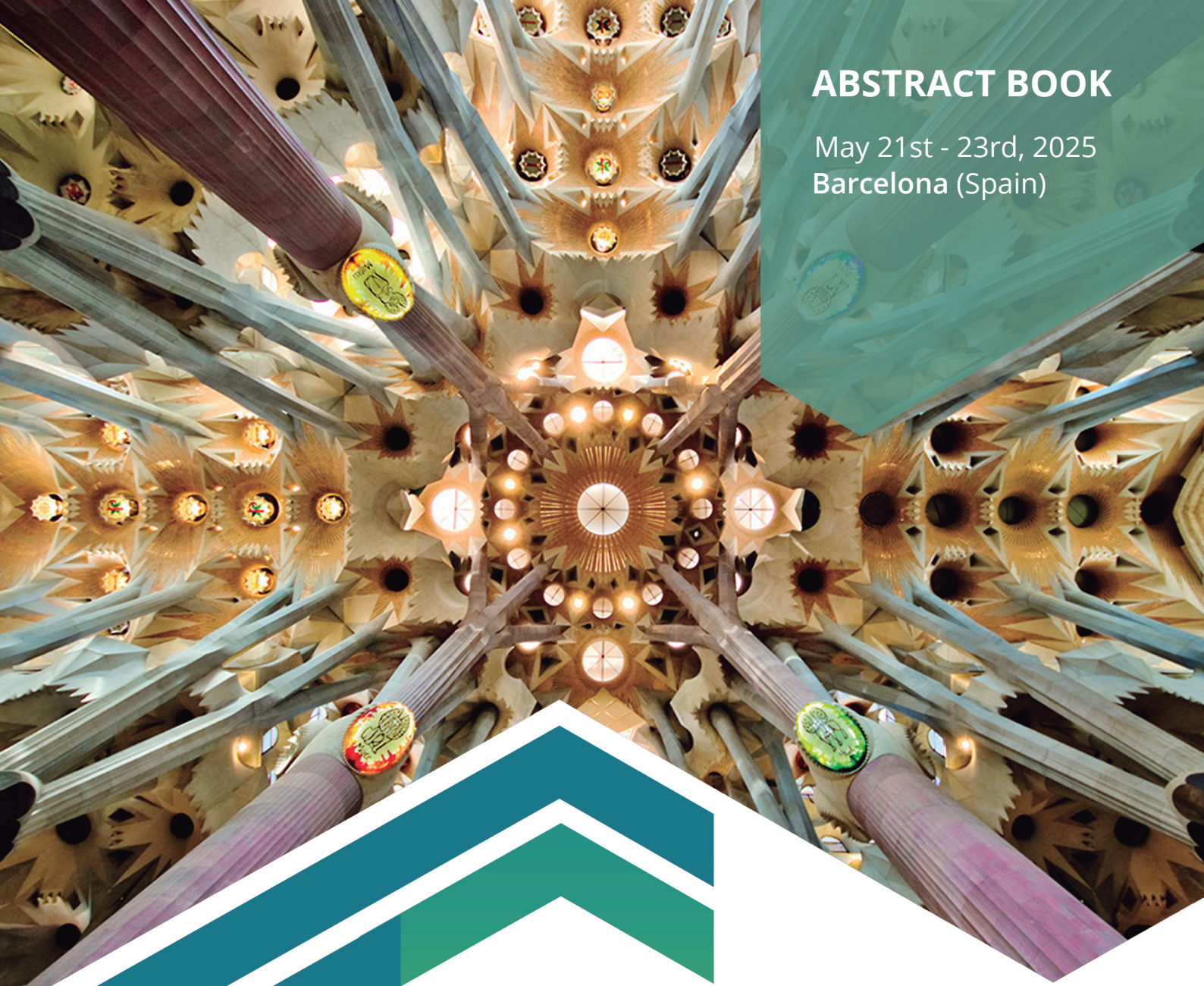

ABSTRACT BOOK

May 21st - 23rd, 2025
Barcelona (Spain)

SEMA | 2025

29th Spanish Environmental Mutagenesis and Genomics Society (**SEMA**) meeting

https://ojs.diffundit.com/index.php/sema/issue/view/95

sema
Sociedad Española de Mutagénesis y Genómica Ambiental

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16:00 – 16:15 Hannes Van Goethem (UAB – ES) **Studying the effects of nanoplastics in the gastrointestinal microbiota of *Mus musculus***

16:15 – 16:30 Zhou Keer (UAB – ES) **Studying the effects of Teflon® micro- and nanoparticles on the gastrointestinal microbiota**

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KEYNOTE LECTURES

Genome plasticity in three dimensions: exploring the dynamics of chromatin folding across time-scales and cell types

Aurora Ruiz-Herrera[1,2*]

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Studies examining the evolution of genomes have been mainly focused on sequence conservation. However, the inner working of a cell implies a tightly regulated crosstalk between complex gene networks, controlled by small dispersed regulatory elements of physically contacting DNA regions. How these different levels of chromatin organization crosstalk in different species underpins the potential for genome evolutionary plasticity. I will provide an overview on the evolution of chromatin organization, discussing general aspects of the mode and tempo of genome evolution to later explore the multiple layers of genome organization. We propose that both genome and chromosome size modulate patterns of chromatin folding and that chromatin interactions facilitate the formation of lineage-specific chromosomal reorganizations. Overall, analyzing the mechanistic forces involved in the maintenance of chromatin structure and function of germ line is critical for understanding genome evolution, maintenance, and inheritance.

Physiopathology of frailty in older adults: development of biomarkers for its early identification

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

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[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
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The ageing process, characterised by a progressive accumulation of a varied range of molecular and cellular alterations, is widely heterogeneous, and may lead to a large discrepancy between “chronological age” and “biological age”. In the pathway from robustness to disability and dependence related to ageing, frailty is an intermediate stage that has emerged as a more accurate measure of biological age. Frailty is a geriatric clinical syndrome encompassing multisystem age-associated physiological decline, reduced homeostatic reserves, and increased vulnerability to stressors, which increases the risk of negative health consequences such as falls, hospitalization, disability, dependency, and death. Although the pathophysiological mechanisms underlying frailty are not fully understood yet, it is known that frailty can be delayed, or even reversed, if detected in its early stages. Therefore, the development of biomarkers that allow the early detection of this syndrome, before the onset of its clinical manifestations, is crucial for implementing preventive actions and specialized geriatric care that improve the health and quality of life of older adults, as well as reduce associated social and healthcare costs. In this study, a set of parameters related to the ageing process or to age-related diseases were investigated in a population of older adults classified according to their frailty status, to determine their potential validity as biomarkers of frailty. The results obtained support the involvement of genomic instability, hypothalamic-pituitary-adrenal axis dysregulation, Th1-type immune activation and inflammageing in the pathophysiology of frailty, as important driving forces of this geriatric syndrome. Furthermore, certain parameters related to these processes have emerged as promising biomarkers of frailty and may be useful for its early identification.

Funding: This work was supported by the Spanish Ministry of Science and Innovation: MCIN/AEI/https://doi.org/10.13039/501100011033 [Grant PID2020-113788RB-I00], and Xunta de Galicia (ED431B 2022/16).

Chemical exposure in early-life: epigenetic mechanisms and genetic susceptibility

M. Bustamante[1,2,3*], H. Vespalcová[1,2,3], L. Balagué-Dobón[1,2,3], B. Knox[1,2,3], S. Aguilar[1,2,3], M. Cosin[1,2,3], A.K. Sakhi[4], C. Thomsen[4], P. Dadvand[1,2,3] & M. Vrijheid[1,2,3], on behalf of BiSC, HELIX and PACE researchers

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There is growing concern about the health effects of chemical exposure, particularly during early life, a critical period marked by organ development and immature detoxification systems. Using data from population-based birth cohorts, our studies aim to (i) investigate the epigenetic mechanisms underlying early-life exposure to toxic chemicals, and (ii) identify genetic variants involved in detoxification processes. This presentation focuses on phthalates, non-persistent chemicals widely used as plasticizers in consumer and industrial products.

Phthalate metabolites were quantified using mass spectrometry in pools of urine samples collected during pregnancy and childhood. Placental genome-wide DNA methylation and genetic variation were assessed using the Illumina EPIC and GSA arrays, respectively. Associations were examined using linear regression models adjusted for relevant covariates, and significant loci were annotated using various bioinformatics tools. In the Barcelona Life Study Cohort (BiSC, N=469 mother-child pairs), in utero phthalate exposure was associated with differential DNA methylation in the placenta, a key organ for foetal development. The affected pathways included immune and vascular regulation, sex hormone receptor response, and steroid biosynthesis, consistent with the known endocrine-disrupting properties of phthalates. Methylation patterns varied depending on the timing of exposure and the sex of the foetus. These analyses are currently being extended to blood tissue within the Pregnancy and Childhood Epigenetics (PACE) consortium. In relation to phthalate detoxification, we identified four genetic loci and two copy number variants associated with urinary phthalate levels and their metabolic ratios in children from the Human Early Life Exposome (HELIX) cohort (N=1,044). These variants mapped to genes involved in phase I and phase II metabolism and renal excretion.

Our findings suggest that prenatal phthalate exposure can alter the placental epigenome in a sex- and timing-specific manner and that genetic variants may influence individual detoxification capacity, with potential implications for child health.

Acknowledgements: BiSC, HELIX and PACE families

Funding: FP7/2007-206 no. 308333; H2020-EU.3.1.2. no. 874583; PI17/01225, PI17/01935, PI20/00190, funded by the ISCIII and co-funded by European Union (ERDF, “A way to make Europe”); JPI HDHL and ISCIII AC18/00006.

Exploiting C. elegans to assess Nanotechnology and nanomedicine

A. Laromaine

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Caenorhabditis elegans (*C. elegans*) is a transparent invertebrate worm that shares 60% genetic homology with humans, and it offers a platform for amenable experiments in various fields. In our group, we use this small organism as an *in vivo* metrology to reduce the number of higher animals used, comply with the 3R principles, and speed up the translation process of nanoparticles (NPs) or biopolymers to the market.

NPs have been suggested as promising as drugs, drug carriers, and therapies. Hundreds of NPs have been produced. However, the lack of time—and batch-efficient methods to evaluate NPs and processes prevents establishing general fundamental principles and impedes the progress of these future drugs and therapies unless high-throughput methods advance. Using this worm, we evidenced how nanoparticles' distinct chemical and structural properties could modulate their interaction with small organisms.

Biopolymers are also highly sought in nanomedicine since they offer a biocompatible platform for cell scaffolds, drug carriers, or tissue regeneration. We will present the evaluation of bacterial nanocellulose and focus on the worm's gastrointestinal tract, allowing us to elucidate lipid metabolism changes.

These simple experiments can potentially revolutionize the engineering of NPs and biopolymers by decreasing the time and cost effort required.

Under the Surface: how the cells and tissues from aquatic organisms respond to Polystyrene Nanoplastic Exposure

M. Garcia[1,2], I. Brandts[2], M. Teles[2], F. Chauvigné[1], J. Cerdà[1], P. De Oro-Carretero[3], J. Sanz-Landaluce[3], G. Pujol[1,2], A. Ruiz-Herrera [1], & N. Roher[1,2*]

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The interaction between polystyrene nanoplastics (PS-NPs) and the cellular machinery remains poorly understood, particularly regarding the effects of PS-NPs on different tissue/cell types in aquatic organisms. Our studies focus on understanding how cells interact with nanoplastics, how they process or manage these particles, and which cellular mechanisms are affected. We have specifically targeted macrophages, liver cells, intestinal cells, and gonad cells of relevant teleost species to explore these interactions across various biological contexts, including co-exposure scenarios with other prevalent aquatic pollutants such as phenanthrene. Disruption of cell and tissue biology by pollutants may represents a tremendous threat for aquatic life and in consequence for human health.

Acknowledgements: Dr. M. Costa and Dr. H. Montón from the central UAB scientific services (SCAC and SMiDRX)

Funding: Spanish Ministry of Science, European commission and AGAUR funds to NR (RTI2018-096957-B-C21 MINECO/FEDER & PID2021-126710OB-C21 and 2021-SGR-00068 (AGAUR)).

SESSIONS

01. Genomics, epigenetics and evolution

Studying the effects of nanoplastics in the gastrointestinal microbiota of *Mus musculus*

H. Van Goethem[1*], A. Rocabert[1], J. Cabrera[2], M. Alaraby[1], A. García-Rodríguez[1], J. Martín-Pérez[1], J. Martínez-Urtaza[2], C. Bussy[3], A. Kuttykattil[3], R. Marcos[1], & A. Hernández[1]

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The persistent nature of plastics in the environment has raised significant concerns about their potential impact on human health. A vast amount of studies have looked into the adverse effects of microplastics on various animal species, including humans, but little research has been done on the effects of even smaller plastic particles: nanoplastics. Previous studies have quantified the toxic effects of nanoplastics, mainly mediated by their small size which allows for the penetration of biological membranes, but none has been able to establish their effects on the gut microbiota. To assess the potential effects of nanoplastics on the human gut microbiota, this work investigates the effects of polystyrene (PS) and polyethylene terephthalate (PET) nanoplastics on the gut microbiota of *Mus musculus*, a model organism that is closely related to humans.

The main objective is to assess the short-term and long-term effects of *in vivo* nanoplastic particle exposure on the gastrointestinal tract microbiota of *Mus musculus.* To accomplish this, two different types of nanoplastic treatments at 2 µg/µl and at three different time points (1, 7 and 28 days) are assessed. The results analyses are performed using MinION Next Generation Sequencing techniques and subsequent bioinformatic analyses. The findings in this research contribute to a better understanding of the positive/adverse effects of nanoplastics on the gut microbiota of mice, and thus also humans, potentially opening up new research avenues for nanoplastic research in humans.

Funding: This project (PlasticHeal) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 965196, the Generalitat de Catalunya (2021-SGR-00731), the ICREA-Academia programme (Ac2232418) to A. Hernández, the Projectes Pre-Competitiu (PPC2024-36) of Universitat Autónoma de Barcelona to A. García-Rodríguez. H. Van Goethem is funded by the Spanish Ministry of Science, Innovation and Universities (PREP2023-000061). A. Rocabert is funded by the Generalitat de Catalunya (2023 FISDU 00288).

Studying the effects of Teflon® micro- and nanoparticles on the gastrointestinal microbiota

Z. Keer[1*], A. Rocabert[1], M. Alaraby[1], R. Egea[1], R. Marcos[1], A. García-Rodríguez[1], & A. Hernández[1]

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One of the most popular synthetic polymers nowadays is polytetrafluoroethylene (PTFE), also known by its brand name Teflon®, which is a fluoroplastic with excellent chemical stability and heat resistance widely used in non-stick cookware. Due to its vast industrial applications and its difficulty in environmental degradation, Teflon® may accumulate in the ecosystem as well as living organisms as micro- and/or nanoplastics (MNPLs). In recent years, the potential impact of plastic pollution on the gastrointestinal (GI) system has attracted attention, especially its effect on the intestinal microbiota. The human GI system is the most densely populated part of the body with microorganisms. The intestinal microbiota plays a key role in host nutrient metabolism, immune regulation, and maintaining intestinal homeostasis. Its imbalance is closely related to the development of several GI diseases such as irritable bowel syndrome and colon cancer.

Our research group has used *Drosophila melanogaster* as a model organism suitable to study the dynamics of GI microbiota. Because of its many advantages (i.e., short life cycle, known genetic background, easy to gene editing, similar intestinal structure and function as humans capable to host complex microbial communities, etc.) fruit flies are an ideal model for studying the effects of environmental pollutants on intestinal microbial communities.

Therefore, in the present study, *Drosophila melanogaster* was used to study the effects of particulate PTFE (50-100 nm and 1 μm) on the homeostasis of GI microbiota. Briefly, flies were fed with PTFE-MNPLs during 7 days at different concentrations (200, 400, 800 μg/mL). Later, flies were dissected under sterile conditions, the midgut extracted for the isolation of GI bacteria, and finally bacterial DNA was purified and sequenced using the MinION system (Oxford Nanopore Technologies). Through the analysis of microbial diversity, community composition, and predicted functional alterations, this study aims to shed light on how exposure to Teflon® nanoparticles impacts the gut microbiome and therefore the overall health of organisms.

Funding: This project (PlasticHeal) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 965196, the Spanish Ministry of Science, Innovation and Universities (PID2020-116789RB-C43), the Generalitat de Catalunya (2021-SGR-00731), the ICREA-Academia programme to A. Hernández (Ac2232418), the Projectes Pre-Competitiu (PPC2024-36) of Universitat Autónoma de Barcelona to A. García-Rodríguez.

Single-cell transcriptomics analysis of *ex vivo* nanoplastic exposed human whole blood as a model to understand their impact on human health

R. Egea[1*], J. Arribas[1], C. Pommerenke[2], R. Marcos[1], & A. Hernández[1]

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Micro/nanoplastics (MNPLs) have emerged as an environmental concern due to their extensive use and ubiquitous presence. Living organisms, including humans, are exposed to them through different routes, being ingestion and inhalation the major ones. Since MNPLs can cross both the intestinal and pulmonary barriers, their presence in the blood compartment is expected. This potential for systemic distribution raises significant concerns about their impact on human health. Consequently, understanding the interactions between MNPLs and human blood is of great value. In this study, human whole blood was exposed *ex vivo* to better mimic real conditions using five different MNPLs: three polystyrene NPLs of around 50 nm (aminated, carboxylated, and pristine forms), together with two real-life MNPLs from polyethylene terephthalate (PET) and polylactic acid (PLA) of around 150 nm. Single cell RNA sequencing (scRNA-seq) was performed and analysed over different blood cell types and treatments. A high number of differentially expressed genes (DEGs) were identified and values from different blood cell types and treatments were compared. A functional analysis of DEGs was performed and enriched pathways and terms were identified. Results were compared to previous biological assays results finding concordances among them. Results showed a broad response involving different molecular mechanisms. For the first time, the response to a variety of nanoplastic particles has been analysed at the single cell transcriptomics level in humans.

Funding: This project (PlasticHeal) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 965196, the Spanish Ministry of Science, Innovation and Universities with project code PID2020-116789RB-C43, the Generalitat de Catalunya (2021-SGR-00731), and the ICREA-Academia programme to A. Hernández.

SESSIONS

02. DNA damage and repair, genome instability and human desease

Base and nucleotide excision DNA repair mechanisms activity in equines: Insights into disease resistance, longevity, and lifestyle

I. Gaivão & A. Rita Guedes

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Horses doesn't develop internal organ cancers in opposite to humans and other animals, although rarely can develop sarcoid, melanoma (common in gray horses), and squamous cell carcinoma (often due to UV exposure). This apparent cancer resistance can be due to a combination of efficient DNA repair, strong immune defenses, tumor suppression mechanisms, and lifestyle factors. Base excision repair (BER) and nucleotide excision repair (NER) are the two main pathways of DNA excision repair. This work aims to infer the inter individual repair activity variability for BER and NER pathways in 20 horses aged between 3 and 23 years, using the comet-based *in vitro* DNA repair assay, our goal was also to determine whether horses exhibit higher repair capacity than the average reported in humans and to explore potential associations with factors such as gender, age, breed, and lifestyle. DNA repair activity was measured as incision activity (% of DNA in tail) using lymphocyte protein extracts. We found a considerable variability between animals, for both BER (6.06±9.28) and NER (4.72±8.09), although both values were lower than those typically reported in literature for healthy humans. Since the DNA repair capacity is a quantitative characteristic, we tried to find associations with several possible host factors effects. At large, results show that NER is significantly higher in young horses and BER is significantly higher in Lusitanos than in warmbloods. Horses with melanomas showed markedly low repair activity for both BER and NER, which is a very interesting result in terms of prognosis. We did not find differences between genders, stabled horses *vs* horses kept in pasture, or coat color (grey *vs* other coats). These preliminary results suggest that there are other factors contributing to the low cancer rates in horses, as tumor suppressor genes, immune system efficiency, and a low-inflammatory lifestyle.

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Expression of DNA repair enzyme APE1 in non-cultured and organ cultured corneal- and limbal epithelium

Yolanda Lorenzo[1*], Bjørn Otto Nicolaissen[1,3], Giang Nguyen[2], Kahsai Beraki[1], Morten C. Moe[1,2], Goran Petrovski[1,2], Dag Krohn-Hansen[1], & Bjørn Nicolaissen[1,2]

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Loss of corneal transparency observed when cellular and molecular defense mechanisms are overwhelmed by stressors is one of the leading causes of blindness worldwide. Treatments to restore vision include transplantation of full thickness or laminar corneal donor tissue. Before surgery, donor corneal tissue is maintained either in cold medium or in organ culture in an Eye Bank. To human tissues and cells, organ and cell culture systems are foreign environments, and transfer of cells to such systems may increase oxidation levels. Apurinic/apyrimidinic endonuclease 1 (APE1) is a protein involved in oxidative stress. Our aim is to examine distribution and expression of APE1 in non-cultured and organ cultured human limbal epithelium.

Samples of non-cultured and Eye-bank organ-cultured corneo-limbal tissue were processed for immunohistochemistry. By semiquantitative evaluation and using Fiji (Image J), distribution and intensity of reactions were examined. In control samples high density of staining for APE1 was seen in nuclei in basal layers in cornea (Superficial (S): 10.3, Intermediate (I): 17, Basal (B): 28.4) as well as in limbus (S: 6. I: 13.8, B:21.6), although nuclei in all layers showed varying degrees of positive reaction. The same gradient was observed in organ cultured samples where high-density staining was mostly observed in basal layers (S: 6.3, I:9.7, B:20.7). In non-cultured limbal samples as well as in organ cultured epithelium, a noticeable crowding of nuclei with high density staining for APE1 was observed in crypt-like formation.

Organ culture is used for storing tissue for transplantation and as a starting point for *ex vivo* production of epithelium. Our experiments show that epithelial cells retain the ability to express APE1 under conditions that are commonly used for such tissue storage/cultivation.

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Evaluating the suitability of the alkaline comet assay in HaCaT cells for predicting photogenotoxicity

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Assessing phototoxicity is crucial for drugs, cosmetics, and other chemicals that absorb UV-vis light, as these substances can initiate photosensitization reactions leading to direct or indirect DNA damage. Current established and validated methods, namely the in vitro 3T3 Neutral Red Uptake (NRU) and Reconstructed Human Epidermis tests, measure only photocytotoxicity (cell death) and are insufficient for detecting a compound's potential to cause photogenotoxicity. Furthermore, due to the limitations of animal models and recent international regulations emphasizing ethical considerations, there is an urgent need to develop *in vitro* assays specifically capable of identifying potential human safety hazards related to photogenotoxicity. This study aimed to establish a rapid *in vitro* method for assessing photogenotoxicity using the HaCaT human keratinocyte cell line. Six chemicals with well-known toxic properties —sodium dodecyl sulfate, chlorpromazine, benzophenone-3, 8-methoxypsoralen, *p*-phenylenediamine and chlorhexidine— were tested. Cells were incubated with the test compounds for 1 hour and irradiated with 4 J/cm² UV-A. After irradiation, treatment was removed and fresh medium was added. Cell viability was measured using the MTT and LDH assays, and an alkaline comet assay was performed both immediately following irradiation and 24 hours later at non-cytotoxic concentrations of the compounds and their vehicles, using a medium-high throughput format with 8 minigels per slide. Sodium dodecyl sulfate, *p*-phenylenediamine and chlorhexidine were classified as non-phototoxic, while the other tested chemicals showed PIF (Photo-Irritation Factor) values above 5 or MPE (Mean Photo Effect) values above 0.15. None of the chemicals showed increased DNA damage 24 hours after irradiation, likely due to DNA repair during this period. The study highlights the importance of assessment timing. Although further optimization is needed to reliably capture transient photogenotoxic effects, this HaCaT-based approach shows promise as a rapid, ethically-aligned method to contribute to the safety assessment of UV-exposed chemicals, addressing current gaps in photogenotoxicity detection.

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Can potassium bromate be used as a positive control in the *in vivo* Fpg-modified comet assay?

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The strategies for genotoxicity testing described by the ICH and EFSA guidelines, include the *in vivo* comet assay, but only in its standard version. This assay, which is covered by the OECD guideline (OECD TG 489), is applied in the detection of DNA strand breaks (SBs) and alkali-labile sites (ALS). However, the mechanistic insights are limited since SBs are nonspecific lesions that can result from various agents and often act as intermediates in the repair of premutagenic damage. On this matter, the comet assay has undergone several modifications in order to enable the detection of other DNA lesions. Among the most commonly applied modifications is the use of repair enzymes, such as formamidopyrimidine DNA glycosylase (Fpg), for the detection of oxidized lesions. By adding this slight modification to the OECD guideline, the information obtained from each animal will increase considerably.

This study aims to evaluate the potential use of potassium bromate ($KBrO_3$) as positive control for the *in vivo* Fpg-modified comet assay. For this purpose, Wistar rats were given two oral doses of $KBrO_3$ (0, 100, 200, and 300 mg/kg bw) or a single dose of 300 mg/kg bw of EMS as the standard positive control. Three hours after the last administration, liver, duodenum, kidney, brain, and whole blood samples were collected and analysed. Histopathology samples were also gathered for further examination.

Results showed an increase in net Fpg-sensitive sites in liver, duodenum, kidney and whole blood, both for $KBrO_3$ and EMS. In addition, an increase in Fpg-sensitive sites was also observed in the brains of the animals administered with EMS. Regarding DNA SBs and ALS, an increase was detected only in the animals administered with EMS, observed in all organs except for the whole blood.

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DNA damage response inhibitors targeting synthetic lethal interactions for cancer treatment

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Targeting synthetic lethal interactions has emerged as a promising strategy for cancer therapeutics, particularly by exploiting DNA damage response (DDR) pathways. A well-known example of this are PARP inhibitors (PARPi), that selectively kill homologous recombination-deficient (HRD) tumors, especially those harboring BRCA1/2 mutations. PARPi have been approved for breast, ovarian, prostate, pancreatic and endometrial cancer. Clinical studies have demonstrated that these inhibitors can extend progression-free survival in patients with these tumors alone or in combination with other treatments like chemotherapy or immunotherapy. Additionally, ongoing research is exploring their efficacy in other cancer types and in patients harboring mutations other than BRCA1/2, potentially broadening their therapeutic applications. Several other drugs inhibiting key DDR components, such as ATR, ATM and DNA-PK, have already progressed to clinical trials. Continued research is essential to fully understand the intricate network of synthetic lethality within DDR and maximize the therapeutic potential of these therapies. Another promising DDR pathway that can be targeted for cancer is the Fanconi anemia/BRCA (FA/BRCA) pathway, responsible for the repair of DNA interstrand cross-links. A key event in this pathway is the monoubiquitylation of the FANCD2-FANCI complex. With the goal to identify a FA/BRCA pathway inhibitor we have performed two high-content virtual screenings that have led to the identification of several compounds that have been further optimized obtaining several hits.

SESSIONS

03. Health hazard assessment of emerging pollutants

Genotoxic characterization of emerging mycotoxins *in vitro*

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Among the many contaminants affecting human and animal health, mycotoxins (MTX) are of special concern as they naturally contaminate food and feed. MTX cause toxic effects, being of particular interest their potential genotoxicity and/or carcinogenicity due to continuous, lifelong intake, even at low doses. To mitigate these risks, maximum concentration levels for certain MTX in specific matrices have been established. However, most identified MTX remain uncharacterized. A bottleneck in MTX mutagenicity testing is the limited commercial availability and high cost of some of them. Moreover, a considerable amount of high purity is required to carry out standardized mutagenicity assays like the Ames test (OECD Test 471).

The SOS/umu test is a medium-throughput assay requiring a small amount of substance while keeping high concordance with the Ames test results. It determines genetic damage in *Salmonella typhimurium* through a colorimetric reaction. The assay is done in the presence or absence of external metabolic activation, and up to six substances can be tested simultaneously without extending the experimental time—unlike the Ames test, where testing multiple substances significantly increases the workload. Therefore, the SOS/umu test serves as a screening tool and even as a first step in genotoxicity testing.

In this work, 20 under-evaluated, not regulated MTX were tested in the SOS/umu test. Nine MTX had published data on the Ames test, and their results in the SOS/umu test were in line with the available bibliography. Genotoxicity testing on bacteria has been performed for the first time for 11 MTX. Two MTX, aflatoxicol and kojic acid, were positive in presence of metabolic activation; while one, o-methylsterigmatocystin, was a weak positive with presence of metabolic activation. The remaining MTX gave negative results in all conditions: 3-nitropropionic acid, andrastin A, apicidin, asperglaucide, asperphenamate, aurofusarin, averantin, averufin, bikaverin, butanolide, cyclo-(L-Pro-L-Tyr), cyclo-(L-Pro-L-Val), cyclopiazonic acid, fusaric acid, mycophenolic acid, skyrin and tryptophol.

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Biocompatibility and antioxidant potential of nanoceria in human nervous cells

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Cerium dioxide nanoparticles (CeO_2 NP), also known as nanoceria, have emerged as promising materials in biomedical and pharmacological applications due to their unique redox properties and antioxidant capacity. Due to these and other specific characteristics, they have raised high attention for their potential use in drug delivery, radioprotection, tissue regeneration or diagnostic imaging, particularly in neurological pathologies associated with oxidative stress. However, previous works have reported that nanoceria may also induce reactive oxygen species (ROS) production under certain conditions, leading to oxidative stress, cellular damage and cell death. This study aimed to investigate the CeO_2 NP effects on cell viability and morphology, as well as their influence on oxidative stress (both oxidizing and ROS scavenging activity) in human nervous cells (SH-SY5Y neurons and A172 glial cells) treated with different CeO_2 NP concentrations (1–100 µg/mL) for 3, 24 and 48 h. Results obtained showed that CeO_2 NP exhibited good biocompatibility, as there was no significant decrease in cell viability, morphological alterations or intrinsic acellular ROS production at any of the conditions tested, despite being stable over time and effectively internalized by both cell types. However, a significant increase in cellular ROS (mainly limited to the longest exposure period) and a slight induction of oxidative DNA damage (limited to the highest concentration after 3 h) was observed, predominantly affecting SH-SY5Y cells. Notably, nanoceria demonstrated a remarkable intrinsic capacity to scavenge H_2O_2-generated ROS, showcasing their antioxidant properties, more pronounced in neuronal cells. In conclusion, this study confirms the biocompatibility of CeO_2 NP within human nervous system cells and highlights their potential as therapeutic agents in neuroprotective strategies against oxidative stress-induced damage. These findings warrant further investigation into the applications of nanoceria in medical fields, especially for treating neurodegenerative diseases and as diagnostic tools in neurology.

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Biological impact of the sweetener sucralose – an in *vivo* study in *Drosophila melanogaster*

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Sucralose is one of the most widely used sugar substitutes in the world, being almost sweeter than sugar. Sucralose is often used in beverages together with sugar to increase their sweetness. However, being a chlororganic compound, sucralose is potentially genotoxic and spermotoxic. For this reason, the investigation of the impact of both substances on the genome is truly up to date, being *Drosophila melanogaster (D. melanogaster)* a suitable model for the study of the sucralose-sugar diet impact. The objectives of this study were to analyse the effects of a diet with the addition of sucralose-sugar mixtures, including behavioural alterations and DNA damage in *D. melanogaster*. The effects on longevity (average and maximum), negative geotaxis, spatial exploration, and genotoxicity (basal DNA damage) were evaluated. Young males (0 to 2 days old) were divided into 2 groups; group one, with sucrose concentrations of 0, 5, 10, 15 and 20%, and group two with sugar concentrations at 10% (control) and additionally sucralose at concentrations of 0.25, 0.5, 1 and 2%, placed on this diet for 72 h. The results were analysed in the F_1, where, compared to the control group, diets with 15% and 20% sucrose showed a 17% decline in average longevity (114 *vs* 95 days), impact on locomotion with an 8% increase in displacement, a 9% decline in exploration (43 *vs* 39 cm^2) and a 47% decrease in the number of descendants (277 *vs* 146 descendants). Concentrations of 15% and 20% sucrose showed a significant increase in DNA damage with percentages of DNA in tail of 39.5% (±3.29) and 42.5% (±2.35), respectively, compared to the control group value of 6.5% (± 0.47).

The data suggest significant influence of increased sucralose-sugar consumption on *D. melanogaster*, affecting fertility, survival and genetic damage.

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In vitro bioassays as a tool to evaluate risk assessment of micro and nanoplastics

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The concern regarding micro and nanoplastics (MNPs) has significantly increased as they can be present in food, water and environment. Once the particles enter the food chain, they can cross the biological barriers, as well as cell membranes, leading to different molecular effects.

To conduct a robust risk assessment of MNPs, two key challenges must be addressed: the **availability of well-characterized MNPs standards**, and the **establishment of a standardized battery of bioassays** to assess their effects across different cellular levels. Non-animal approaches, such as in vitro bioassays based on cell cultures, are especially relevant for evaluating complex mixtures of low-level contaminants. For particulate substances, it is essential to consider properties such as surface chemistry, impurities, dissolution rate, and stability under biological conditions.

The aim of the study is to develop standards of micro and nanoplastics to be used, first of all, in an in vitro gastrointestinal digestion to evaluate their stability and dissolution under biological conditions and, finally, to assess cytotoxicity (Alamar Blue), oxidative stress and genotoxicity (Micronucleus and Comet assay) in different cell lines.

The results showed no cytotoxic effects or induction of reactive oxygen species. However, certain plastic particles induced genotoxic responses in specific conditions, highlighting the need for further investigation. These findings underscore the importance of using well-characterized reference materials and standardized in vitro methods for hazard assessment.

In conclusion, this study reinforces the necessity of developing harmonized protocols for the preparation and testing of MNPs to generate reproducible data and ensure consumer safety.

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Intestinal impact of PTFE-derived micro- and nanoplastics

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The increasing prevalence of micro- and nanoplastics (MNPLs) due to widespread plastic use poses a potential health threat, particularly via ingestion. Polytetrafluoroethylene (PTFE) MNPLs are a significant source of human exposure, yet their biological impacts remain underexplored.

The aim of this project is to assess the potential intestinal hazards posed by MNPLs released from PTFE using human-relevant in vitro intestinal models. Two PTFE-MNPL types- heterogeneous (HT) and homogeneous (HO)- were characterized and tested on undifferentiated and differentiated Caco-2/HT29MTX cell co-cultures. Cells were exposed to 50, 100, and 200 µg/mL of each MNPL type for 24 and 48 hours. Transmission electron microscopy (TEM) and confocal microscopy were used to analyze particle uptake and localization. Toxicity was evaluated through cell viability assays, ROS induction, IL-8 secretion, mitochondrial membrane potential, membrane integrity, and genotoxicity assessments.

Fourier Transform Infrared (FTIR) confirmed that both MNPL types shared identical functional groups despite their distinct morphology. Both PTFE-MNPL types exhibited cellular internalization without disrupting membrane integrity or barrier permeability. However, dose-independent oxidative stress and DNA damage were observed, with PTFE(HO)-MNPLs inducing stronger oxidative responses at higher concentrations.

In conclusion, PTFE-derived MNPLs, while not overtly cytotoxic, can cross intestinal barriers and induce oxidative and genotoxic stress. These findings raise concerns about long-term health impacts associated with chronic exposure to MNPLs from consumer products like non-stick cookware.

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Size-dependent effects of polystyrene nanoplastics on human primary endothelial cell function

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Micro- and nanoplastics (MNPLs) have emerged as pervasive environmental pollutants, raising growing concerns about their potential health risks. Their documented presence in the human bloodstream highlights the need to understand their interactions with endothelial cells and their role in cardiovascular diseases such as atherosclerosis. However, the full implications of these interactions remain largely unknown. To address this, we investigated the impact of three sizes (30, 50, and 100 nm) of polystyrene carboxylated nanoplastics (PS-C-NPLs) on human umbilical vein endothelial cells (HUVECs) at 100 µg/mL concentrations. By integrating *in vitro* assays with bioinformatic transcriptomic analyses, we employed a multimodal approach to comprehensively investigate the effects of PS-C-NPLs on endothelial cells. *In vitro* assessments included nanoplastics (NPLs) internalisation (flow cytometry, confocal microscopy, TEM), morphological and internal complexity changes (flow cytometry), genotoxicity (comet assay), and functional alterations, such as cholesterol biosynthesis (Filipin III staining), migration (wound healing assay), angiogenesis, and inflammation (IL-6 ELISA). Additionally, RNA sequencing provided transcriptomic insights into the cellular response to PS-C-NPLs, complementing the *in vitro* findings and revealing molecular pathways underlying the observed effects. All three PS-C-NPL sizes were rapidly internalised by HUVECs within 20 minutes, inducing significant alterations in cell morphology, internal complexity, and function. PS-C-NPLs caused genotoxic damage and disrupted cholesterol metabolism, migration, angiogenesis, and inflammatory responses. Notably, some effects exhibited a size-dependent trend, with similarities emerging between carboxylated polystyrene (PS-C) 50 and 100 nm NPLs, while the smallest 30 nm NPL showed slightly distinct responses. Transcriptomic analyses reinforced these findings, revealing shared pathways across all three PS-C-NPLs — linked to cholesterol metabolism, endothelial-to-mesenchymal transition, DNA damage, and inflammation — alongside size-specific molecular signatures. This study is the first to comprehensively link transcriptomic changes to size-dependent functional alterations in endothelial cells induced by PS-C-NPLs. Our findings demonstrate that PS-C-NPLs significantly impair endothelial cell function and integrity in a size-dependent manner, underscoring their potential cardiovascular risks.

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SESSIONS

04. Human biomonitoring I

Inflammation and frailty: association of immunological biomarkers with physical and cognitive impairment in older adults

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Frailty is a clinical condition coined to describe the heterogeneity in health status among older individuals of the same chronological age, characterized by loss of physiological and cognitive reserves and increased susceptibility to adverse outcomes. Arising from a multifactorial and complex aetiology, it involves cumulative damage across physiological systems, progressively reducing the body's capacity to preserve homeostasis. Physical frailty is commonly assessed using the phenotype model established by Fried, which relies on five clinical criteria. An alternative approach, the Frailty Index (FI), offers a broader evaluation based on the accumulation of health deficits, encompassing clinical signs, functional limitations, and cognitive decline. In addition, the concept of cognitive frailty has emerged to describe the simultaneous presence of physical frailty and mild cognitive impairment, in the absence of overt dementia, representing a state of increased vulnerability to neurodegenerative processes. The immune system is suspected to play a fundamental role in the development of both physical and cognitive frailty. Thus, the aim of this study was to explore the potential association between immunological biomarkers and frailty status considering physical and cognitive components. To this end, a cross-sectional study was conducted involving 155 Spanish older adults (aged 65 and above). Frailty was evaluated according to the phenotype criteria and the FI, cognitive impairment was assessed using the Montreal Cognitive Assessment (MoCA) test, and circulating levels of the inflammatory markers IL-6, CRP, TNFα, sTNF-RII, GDF-15, and HTRA1 were determined. The results demonstrated significantly higher levels of CRP, TNFα, sTNF-RII, and GDF-15in the frail group compared to the non-frail group (both for phenotype frailty and FI). Regarding cognitive frailty, significant associations were found for all biomarkers, except for IL-6. Additional studies with a wider range of immunological biomarkers are essential to gain deeper insight into their contribution to both physical and cognitive frailty development.

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Analysis of DNA damage by e-cigarettes exposure in a youth population

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The global consumption of electronic cigarettes has significantly increased, particularly among the youth population, while the genetic damage associated with their use remains poorly understood. Consequently, this study aimed to evaluate the genotoxic effects and susceptibility related to the consumption of both electronic and conventional cigarettes.

A total of 156 university students were categorized into control, vaper, and smoker groups. Serum cotinine levels were measured as a biomarker for nicotine exposure. DNA damage was assessed using the comet assay (measuring the percentage of tail DNA and tail length) and a competitive ELISA for detecting 8-hydroxy-2'-deoxyguanosine (8-OHdG). The variant rs16969968 (G>A) of the *CHRNA5* gene was identified, which is associated with increased susceptibility to nicotine consumption frequency. Null genotypes of *GSTM1* and *GSTT1*, which are linked to reduced xenobiotic detoxification, were also evaluated.

Cotinine levels confirmed the classification of groups. The percentage of tail DNA and tail length were significantly higher in vapers (7.33 ± 2.01 and 23.09 ± 7.98) and smokers (7.65 ± 2.67 and 24.59 ± 11.03) compared to controls (4.64 ± 1.79 and 14.00 ± 5.88). The 8-OHdG levels were elevated in vapers (124.66 ± 53.86) and smokers (114.05 ± 40.86) compared to controls (96.51 ± 42.14). The *CHRNA5* polymorphism showed a tendency toward higher cigarette/puff consumption. Additionally, the null genotypes of *GSTM1* and *GSTT1* were associated with increased tail length and 8-OHdG levels, respectively.

These findings suggest that electronic cigarettes induce clastogenic damage to DNA and that certain genetic variants may increase individual susceptibility. Further research is needed to understand their impact better and inform policies countering the perception of e-cigarettes as harmless.

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Integrating microbiota profiling in biomonitoring of occupational exposure to micro- and nanoplastics

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The widespread generation of micro- and nanoplastics (MNPLs) during industrial and manufacturing processes has raised concerns about their potential impact on human health, particularly regarding chronic occupational exposure. While research has primarily focused on the toxicological effects of MNPLs on respiratory and systemic health, their influence on the human microbiota remains largely unexplored. Therefore, this study aims to assess the gut microbiota composition of workers potentially exposed to MNPLs in occupational environments, using high-throughput sequencing techniques.

A cohort of exposed workers from various industrial sectors was recruited, alongside a matched control group with no known occupational MNPL exposure. Fecal samples were collected to evaluate microbial diversity and community composition through 16S rRNA nanopore sequencing, followed by bioinformatics analyses to identify potential dysbiosis. Preliminary data suggests that alpha and beta diversity metrics might significantly shift in microbial diversity between control and exposed group. The observed microbial imbalances suggest that chronic MNPL exposure may contribute to microbiome dysregulation, potentially influencing immune responses, metabolic health, and systemic inflammation. These findings highlight the importance of including microbiome analysis in occupational health risk assessments.

This study underscores the need for further research on MNPL-microbiota interactions and the development of preventive measures for workers in high-risk environments. Future investigations should explore the mechanistic pathways linking MNPL exposure to microbiome-associated health outcomes, as well as the potential for microbiota-based biomarkers in biomonitoring strategies.

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SESSIONS

05. New approach methodologies in toxicity and risk assessment

In vitro evaluation of genetic instability and cellular transformation capacity as potential biomarkers of non-genotoxic carcinogens

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Non-genotoxic carcinogens (NGTxCs) present a major challenge in chemical risk assessment because they do not directly damage DNA, making them undetectable by standard genotoxicity testing strategies. Unlike genotoxic carcinogens, NGTxCs act through less well-understood mechanisms and are currently identified mainly through long-term *in vivo* carcinogenicity studies. These bioassays typically require two years of exposure, large numbers of animals, and significant financial resources. Moreover, they raise ethical concerns and are considered to have limited human relevance. 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 identifying NGTxCs.

In our laboratory, we aim to test various methods, including the standard and enzyme-modified comet assay, as well as two versions of the Cell Transformation Assay (CTA).

In a first phase, 8 compounds classified as either non-carcinogens or NGTxCs were tested using both standard and enzyme-modified comet assays in 2D and 3D HepG2 models, after short and long exposures. All results were negative under the tested conditions. A second phase was launched with 13 additional compounds representing diverse carcinogenic mechanisms. This phase was performed using a 2D HepG2 model and the Fpg-modified comet assay after short exposure. Among the 13 compounds, only potassium bromate produced a clear dose-response when using Fpg. New experiments are currently being conducted on agents associated with oxidative stress, employing different experimental designs.

As part of this project, we have implemented the CTA following the corresponding OECD guidance. This method assesses carcinogenic potential through both initiation and promotion stages. We are also implementing a high-throughput, soft agar-based CTA, which detects anchorage-independent cell growth. Using both assays, we will analyze a selection of compounds.

So far, our results suggest that the comet assay, in both its standard and enzyme-modified versions, is not able to detect NGTxCs under the tested conditions.

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Long-term exposure to secondary polyethylene terephthalate nanoplastics induces carcinogenesis *in vitro*

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Micro-/nanoplastics (MNPLs) are environmental contaminants originated mainly from plastic waste degradation that pose potential health risks. Inhalation is a major exposure route, as evidenced by their detection in human lungs, with polyethylene terephthalate (PET) among the most abundant particles in respiratory airways. However, the harmful effects of particle bioaccumulation remain unclear, as chronic effects are understudied. To assess long-term effects, specifically carcinogenic effects, BEAS-2B cells were exposed to PET-NPLs for 30 weeks. Genotoxicity, carcinogenic phenotypic hallmarks, and a panel of genes and pathways associated with cell transformation and lung cancer were examined and compared across three exposure durations. No significant effects were observed after 24 hours or 15 weeks of exposure. However, 30-week exposure led to increased genotoxic damage, anchorage-independent growth, and invasive potential. Transcriptomic analysis showed upregulation of several oncogenes and lung cancer-associated genes at the end of the exposure. Further analysis revealed an increase in differentially expressed genes over time and a temporal gradient of lung cancer-related genes. Altogether, the data suggest PET-NPLs' potential carcinogenicity after extended exposure, highlighting serious long-term health risks of MNPLs. Assessing their carcinogenic risk under long-term chronic, real-life conditions is crucial to address knowledge gaps and eventually develop preventive policies.

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Stem cell models to study long-term effects of PS and PET nanoplastics: a focus on cell transformation

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The wide diversity of human exposures across individuals and throughout their lifetime calls for new approaches and models to implement risk assessment strategies. Stem cells provide a unique possibility to study specific functionalities of immature cells such as cell fate and cell transformation. Due to their long lifespans, stem cells can be particularly susceptible to long-term exposures and the accumulation of abnormalities could lead to a differential impact compared to short-lived cells, including the emergence of cancer stem cells. Therefore, we propose stem cells as a relevant model for the evaluation of the potential impact of micro- and nanoplastics (MNPLs) on human health. The population is continuously exposed to these small plastic particles that can translocate through physiological barriers and cause cytotoxicity, oxidative stress, DNA damage, or inflammation. The information regarding the bioaccumulation of MNPLs is still limited but the extended exposure is expected to induce accumulative adverse effects such as mutagenesis and carcinogenesis, aspects insufficiently explored until now.

Here we present a comparative study of the long-term effects of polystyrene (PS) and polyethylene terephthalate (PET) nanoplastics on stem cells. We continuously exposed mammary stem cells (MCF10A) to both nanoplastics for 20 weeks. Using a battery recognized assays, we characterized the stemness status, transformed phenotype and molecular changes induced by the exposure. Results show that, while the stem functionalities of the cells are not altered, both PS and PET trigger comparable pre-neoplastic phenotypes. Furthermore, transcriptomic analysis has shown that the mechanism behind the cell transformation process is different for each type of nanoplastic.

This work underscores the interest of incorporating stem cells in risk assessment strategies, providing relevant data on the impact of MNPLs of cell transformation and highlighting the need of understanding which mechanisms determine the cellular response to long-term exposures.

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Establishment of a human pulmonary *in vitro* co-culture model for nanoplastic toxicological assessment: Calu-3 and THP-1 macrophages

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Plastic can be degraded with weathering into micro and nanoplastics (MNPLs) and be spread worldwide, ultimately reaching living organisms through air, water, and food sources. Apart from ingestion and dermal absorption, inhalation is one of the main routes of human exposure to MNPLs. Despite its particular concern, limited information has been collected regarding the interaction of MNPLs with the lung epithelium, leaving critical gaps in our understanding of their potential effects on respiratory health. Therefore, there is an urgent need to develop new approach methodologies (NAMs) capable of better mimicking human epithelia *in vitro* as a model to test MNPLs. Calu-3 is a lung adenocarcinoma cell line that can be cultured under air-liquid interface (ALI) conditions, exhibiting respiratory tract features that remain stable for extended periods. However, airway epithelial cells do not function in isolation, the immune system is also present in the lungs. To enhance the reliability of this model assessing MNPLs pulmonary effects, a co-culture of Calu-3 cells with THP-1 macrophages are established under ALI conditions. To test the new model robustness several endpoints were assessed, including the capability of generating stable tight junctions, barrier permeability, microvilli formation, macrophage-epithelial cell interaction and cytokine release. Following the standardization of the model, it was used to assess the effects of MNPLs on the barrier after two weeks of repeated exposures. Preliminary results demonstrated the impact of polyethylene terephthalate nanoplastics (PET-NPLs) on the barrier, highlighting the coculture model as a reliable NAM for testing the effects of repeated MNPL exposures on the lung epithelium.

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SESSIONS

06. Ecotoxicity and reproductive toxicity

Quality evaluation and review of *in vivo* mammalian reproductive and developmental toxicity studies on micro- and nanoplastics

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Despite increasing evidence of human exposure to micro- and nanoplastics (MNPLs), their potential impact on reproductive and developmental health remains poorly understood. Studies have shown systemic translocation of MNPLs within organisms, including accumulation in reproductive organs, raising concerns among regulators and researchers. However, existing toxicity data vary in quality and regulatory relevance. This study aims to systematically evaluate current evidence on the effects of MNPLs on reproductive and fetal health to address these gaps.

We pursued two main objectives. First, we conducted a systematic review of *in vivo* mammalian studies assessing MNPLs effects on reproductive and developmental health. Second, we evaluated the quality of these studies in terms of Reliability and Relevance for regulatory purposes, using the Science in Risk Assessment and Policy (SciRAP) *in vivo* tool (version 2.3). To our knowledge, this is the first systematic application of SciRAP to this topic.

A total of 102 studies were included, categorized into three themes: Biodistribution, Reproductive System, and Fertility and Development. We found evidence of MNPLs accumulation in reproductive organs, leading to impairments in testicular, ovarian, and uterine functions. Both the quantity and quality of male and female gametes were reduced. Evidence also indicated maternal transfer via the placenta and breastfeeding, negatively affecting fetal development and offspring health.

Quality assessment revealed frequent gaps in reporting, particularly in dose and particle characterization. Since SciRAP was designed for chemicals, it does not fully address MNPLs-specific aspects. To adapt it, we included additional parameters such as polymer type, particle size, and shape under the tool's "Other" criterion and introduced a "Non-evaluable" option in the Relevance evaluation.

In conclusion, while several studies indicate potential reproductive and developmental toxicity of MNPLs, methodological limitations and inconsistent reporting hinder interpretation and weight-of-evidence assessments. Instead of calling for more studies, we emphasize improving study quality to strengthen the evidence base and support informed regulatory decisions.

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The impact of nanoplastics on mammalian reproductive function: an *in vitro* study with gametes, embryos and placental cells

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Plastic pollution is a growing global environmental challenge. In the environment, plastics break down into massive amounts of microplastics (MPLs, <5 mm) and nanoplastics (NPLs, <1 μm). Their widespread distribution, persistence, and minute size facilitate their uptake by living organisms, raising concerns about their potential harmful effects on ecosystems, wildlife, and human health. Given that reproductive function is highly susceptible to pollutants, exposure to MPLs and NPLs may negatively impact the fertility of current individuals and pose risks to future generations.

To elucidate the impact of NPLs on various mammalian reproductive cellular components, we exposed CD-1 mouse gametes and preimplantation embryos and human placental cells (JEG-3 and BeWo) to polystyrene NPLs *in vitro* (100 nm; 100 μg/ml). We found that NPLs adhered to sperm plasma membranes, leading to a significant reduction in motility and membrane integrity and compromising acrosome reaction. However, no significant increases in oxidative stress or DNA fragmentation were observed. NPLs also attached to the zona pellucida of oocytes and embryos, but their slow internalization prevented adverse effects on oocyte maturation and embryonic development, with oxidative stress levels remaining stable. Notably, rapid NPLs internalization occurred in zona pellucida-free embryos, triggering toxicity characterized by increased oxidative stress, impaired embryonic development, and diminished blastocyst quality. Placental cells rapidly trafficked NPLs to lysosomes. NPLs internalization did not disturb metabolic efficiency, but had a genotoxic effect, resulting in an increased frequency of DNA double-strand breaks and micronuclei in both cell lines.

In conclusion, our results indicate that gametes, embryos and placental cells are vulnerable to NPLs exposure, although the zona pellucida acts as a crucial protective barrier for oocytes and embryos. These findings emphasize the potential threats of plastic pollution to reproductive health and highlight the need for further research into the long-term consequences of MNPLs exposure on fertility and embryo-fetal development.

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Reproductive toxicity of nanomaterials. Silver nanoparticles and *Drosophila* as models

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Reproductive toxicity is a significant concern among the harmful effects induced by environmental pollutants. To reduce the use of mammalian models, lower eukaryotes like *Drosophila melanogaster* serve as viable alternatives. This study addresses the gap in understanding the link between reproductive adverse outcomes and the physical presence of pollutants in reproductive organs.

Silver nanoparticles (AgNPs) were selected for their ease of internalization, detection, quantification, and widespread environmental presence due to diverse applications. A novel exposure method was developed in which adult flies were fed peach-grape juice via sponge plugs in rearing tubes. Both male and female flies were exposed to AgNPs (28±4 nm, 100 and 400 µg/mL) for one week. Internalization and bioaccumulation of AgNPs in organs were assessed using transmission electron microscopy (TEM), confocal microscopy, and inductively coupled plasma mass spectrometry (ICP-MS). Results showed substantial accumulation of AgNPs in the gastrointestinal tract, Malpighian tubules, hemolymph, reproductive organs (ovaries and testes), and gametes (eggs and sperm). The highest AgNPs content was observed in the testes. AgNPs were also detected in eggs, indicating transgenerational transfer. Exposure to AgNPs reduced ovary size and fecundity, particularly at higher concentrations, though fertility and gender ratios of the offspring were unaffected. At the molecular level, significant deregulation of reproductive-related genes was observed, particularly in males.

These findings underscore the utility of *D. melanogaster* as a model for evaluating reproductive hazards posed by AgNPs exposure. The ease of AgNPs internalization in *D. melanogaster* reproductive targets suggests potential implications for mammalian reproductive toxicity, raising concerns about the broader impacts of nanoparticle exposure.

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SESSIONS

07. Human biomonitoring II

Biomonitoring of human population exposed to micro- and nanoplastics in clear aligner orthodontic treatment. Novel approach using MinION sequencing to detect effects on the oral microbiota

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Clear aligners (CAs) are a widely used therapeutic option for the treatment of dental malocclusion. These appliances are manufactured from various thermoformed materials, which are susceptible to degradation into micro- and nanoplastics (MNPLs), particularly in the harsh environment of the oral cavity. Contributing factors include pH fluctuations, temperature changes, mechanical attrition, and the presence of saliva, among others. The use of CAs and its associated MNPLs release may influence oral bacterial communities and potentially impact oral health. Currently, only a limited number of studies have investigated the oral microbiota using the MinION device (*Oxford Nanopore Technologies*), and none have done so within the specific context of CA treatment and MNPLs exposure.

The objective of our study is to assess the changes in the oral microbiota of individuals undergoing CA orthodontic treatment, as well as after treatment completion, in order to assess the reversibility of any observed changes. Understanding how CAs affect oral microbial communities will help clarify their potential role in the development of caries and periodontal disease. To address this, we are conducting a prospective longitudinal study.

At present, we have fully developed and optimized a protocol to extract bacterial DNA from buccal swabs using PureLink Microbiome DNA Purification Kit (*Invitrogen, Thermo Fisher Scientific),* followed by sequencing with the MinION system. All samples have been successfully sequenced, confirming the reliability of MinION for detecting and characterizing microbial changes in the oral microbiota.

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Assessment of micro- and nanoplastic exposure risks using clear dental aligners as a model and salivary white blood cells as biomarkers

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One of the most pressing environmental and public health challenges today is the continuous exposure of living organisms to micro- and nanoplastics (MNPLs). Due to their widespread presence, human contact with these particles is virtually unavoidable. The lack of regulation—largely stemming from limited knowledge about their effects—makes human biomonitoring studies crucial for assessing the risks associated with MNPL exposure.

To contribute to this understanding, the Mutagenesis Group is carring out a project involving individuals with continuous and direct plastic exposure: patients undergoing invisible orthodontic treatment.

To facilitate volunteer participation, minimally invasive sample collection methods will be used. To this end, during my master's program, we set out to validate in the laboratory a simple, fast, and non-invasive method for detecting DNA damage using salivary lymphocytes in the comet assay.

Preliminary findings support the suitability of salivary lymphocytes for use in human biomonitoring, particularly for field studies where minimally invasive approaches are critical. These results contribute to the growing need for practical and reliable tools to evaluate MNPL exposure in the general population. Additionally, we believe that the use of salivary lymphocytes *in vitro* studies is an area of growing interest, as they may serve as a primary cell source and will complement *in vivo* research.

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Genotoxic damage, immunotoxicity, gene expression signature, and circulating miRNAs as biomarkers of nanoplastic exposure: A pilot study in human-exposed population

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Nanoplastics (NPLs) are widespread environmental contaminants that can enter the human body through inhalation, ingestion, or dermal contact. Once internalized, they may cross biological barriers and accumulate in tissues, potentially inducing oxidative stress, inflammation, and genotoxicity. However, the health risks associated with NPL exposure remain unclear due to the lack of specific biomarkers and the analytical challenges related to their small size and chemical diversity.

To address this gap, we aimed to identify and validate novel molecular biomarkers of NPL exposure. *Ex vivo* experiments were conducted using blood from healthy donors to detect changes in gene expression in peripheral blood immune cells and alterations in circulating miRNAs in plasma. These candidate biomarkers were then evaluated in a pilot study involving textile workers occupationally exposed to NPLs and compared to non-exposed individuals. Complementary assessments included the comet assay to measure DNA damage and analysis of inflammatory cytokines in plasma.

Our findings revealed distinct gene expression signatures and altered miRNA profiles in both *ex vivo* exposed samples and in occupationally exposed individuals. In addition, exposed workers exhibited significantly increased DNA damage and higher levels of inflammatory cytokines compared to controls.

These results support the relevance of the identified genes and miRNAs as potential biomarkers of NPL exposure and related biological responses.

This study provides novel molecular evidence of NPL-related effects and proposes new tools for biomonitoring in both environmental and occupational health contexts. The findings underline the urgent need for preventive strategies and regulatory policies aimed at reducing NPL exposure and protecting public health.

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EDUCATIONAL RESOURCES

RALDE: Re-thinking Active Learning for Distance Education

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The COVID-19 pandemic challenged higher education institutions (HEIs) to rapidly transition from traditional face-to-face teaching to fully online formats, often without adequate tools or digital pedagogy training. The Erasmus+ RALDE project (2020-1-FR01-KA226-HE-095581; https://main-site.ralde.eu) responded to these challenges by developing innovative and accessible digital teaching resources aimed at Biology and Environmental Sciences in HE. The main goal was to enhance digital education quality, promote active learning methodologies, and support educators and students in acquiring relevant digital competencies.

RALDE adopted a multipronged strategy that included: (i) a European survey to assess pedagogical responses during the pandemic; (ii) the design of virtual practicals—such as a rodent necropsy and a cell culture lab simulation; (iii) the development of problem-based learning (PBL) modules; and (iv) gamified tools, including a serious game on cell culture techniques. Additionally, the project produced Do It Yourself tutorials to empower teachers to create their own digital content, and a dedicated module to improve scientific communication skills among MSc and PhD students. These outputs are freely accessible through the RALDE platform, including a podcast series and video materials hosted on YouTube.

Overall, the project generated more than 150 hours of online teaching content and provided practical training for educators and learners. RALDE demonstrates how targeted, high-quality digital tools can not only bridge the gap left by traditional methods during crises, but also permanently enrich teaching practices by fostering engagement, autonomy, and collaborative learning.

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Engaging the youngest minds; communicating environmental mutagenesis through microplastic pollution education at the European Researchers' Night

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The *European Researchers' Night* is a pan-European science communication initiative funded by the European Commission under the Marie Skłodowska-Curie Actions, celebrated annually in over 400 cities. This event aims to bring science closer to the general public, especially young audiences, through interactive and entertaining activities. By offering hands-on experiences and personal interaction with researchers, it fosters curiosity, builds trust in science, and highlights the societal impact of scientific work. For researchers, it also provides a valuable opportunity to develop communication skills and to promote the relevance of their projects in an accessible way. Our participation in this event allowed us to creatively engage with children and families on key environmental health challenges.

Our contribution was framed within the H2020-funded project PLASTICHEAL, which investigates the health effects of micro- and nanoplastics (MNPLs) on the human body. The project aims to characterize the toxicological mechanisms of MNPLs, assess their risks through advanced in vitro and in vivo models, and develop innovative tools for risk assessment and mitigation. Given the widespread environmental presence of plastic particles and the growing concern about their potential mutagenic and toxic effects, communicating these risks to a broad audience is critical, especially to younger generations who will face the long-term consequences of plastic pollution.

To reach the youngest participants, we designed an activity that combined environmental awareness, hands-on experimentation, and basic human biology. Children were given a mixture of sand and plastic particles of various sizes and challenged to isolate them using a colander, simulating the difficulty of environmental remediation. After this, the isolated particles were introduced into a transparent tubing system representing both the respiratory and gastrointestinal tracts. This allowed participants to understand how different particle sizes travel through the body and can impact different organs. The setup offered an accessible and engaging way to explain the health hazards of micro- and nanoplastics while introducing basic anatomical concepts. This educational strategy effectively combined environmental science, toxicology, and public health in an age-appropriate and interactive format.

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Activities in Ibero-America of the Ibero-American Network of Toxicology and Chemical Safety

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Our research activity focuses on mutagenic evaluation studies, including carcinogenic evolution through assays with Salmonella/microsomal and with cell cultures, using human peripheral blood lymphocytes and in vitro cell lines, evaluating natural and synthetic products, mainly for phytosanitary use and as a cytostatic agent. In the poster, we show the close collaboration between the Environmental Mutagenesis Group of the CSIC and the members of the Toxicological Centers of Mexico, under the direction of the Toxicological Center of the Juárez Hospital of Mexico and the Toxicological Center of Ángel Hospital of Morelia, and with the Chilean Universities of Valparaíso, the University of Concepción, the Catholic University of the North in Coquimbo, the University of Antofagasta, Andrés Bello University, and RITA-Chile.

RITSQ was created by a joint initiative of the University of São Paulo, a member of IUTOX, and the President of the Spanish Association of Toxicology of the Spanish National Research Council. Its activities began in 2006, and since then, we have held RITSQ meetings in 2007 in Montreal, Canada; in 2010 in Barcelona, Spain; in 2016 in Mérida, Mexico; and in 2024 in Santiago de Chile, Chile. RITSQ is part of the sister organizations of the Hispanic Organization of Toxicologists (HOT) of the Society of Toxicology (SOT).

In the eighteen years of RITSQ's existence, we have maintained a website and published data annually, including: news, 4 conferences, 5 courses, 6 posters, 85,362 users, 521,756 sessions, and 283,563 page views. To date, 178 posters have been presented at conferences, seminars, and workshops. Therefore, we ask potential event organizers to kindly send us relevant information in advance so that we can publish, disseminate, and advertise it on our website (https://www.ritsq.org).

Collaborators:
From México: Drs. P. Escalante, J. Rodríguez, J. Madrigal, M. Canul; and G. López-Orozco.
From Chile: Drs. F. Cavieres, B. Schulz, C. Müller, F. Pansetti, S. Zuñiga, E. Benelli; C. Santibañez, L. Bórgell.
From Colombia: Dres, N. Patiño, D. Comboriza, L. Peña, P. Acosta, E. Sánchez, J. Tellez, M. Gutiérrez.

The PELAGOS expedition: science beyond the lab

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Science communication strategies are essential for effectively disseminating research findings to diverse audiences, from fellow scientists to the general public. In the context of the H2020 project PLASTICHEAL, which aims to evaluate the health impacts of micro-/nanoplastics, we have tailored communication actions to engage all potential stakeholders. As part of our outreach efforts, we collaborated with the Pelagos Institute in Greece to conduct a unique scientific expedition. This initiative sought to bridge the gap between laboratory research and real-world environmental challenges, fostering interdisciplinary collaboration and public engagement.

During the expedition, PLASTICHEAL scientists, typically focused on laboratory research, engaged in field activities such as monitoring dolphin and whale populations and tracking their migration routes. This hands-on experience allowed us to observe firsthand the impact of human activities on marine life. Concurrently, Pelagos scientists participated in marine water sampling to assess micro/nano-plastic levels in the region, aiming to better understand their potential effects on marine ecosystems.

This collaborative effort not only allowed us to effectively disseminate our research findings to a broader audience but also highlighted the interconnection between different scientific disciplines. It demonstrated that interdisciplinary collaboration and direct engagement with environmental challenges can significantly enhance the impact and relevance of scientific research.

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PlasticHealers: an interactive journey through the health effects of micro- and nanoplastics

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Effective science communication with younger audiences requires adaptable tools that bridge knowledge gaps while remaining accessible, engaging, and inclusive. Interactive digital activities offer a powerful solution, as they can be distributed widely (i.e., online or offline), translated into multiple languages, and used both in formal education settings and informal home environments. These tools can spark curiosity, encourage critical thinking, and make complex topics such as environmental mutagenesis understandable to children. However, their development also poses challenges, including the need to balance scientific rigor with user-friendly design, ensure digital accessibility, and foster meaningful reflection, not just passive learning.

Our initiative is framed within the H2020 project PLASTICHEAL, which aims to evaluate the health impacts of micro- and nanoplastics (MNPLs) through advanced mechanistic studies. The project explores how MNPLs interact with human biological systems, using in vitro and in vivo approaches to assess their toxicity and potential mutagenic effects. As plastic pollution grows, especially at microscopic levels, raising awareness about its potential health risks becomes essential, particularly among the younger generations, who will face its long-term consequences.

To address this, we developed an interactive digital activity designed for students aged 10–12 years. The storyline follows *Martina*, a young researcher from the Universitat Autònoma de Barcelona, who is investigating how MNPLs affect human health. Students assist Martina by choosing among various MNPL sources (such as glitter in cosmetics, synthetic fabrics, or tire abrasion) and follow the journey of plastic particles through different environmental pathways. Along the way, they explore how particle size determines the likely entry route into the human body (ingestion, inhalation, dermal exposure), and how researchers study these effects using laboratory models. The activity includes interactive tasks such as concept matching, size sorting, and pop-up quizzes, encouraging students to think critically and apply what they've learned in real time.

At the end of the activity, students are prompted to perform a group reflection, discussing strategies to reduce and prevent MNPL pollution and its health consequences. In parallel, we provided printable technical sheets for each participant to record their findings, answer guided questions, and consolidate learning outcomes. This blended approach of storytelling, interactivity, and hands-on reflection fosters meaningful engagement with science and empowers the next generation to take part in the environmental challenges of our time.

Funding: This work was supported by the European Union's Horizon 2020 research and innovation programme (PlasticHeal, Grant Agreement No. 965196), the Spanish Ministry of Science, Innovation and Universities (PID2023-146489OB-I00), and the Generalitat de Catalunya (2021-SGR-00731). Additional support was provided by the ICREA-Academia programme (grant Ac2232418 to A. Hernández) and the *Projectes Pre-Competitius* of the Universitat Autònoma de Barcelona (PPC2024-36 and PPC2024-28 to A. García-Rodríguez and L. Rubio). A. Rocabert is funded by the Generalitat de Catalunya (2023 FISDU 00288). I. Barguilla 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 (2023-BP-00212).

ToxLearn4EU: Toxicology Innovative Learning for Europe

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Environmental pollution, climate change and biodiversity loss are major health threats identified by the EU Action Plan "*Towards Zero Pollution for Air, Water and Soil*". Addressing these challenges requires highly qualified toxicologists and ecotoxicologists, capable of evaluating emerging pollutants, complex mixtures and associated risks using innovative methods. The Erasmus+ Project *ToxLearn4EU* (2021-1-FR01-KA220-HED-000030081; https://toxlearn4eu.eu) was launched to modernise higher education in toxicology across Europe by designing and implementing open-access digital resources.

The project brings together ten higher education and research institutions from eight European countries. It focuses on three key areas: emerging pollutants, new toxicological methodologies and models, and risk assessment and communication. ToxLearn4EU has produced a series of online training materials, including core and advanced courses, case studies, and expert lectures—available via YouTube and the project website. These resources target both students and teachers, aiming to enhance competencies, promote digital literacy, and support curriculum renewal in line with the European Green Deal.

The pedagogical approach combines interactive learning and real-world applicability, promoting engagement through problem-based learning and thematic integration. Teachers are also supported with implementation guidelines to incorporate these resources into regular teaching practices. The courses contribute to life-long learning and academic-professional transitions in human and environmental toxicology.

By aligning digital innovation with sustainability goals, *ToxLearn4EU* strengthens toxicological education across Europe, improves student motivation and reduces dropout rates, and reinforces cross-border collaboration in science education.

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Mutagens and *Experimental lab* card games: innovative and alternative resources for introducing gamification within education in bioscience

R. Egea*, A. García-Rodriguez, L. Rubio-Lorente, I. Barguilla, J. Gutiérrez-García, J. Martín-Pérez, H. M. Morataya-Reyes, G. Banaei, A. Rocabert, R. Marcos, & A. Hernández

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In the field of education, particularly in the sciences, conveying complex concepts in an engaging and comprehensible manner is a persistent challenge. A couple of educational resources designed to address this challenge have been developed in our group: “Mutagens” and “Experiment al lab” card games.

The primary objective of these card games is to simplify and elucidate intricate scientific concepts, making them accessible and enjoyable for students and, even, for the general population.

Experiment al lab card game guides players through the steps of conducting an experiment in a laboratory setting. It introduces basic concepts about experimental research and integrates several research project situations with classical card games mechanics. *Mutagens* card game introduces players to the concept of mutagenesis, where the genetic information of an organism is changed. Through interactive gameplay, players learn about different types of mutagenic agents and how to prevent or repair their actions while emphasizing the presence of mutations in natural populations.

By integrating gameplay into the learning process, these resources aim to enhance classroom engagement and motivation in students while improving conceptual understanding. They also aim to awaken scientific curiosity and facilitate effective communication of scientific activities.

The *Mutagens* and *Experiment al lab* card games represent a novel approach to science education and communication. By transforming complex concepts into engaging gameplay, these resources not only aid in classroom as a complementary tool but also improve the communication of scientific activities by triggering interest in science among the general population, making scientific knowledge more accessible and appealing.

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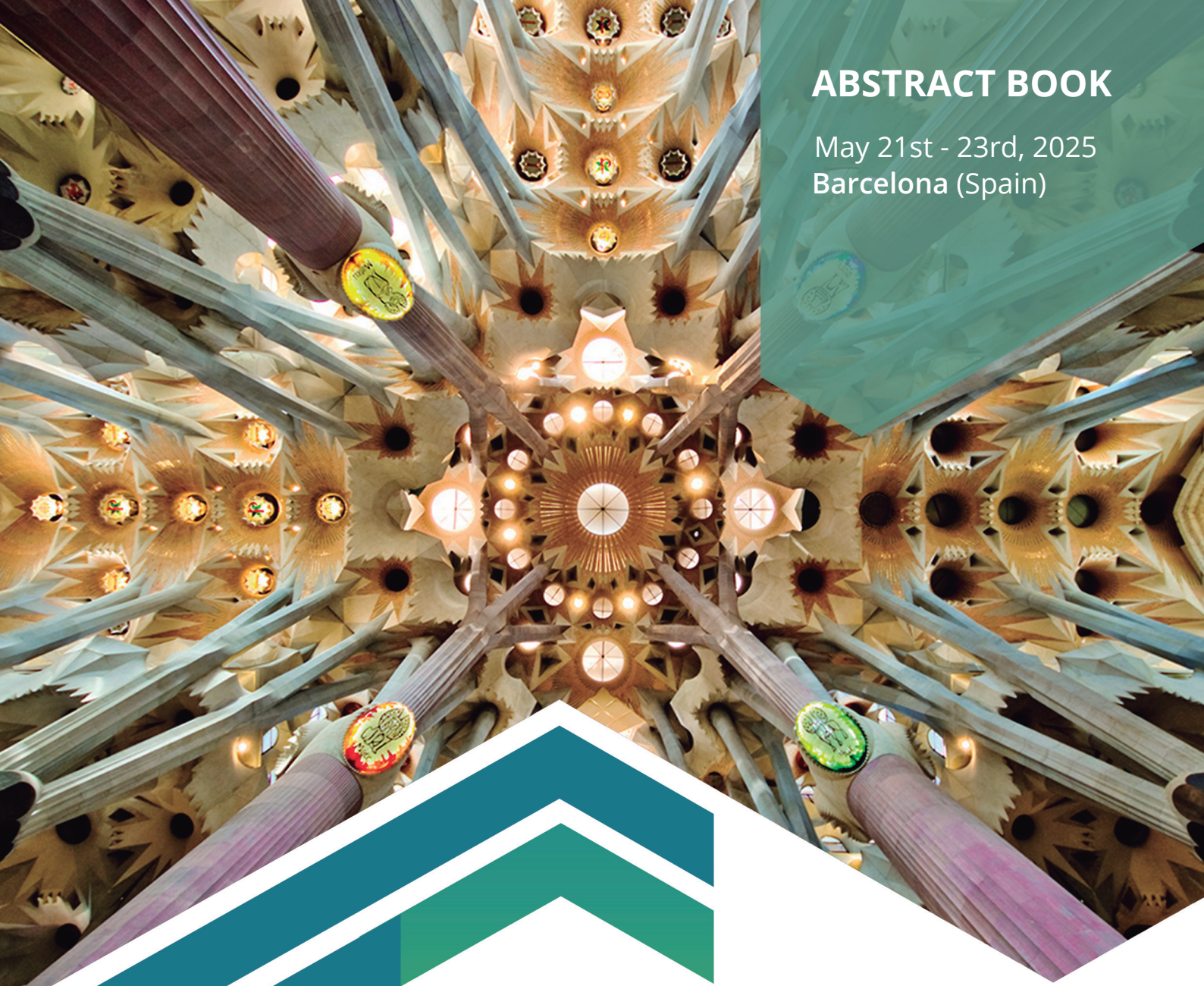

ABSTRACT BOOK

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