SEMA 2022

XXVI Reunión Científica

21-22 junio 2022
Virtual

Libro de Resúmenes

Sociedad Española de Mutagénesis y Genómica Ambiental

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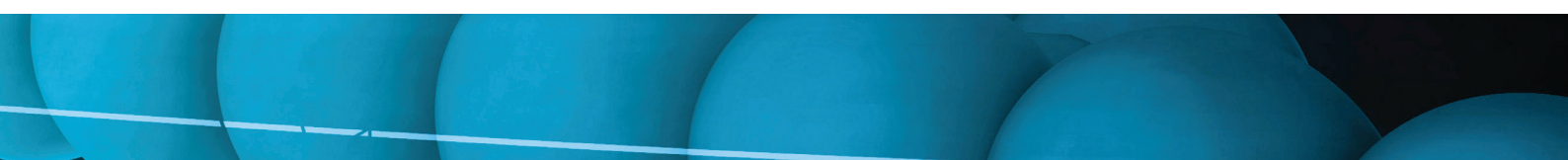

INVITACIÓN
INVITATION

La Sociedad Española de Mutagénesis y Genómica Ambiental (SEMA), tiene el placer de invitarte a la **XXVI Reunión Científica de la SEMA**. El evento se celebrará online los días **21 y 22 de junio de 2022**.

The Spanish Society of Environmental Mutagenesis and Genomics (SEMA), are pleased to invite you to the **XXVI SEMA Scientific Meeting**. The event will be held online on **June 21-22, 2022**.

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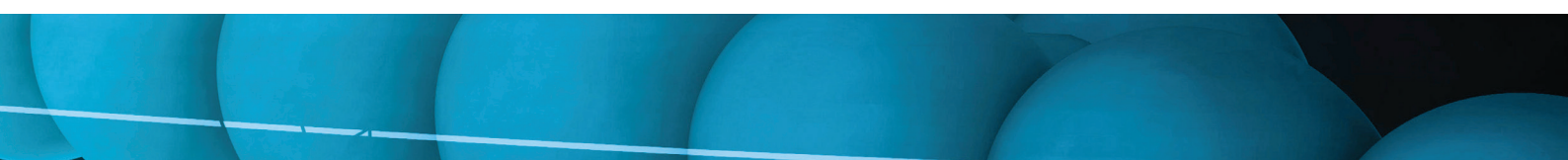

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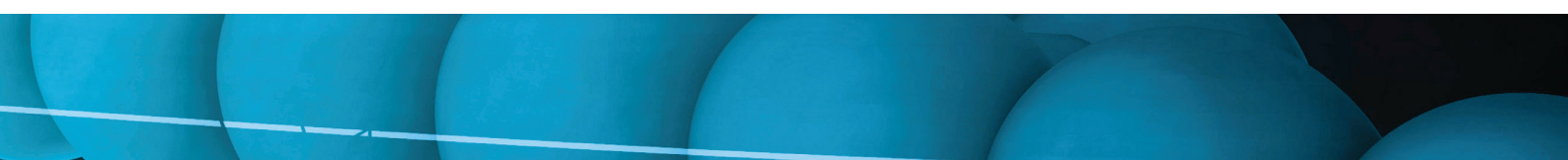
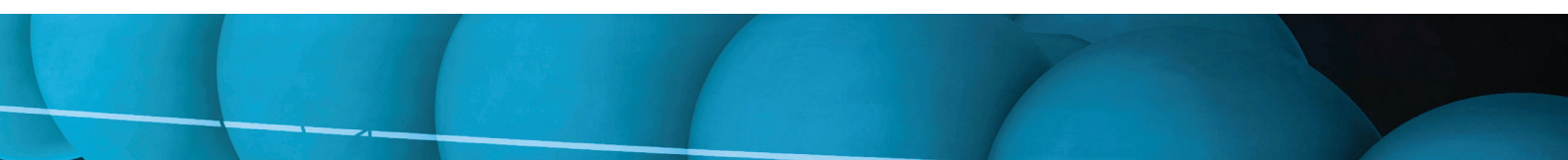


PROGRAMA CIENTÍFICO
SCIENTIFIC PROGRAMME



MARTES 21 DE JUNIO/ TUESDAY, JUNE 21

09:00 – 09:15 Bienvenida / Congress opening

SESIÓN I / SESSION I
CONTAMINANTES EMERGENTES
EMERGING POLLUTANTS
Chairs: **Adela López de Cerain, Ariane Vettorazzi** (Universidad de Navarra)

09:15 – 09:35 *"Stemness imbalance and oncogenic effects induced by in vitro chronic exposure to PS and PET nanoplastics in breast progenitor cells"*
Irene Barguilla Moreno
(Centre de Recherche en Cancérologie de Lyon, France)

09:35 – 09:55 *"Genotoxicity testing of nanomaterials: adequacy of the standardized in vitro mammalian cell micronucleus test"*
Natalia Fernández Bertólez
(Universidade da Coruña, Spain)

09:55 – 10:15 *"Potential harmful effects of nanoplastics derived from teabags in an in vitro model of the intestinal barrier"*
Gooya Banaei
(Universitat Autònoma de Barcelona, Spain)

10:15 – 10:35 *"Size-dependent effects of PSNPLs on human hematopoietic cell lines"*
Alireza Tavakolpournegari
(Universitat Autònoma de Barcelona, Spain)

10:35 – 10:55 *"Biological effects of a mixture of environmental contaminants on mouse. Modulation by selenium and the gut microbiota"*
Paula Victoria Huertas Abril
(Universidad de Córdoba, Spain)

10:55 – 11:15 *"Can the digestion of polystyrene nanoplastics modulate their toxicological profile? Studies in three different human hematopoietic cell lines"*
Lourdes Vela
(Universitat Autònoma de Barcelona, Spain)

11:15 – 11:45 Descanso / Break

11:45 – 12:45 **Conferencia Invitada / Keynote Lecture**
"Addressing the toxic potential of nanoparticles and potentially toxic elements mixtures: state-of-the-art and insight into project NanoLegaTox findings"
Ana Teresa Reis
(Portuguese National Institute of Health, Portugal)

SESIÓN II / SESSION II
CONTAMINANTES EMERGENTES
EMERGING POLLUTANTS

Chairs: **María Teresa Roldán, Rafael Rodríguez** (Universidad de Córdoba)

12:45 – 13:05 *"Preliminary data on the cytotoxic effects of polyethylene terephthalate and polystyrene nanoplastics on Huh-7 hepatocytes"*
Michelle Morataya Reyes
(Universitat Autònoma de Barcelona, Spain)

13:05 – 13:25 *"Long-term exposure to PS and PET nanoparticles in human lung cells"*
Javier Gutiérrez García
(Universitat Autònoma de Barcelona, Spain)

13:25 – 13:45 *"A new source of representative secondary PET nanoplastics. Obtention, characterization, and hazard evaluation"*
Aliro Villacorta
(Universitat Autònoma de Barcelona, Spain)

13:45 – 14:05 *"Sub-lethal in vitro effects of nanoplastics (PET and PS) in primary human nasal epithelial cells (HNEpC) – Role of the autophagy pathway"*
Balasubramanyam Annangi
(Universitat Autònoma de Barcelona, Spain)

14:05 – 14:25 *"Polystyrene nanoplastics: Surface- and Size-dependent effects on human primary endothelial cells"*
Joan Martín Pérez
(Universitat Autònoma de Barcelona, Spain)

14:25 – 14:45 *"The Mechanistic Effects of Human Digestion on Magnesium Oxide Nanoparticles: Implications on Probiotics Lactobacillus rhamnosus GG and Bifidobacterium bifidum VPI 1124"*
Alba García Rodríguez
(Binghamton University, USA)

14:45 – 16:30 Comida / Lunch

SESIÓN III / SESSION III
EDUCACIÓN EN MUTAGÉNESIS Y GENÓMICA
MUTAGENESIS & GENOMICS IN EDUCATION

Chairs: **Rosario Planelló** (Universidad Nacional de Educación a Distancia), **Mónica Aquilino** (University of Birmingham, UK)

16:30 – 16:50 *"Use of cell division graphics as a tool for teaching Environmental Toxicology"*
José Manuel Pérez Martín
(Universidad Autónoma de Madrid, Spain)

16:50 – 17:10 *"Iberoamerican Network of Toxicology and Chemical Safety. Activities carried out from its creation until 2022"*
Eduardo de la Peña de Torres
(Red Iberoamericana de Toxicología y Seguridad Química, Spain)

17:10 – 18:10 Asamblea SEMA / SEMA Assembly

MIÉRCOLES 22 DE JUNIO/ WEDNESDAY, JUNE 22

SESIÓN IV / SESSION IV
DAÑO, REPARACIÓN Y PROTECCIÓN DEL ADN
DNA DAMAGE, REPAIR AND PROTECTION
Chairs: **Ricard Marcos, Alba Hernández** (Universitat Autònoma de Barcelona)

09:00 – 09:20 *"Assessing DNA repair ability in salivary leucocytes with the challenge-comet assay"*
Carlota Lema Arranz
(Universidade da Coruña, Spain)

09:20 – 09:40 *"Resistance to temozolomide in glioblastoma: role of DNA repair mechanisms"*
Mª Isabel Martínez Macías
(IMIBIC, Universidad de Córdoba, Spain)

09:40 – 10:00 *"Opposite base specificity during DNA repair of abasic sites"*
Marina Jordano Raya
(Universidad de Córdoba, Spain)

10:00 – 10:20 *"Flash-comet and Cometchip, a comparison with the standard comet assay"*
Miguel Collía Martín
(Universidad de Navarra, Spain)

10:20 – 10:40 *"The role of succinate and fumarate in the response to cisplatin induced DNA damage"*
Enol Álvarez González
(Universidad de Oviedo, Spain)

SESIÓN V / SESSION V
INESTABILIDAD GENÓMICA, MUTAGÉNESIS Y CARCINOGÉNESIS
GENOMIC INSTABILITY, MUTAGENESIS AND CARCINOGENESIS
Chairs: **Ricard Marcos, Alba Hernández** (Universitat Autònoma de Barcelona)

10:40 – 11:00 *"Genotoxic activity of Pteridium aquilinum in vivo in Drosophila melanogaster"*
Luisa María Sierra Zapico
(Universidad de Oviedo, Spain)

11:20 – 11:50 Descanso / Break

11:50 – 12:50 **Conferencia Invitada / Keynote Lecture**
"Mycotoxins, Parkinson and DNA damage"
Ariane Vettorazzi Armental
(Universidad de Navarra, Spain)

SESIÓN VI / SESSION VI
RESPUESTAS GENOTÓXICAS: ALTERACIONES FISIOLÓGICAS Y DE COMPORTAMIENTO
GENOTOXIC RESPONSES: PHYSIOLOGICAL AND BEHAVIOURAL ALTERATIONS

Chairs: **Blanca Laffon, Vanessa Valdiglesias** (Universidade da Coruña)

12:50 – 13:10 *"Further information about the assimilation and activity of ultra-small, non-magnetic iron oxide nanoparticles with potential use in biomedicine"*
Alonso Rodríguez Pescador
(Universidad de Oviedo, Spain)

13:10 – 13:30 *"Influence of alpha-synuclein overexpression on DNA damage in neuronal cell lines"*
Elba Beraza Ibáñez
(Universidad de Navarra, Spain)

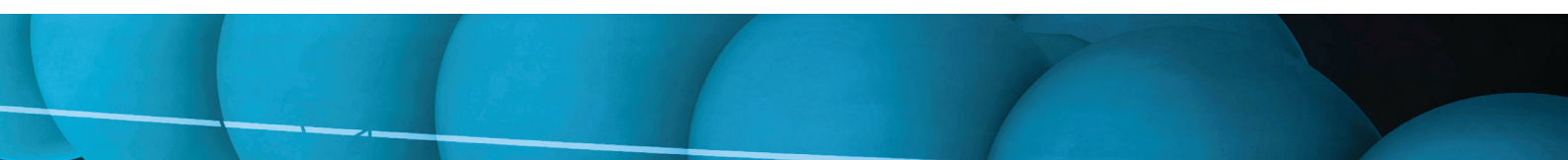
13:30 – 13:50 *"Study of the effects of plasmatic concentrations of sertraline, duloxetine and fluoxetine on THP-1 cells using the comet assay"*
Ainhoa Garayo Larrea
(Universidad de Navarra, Spain)

13:50 – 14:10 *"Wolbachia alters gene expression related to immunity and energy metabolism in Chorthippus parallelus (Orthoptera: Acrididae)"*
Patricia Jiménez Florido
(Universidad Autónoma de Madrid, Spain)

14:10 – 14:30 *"Genotoxicity and DNA methylation patterns associated with Electronic Cigarettes"*
Camila Bernal Forigua
(Pontificia Universidad Javeriana, Colombia)

14:30 – 15:00 Clausura / Closing Ceremony

CONFERENCIAS INVITADAS
KEYNOTE LECTURES



Addressing the toxic potential of nanoparticles and potentially toxic elements mixtures: state-of-the-art and insight into project NanoLegaTox findings

F. Rosário[1,2,3], M. J. Bessa[1,2,3,4], F. Brandão[1,2,3,4], C. Vilaça[3,4], C. Costa[1,2,3], C. B. Lopes[5], A. C. Estrada[5], J.P. Teixeira[1,2,3] and A. T. Reis[1,2,3,6]

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The industrialization and modernization processes, the development of new technologies and products, and the exploitation of natural resources results in a panoply of contaminants released into the environment. Consequently, humans are exposed to mixtures of substances and there is a pressing need to understand the potential adverse health effects resulting from the interactions between these substances. Existing data on mixtures' toxicity is rather limited, since the estimation of health risks from exposure to chemical mixtures is a complex issue, particularly if the chemicals have different modes of action.

One such example would be engineered nanoparticles (NPs) and potentially toxic elements (PTEs), such as metals and metalloids. Mixtures of these substances are of interest since:

I) Due to their growing production and application, NPs are increasingly discharged into the environment. The released NPs can potentially interact with pre-existing contaminants, leading to biological effects (bioaccumulation and/or toxicity) that are poorly understood. Indeed, the limited existing data suggests that NPs-metal(loid) interactions affect the behavior, uptake and toxicity of each individual contaminant (addition, antagonism, potentiation, and synergy, have all been reported).

II) PTEs, such as arsenic, cadmium, lead, and mercury, among others, have a particular affinity for NPs, namely significant accumulation of metal(loid) at NPs surfaces, and considering their persistent and toxic character it is imperative to assess the toxic profile resulting from these interactions.

Assessing the risk of NPs and PTEs mixtures is challenging and requires a dedicated approach. This presentation will: 1) summarize the current state-of-art and available data on toxicity resulting from NPs co-exposed with PTEs; 2) review *in vitro* and *in vivo* methods for investigating and evaluating joint effects of NPs and PTEs; 3) discuss knowledge gaps and future research directions to better understand the risk associated with NPs and PTEs co-exposure.

NanoLegaTox (PTDC/SAU-PUB/29651/2017) cofinanced by COMPETE2020, Portugal2020 and European Union, through FEDER. A.T.Reis, F.Brandão and M.J.Bessa financed by FCT Grants SFRH/BPD/122112/2016, SFRH/BD/101060/2014 and SFRH/BD/12046/2016. A.C.Estrada and C.B.Lopes funded by national funds (OE), in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of article 23, Decree-Law 57/2016, August 29, changed by Law 57/2017, July 19.

Mycotoxins, Parkinson and DNA damage

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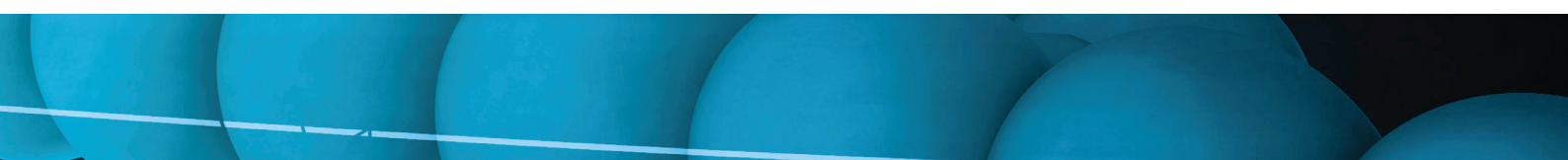
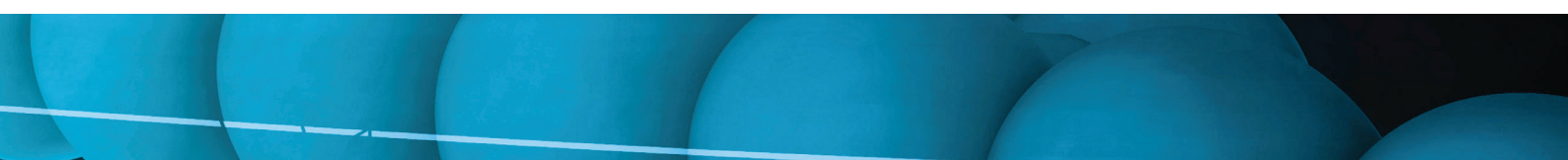
Mycotoxins (MTX) are a group of naturally occurring fungal metabolites contaminating a huge variety of crops worldwide. The current climate change scenario might also modify human exposure as new emerging MTX or mixtures might appear. The main health concern is related to their genotoxic and carcinogenic potential and different regulations have been laid down to diminish human exposure. In the last decades, the MTX ochratoxin A (OTA) has also been described to be neurotoxic. On the other hand, although some environmental factors, such as living in rural areas, have been associated with an increased risk of getting Parkinson´s disease (PD), etiological agents for the disease have not yet been identified.

Our group has demonstrated preliminary data on long-term effects of OTA on PD related molecular features (alpha-synuclein pathology, dopaminergic dysfunction and motor deficits) six months after the end of a 28 day oral treatment in mice (0.21 or 0.5 mg/kg b.w.). Moreover, the mechanism was further validated *in vitro* in a human SH-SY5Y neuroblastoma cell line. In this lecture, the preliminar results obtained within the funded project “Mycotoxins and Parkinson: the missed link“ (Gobierno de Navarra, 2019-project 43), as well a new insights linking DNA damage and PD will be presented.



SESIÓN I: Contaminantes emergentes
SESSION I: Emerging pollutants



Stemness imbalance and oncogenic effects induced by *in vitro* chronic exposure to PS and PET nanoplastics in breast progenitor cells

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The widespread presence of micro- and nanoplastics (MNPLs) in the environment is rising concerns regarding their potentially harmful effects on human health. They easily enter the human body and have the ability to translocate through physiological barriers. Indeed, MNPLs have already been detected in blood and lung tissue. The number of studies focused on their effects on health is progressively increasing, generally linking MNPLs exposure with mild but relevant effects in terms of cytotoxicity, ROS generation, DNA damage, and pro-inflammatory response alterations. These endpoints have been mainly explored at the short term; however, plastic particles are well-known for their persistence in the environment and this feature is expected to also occur in tissues. MNPLs' bioaccumulation extended in time could lead to molecular adverse effects such as mutagenesis and carcinogenesis, an aspect insufficiently explored until now.

Stem cells are among the most persistent cell types, with lifespans ranging from months to several years. This makes them likely targets of persistent pollutants as is the case of MNPLs. Therefore, this study aims to evaluate the long-term effects of polystyrene- (PS-NPLs) and polyethylene terephthalate (PET-NPLs) nanoplastics on MCF10A, a model of breast progenitor cells with known potential to go through malignant transformation. We have developed an *in vitro* approach in which we continuously exposed the cells for 12 weeks to PS- or PET-NPLs in combination with BMP_2 and IL-6, which mimic an inflammatory microenvironment. At several time points during the chronic exposure, we have monitored the cell's differentiation and transformation status. The colony-forming-cell (CFC) assay and the mammosphere formation assay have shown a differential effect of PS- and PET-NPLs on MCF10A differentiation, while no significant indications of cell transformation have yet been observed with the soft agar assay. Ongoing work is focused on the in-depth characterization of the exposed cells, analyzing several differentiation markers which will contribute with relevant data regarding the impact of MNPLs in cell fate deregulation.

Genotoxicity testing of nanomaterials: adequacy of the standardized *in vitro* mammalian cell micronucleus test

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Common toxicity tests might not be fully adequate for evaluating nanomaterials since their unique features are also responsible for unexpected interactions with assay components or detection systems. The *in vitro* cytokinesis-block micronucleus (CBMN) test is recommended by OECD (Test Guideline No.487) for genotoxicity assessment. Nevertheless, cytochalasin-B (Cyt-B) used to inhibit cytokinesis may affect nanoparticle (NP) uptake, leading to inaccurate results. This study evaluated whether the presence of Cyt-B influences cellular uptake and MN production by TiO_2 NP in SH-SY5Y cells. Following OECD recommendations, two options to prevent interference were applied: (1) post-treatment (application of NP for 3-24h, removal of the NP, and addition of Cyt-B), and (2) delayed co-treatment (application of NP for 3–24h, addition of Cyt-B 3-6 h later, and further incubation for 24h). These options were compared to the traditional co-treatment. TiO_2 NP were significantly internalized by the cells after 3-24h treatments, but no differences were observed between the presence or absence of Cyt-B. CBMN test showed progressive increases in the MN frequencies after 6 or 24h treatments in the three treatment options; however, no differences were obtained in the comparisons between treatment options. Despite previous studies highlighted the possible interference of Cyt-B on NP cellular uptake and, consequently, on MN detection by CBMN test, current data do not support that hypothesis. Additional experiments are necessary to define the most suitable protocol of CBMN test for assessing genotoxicity of nanomaterials.

FUNDING. This work was funded by Spanish Ministry of Science and Innovation: MCIN/AEI/10.13039/501100011033 (Grant PID2020-114908GA-I00), and Ministry of Education, Culture and Sport [BEAGAL18/00142 to V.V.]. This communication is based upon work from COST Action CA17140 "Cancer Nanomedicine from the Bench to the Bedside" supported by COST (European Cooperation in Science and Technology).

Potential harmful effects of nanoplastics derived from teabags in an *in vitro* model of the intestinal barrier

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The increasing presence of micro- and nano-sized plastics in the environment, and in the food chain, is of growing concern. Plastics from consumer goods can break down into microplastics and nanoplastics (MNPLs) complicating their detection and quantification. One of the most popular goods used universally is the teabags, which is a new source of MNPLs since the traditional paper bags were substituted by plastic bags.

Among the different studies on the potentially hazardous effects of MNPLs, those related to the MNPLs released from teabags are practically inexistent. To cover this gap, we have designed a new method to first obtain, identify and characterize the plastic particles released from teabags, and then determine their harmful effects on human cells. Since humans are exposed to teabags-MNPLs via ingestion, we have used a well-stablished model of *in vitro* intestinal barrier as a target. The model consists in a triple coculture containing enterocytes (Caco-2 cells), goblet cells (HT-29 cells), and lymphocytes B (Raji-B cells).

To obtain the released MNPLs, we have used tea bags available from the supermarket. After removing the tea fraction, the teabags were washed 3 times with Milli-Q water and boiled in water at 95 ºC for 30 min. Finally, the sample was sterilized with 95 % ethanol and ultra-centrifuged to have enough volume and concentration to carry out the characterization and cell treatment procedures. The particles were fully characterized by TEM (Transmission Electron Microscopy), SEM (Scanning Electron Microscopy), SEM-EDX, Nano Z-sizer, and FTIR (Fourier Transform Infrared Spectroscopy).

The results from SEM-EDX and FTIR confirmed that the particles derived from teabags were mostly polylactic acid (PLA). Moreover, using the Nano Z-sizer we could detect a hydrodynamic size of approximately 100 nm. Finally, TEM and SEM images showed the spheric shape and confirmed the size from both PLA in suspension and in the teabag tissue.

Once the protocol for MNPLs has shown to be robust, and the overall characterization has been done, we aimed to test our own teabag-derived PLA-NPs on the *in vitro* model of the intestinal barrier Caco-2/HT29/Raji-B. Our hazard approach involves determining the toxicity, evaluating the effects on the barrier integrity and permeability, determining cell uptake and translocation, as well as potential induction of oxidative stress and genotoxicity.

FUNDING. This work was funded by the EU Horizon 2020 (965196, PlasticHeal) and the Spanish Ministry of Science and Innovation [PID2020-116789, RB-C43].

Size-dependent effects of PSNPLs on human hematopoietic cell lines

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Plastics are a serious ecological problem since their wastes pollute any corner of our environment. In the environment, plastic wastes are degraded to microplastics (MPLs, < 5000 nm), and further to nanoplastics (NPLs, < 100 nm). The urgent question, then, is whether these MNPLs can affect human health.

One of the main plastics used for consumer packaging and construction purposes is polystyrene (PS). It is accepted that polystyrene nanoplastics (PSNPLs) can enter the human body translocating epithelial barriers and moving via the blood vessels to inner organs and tissues. Therefore, blood cells can be a direct target of PSNPLs. The hazardous effects of MNPLs may be modulated by various factors, including size. It seems appropriate to consider that size can be a relevant factor affecting cell uptake and, consequently, their potential hazards. Although some studies have focused on the effect of size, most of them involved only the micro range. Thus, there is a lack of data on the modulation of the *in-vitro* toxicological effects caused by PS in the nanometer (nm) range; PSNPLs.

To cover this gap, we have assessed the toxic effects of PSNPLs in three different sizes, 50 nm, 200 nm, and 500 nm on human hematopoietic cell lines. To such end, we have used TK6 (lymphoblast), THP-1(monocytes), and Raji-B (B-lymphocyte) as target cells. After a complete characterization of commercial PSNPLs by TEM (Transmission Electron Microscopy) and Nano Z-sizer, the three cell lines were exposed to different concentrations of all sizes of PSNPLs with various exposure times (24 and 48 hours) to determine cell viability, intracellular reactive oxygen (ROS) production, and cellular internalization (using TEM).

Preliminary results indicate that in exposures lasting for 24 h no changes in cell viability nor in ROS production were observed. These results are relevant if we take into consideration that cell internalization was observed for all sizes and in all three cell lines. Surprisingly, after cell internalization, 50 nm PSNPLs were able to reach the mitochondria of the Raji-B cells. To complete these findings, further mitochondrial function assays, intracellular ROS (at 48 h), and a deeper cell internalization study (by confocal microscopy and flow cytometry) with lab-made labeled PSNPLs are currently in progress.

FUNDING. This work was funded by the EU Horizon 2020 (965196, PlasticHeal) and the Spanish Ministry of Science and Innovation [PID2020-116789, RB-C43].

Biological effects of a mixture of environmental contaminants on mouse. Modulation by selenium and the gut microbiota.

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Environmental contaminants often occur in mixtures where they can interact and suffer changes in their properties. The individual concentrations in the environment of metals and pharmaceuticals, that usually contaminate water and food, are normally harmless, but their joint action must be analyzed and considered when establishing exposure risk factors for the population. This study focusses on a mixture of contaminants formed by three metals/methaloids (As, Cd and Hg), which are widely spread in the environment due to their importance in industry; and two pharmacologically active compounds (diclofenac and flumequine), which are broadly used and highly resistant to degradation in wastewater plants. The aim of this study is to analyze the biological response against this contaminant mixture (CM) using *Mus musculus* as model organism. To this end, we have investigated the effect of the CM on survival, gut bacterial composition and liver metabolism, trying to determine the role that the gut microbiota (GM) or the intake of a selenium (Se) nutritional supplement could play in the toxicity of CM.

The CM significantly decreased the survival of mice, especially of those with an altered microbiota suggesting that the GM is required to metabolize and detoxify this mixture. The results show that the CM caused oxidative stress and metabolic dysfunction in the liver, corroborated by increased levels of bile acids and impaired carbohydrate metabolism. Additionally, the CM provoked a profound alteration in the composition of the GM, which was enriched in acetate- and butyrate-producing bacteria, affecting host metabolism and gene expression. In this line, some transcripts implied in the antioxidant response under the control of NRF2, such as *MT1* and *Hmox1* increased, but key enzymes such as glutathione peroxidase, superoxide dismutase or catalase decreased. This indicates that, pollutants not only generate an oxidative stress situation, but also compromise the defense against this stress. A selenium supplement in the diet during exposure partially prevented the toxic effect of the CM.

In short, the exposure to environmentally relevant concentrations of mixed metals (As, Cd and Hg) and drugs (diclofenac and flumequine) present in water and food constitute a health risk. Thus, it is necessary to minimize their incorporation to ecosystems and to improve water purification procedures to reduce their presence in the environment.

FUNDING. This work was funded by the Spanish MICIN, PG2018-096608-B-C21.

Can the digestion of polystyrene nanoplastics modulate their toxicological profile? Studies in three different human hematopoietic cell lines

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Plastic wastes released into the environment are degraded to micro-nano plastics (MNPLs) by the effects of physicochemical/biological processes. The main exposure route to MNPLs is via ingestion. Once MNPLs enter the human body they must pass through different compartments of the gastrointestinal tract that may affect their physicochemical properties and surface features. Thus, to effectively analyze the toxicity of MNPLs, the role of the digestion processes, must be considered. For this reason, this study aims to determine the influence of the in vitro digestive process on the toxicity of polystyrene nanoplastics (PSNPLs) in three different human leukocytic cell lines: Raji-B (B-lymphocytes), TK6 (lymphoblasts), and THP-1 (monocytes). The *in vitro* digestion process was performed on pristine polystyrene (dPSNPLs) and on its fluorescent counterpart (dFPSNPLs). Using transmission electron microscopy (TEM) and scanning electron microscopy (SEM) we have determined that all particles are spherically shaped, with similar appearance and sizes. Nevertheless, the digested particles show a relevant tendency to agglomerate. The hydrodynamic radius (measured by Dynamic Light Scattering, DLS), shows that digested particles have a larger hydrodynamic size, and the polydispersity index (PdI) indicates that the non-digested particles are more monodisperse. These results agree with the results of the Z-potential showing that the digested particles have less Z-potential.

Cell uptake was evaluated with the fluorescent polystyrene (FPSNPLs) and the digested version (dFPSNPs) by flow cytometry and confocal microscopy. Results show that the three cell lines internalize more dFPSNPs than FPSNPLs, at the same concentration. When cell viability was assessed, only moderate effects were observed at the highest concentration of dPSNPLs in TK6 at exposures lasting for 24/48 h. No intracellular ROS production was observed in any of the cell lines at 24/48 h and, finally, genotoxic damage induction was detected only at 24 h exposure in THP1 cells, and at the highest concentrations. No oxidative DNA damage was detected at any time and in any cell line.

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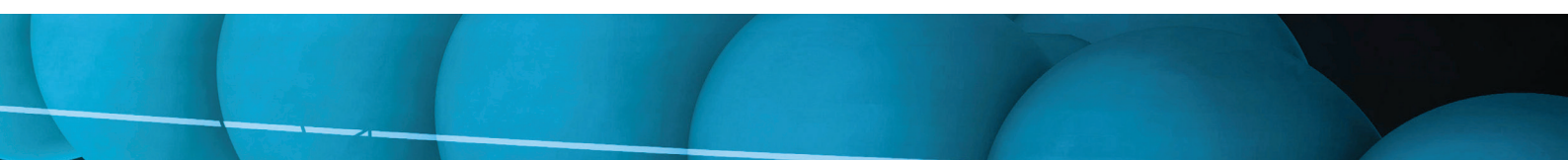
SESIÓN II: Contaminantes emergentes
SESSION II: Emerging pollutants



Preliminary data on the cytotoxic effects of polyethylene terephthalate and polystyrene nanoplastics on Huh-7 hepatocytes

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Increasing demand for plastic products and inadequate waste management have resulted in their accumulation in the environment, where different physicochemical/biological mechanisms cause them a steady fragmentation to reach micro and nanoplastics (MNPLs) size, with structural differences and with potential changes in their ways of action. Although there is evidence of human exposure to MNPLs, their effects on secondary organs after trespassing dermal, respiratory, and gastrointestinal barriers are still not understood. One of these potential target organs is the liver.

To address this gap in knowledge, we are studying the effect of nanoplastics coming from two of the most used plastics, polyethylene terephthalate (PET) and polystyrene (PS), in Huh-7 cells as an *in vitro* model of human hepatocytes. In our experimental approach cells were exposed to 50 µg/mL of PET (100 nm), pristine PS (50 nm), and carboxylate PS (PS-COOH, 50 and 100 nm) for exposures lasting 1, 6, 24, 48, and 72 h. Our results indicate an increase in their internalization with time for PS, 100 nm PS-COOH, and PET. It is interesting to point out the high internalization of 50 nm PS-COOH, which was present in more than 99% of the cells from the first hour of exposure. We confirmed the NPLs internalization by using confocal microscopy, showing all the plastics to be inside the cellular membrane and also into the nucleus.

Furthermore, after exposing cells to 25, 50, 100, and 200 µg/mL of the selected NPLs, we observed a dose-dependent decrease in Huh-7 viability, although in all cases it was above 70%. Finally, we have detected the generation of intracellular reactive oxygen species (ROS) in the exposed cells showing a time- and size-dependent relationship.

After these preliminary results, we plan to follow the hazard assessment of the selected NPLs by determining genotoxic damage, changes in the cytokine profiles, and immunologic response associated with gene expression changes. This data will be used to propose biomarkers to extend our acute experiments to long-term exposure scenarios and to provide relevant information for regulatory authorities.

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Long-term exposure to PS and PET nanoparticles in human lung cells

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Plastic properties, such as chemical stability, easy handling, high malleability, or good resistance, have promoted the massive use of these materials in a vast variety of applications. The exponential production of these polymers, along with an inefficient recycling system, has resulted in an alarming environmental accumulation of plastic waste. The degradability of these polymers in the environment results in the so-called micro and nanoplastics (MNPLs). These ubiquitous tiny particles have been identified in all the ecosystems, ranging from abiotic environments to higher organisms. In humans, besides ingestion which has already been pointed out as an important route of exposure to MNPLs, attention is now paid to inhalation, considered also as a major source of exposure. Although MNPLs have recently been identified in the lungs of living people, very little is known about their chronic effects on human health. The purpose of this study is to identify the long-term adverse effects of two of the most common inhalable MNPLs: polystyrene (PS) and polyethylene terephthalate (PET). For that, Beas-2B (bronchial cell line) and A549 (alveolar cell line) have been exposed up to 15 weeks and different effects were evaluated. By using a battery of i*n vitro* assays, we have determined the ability of MNPLs to reach cell cytoplasm, their genotoxic potential, and some cell transformation hallmarks, like proliferation rate, anchorage-independent growth, migration potential, invasion ability, and tumorsphere generation.

Our results show a high ability of PS and PET NPLs to be uptake by the cell, although no genotoxic nor carcinogenic potential has been anticipated until now. We wish to point out the relevance of the *in vitro* approach used for assessing the carcinogenic potential of the MNPL. We propose our battery of hallmarks as an appropriate way to assess the carcinogenic potential of MNPLs, as it has been previously demonstrated for other agents, including several nanoparticles.

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A new source of representative secondary PET nanoplastics. Obtention, characterization, and hazard evaluation

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Micro and nanoplastics (MNPLs) are emergent environmental pollutants requiring urgent information on their potential risks to human health. One of the problems associated with the evaluation of their undesirable effects is the lack of real samples, matching with those resulting from the environmental degradation of plastic wastes. To such end, we propose an easy method to obtain polyethylene terephthalate nanoplastics from water plastic bottles (PET-NPLs) but, in principle, applicable to any other plastic goods sources. An extensive characterization indicates that the proposed process produces uniform samples of PET-NPLs of around 100 nm, as determined by using a multi-angle and dynamic light scattering methodology. An important point to be highlighted is that to avoid the metal contamination resulting from methods using metal blades/burrs for milling, trituration, or sanding, we propose to use diamond burrs to produce metal-free samples. To visualize the toxicological profile of the produced PET-NPLs we have evaluated their ability to be internalized by cells, their cytotoxicity, their ability to induce oxidative stress, and to induce DNA damage. In this preliminary approach, we have detected their cellular uptake, but without the induction of significant biological effects. Thus, no relevant increases in toxicity, reactive oxygen species (ROS) induction, or DNA damage -as detected with the comet assay- have been observed. The use of real samples, as produced in this study, will generate relevant data in the discussion about the potential health risks associated with MNPLs exposures.

FUNDING. This work was funded by the EU Horizon 2020 (965196, PlasticHeal) and the Spanish Ministry of Science and Innovation [PID2020-116789, RB-C43].

Sub-lethal *in vitro* effects of nanoplastics (PET and PS) in primary human nasal epithelial cells (HNEpC) - Role of the autophagy pathway

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The potential effects of released micro-nanoplastics (MNPLs) into the environment from various sources, as emerging pollutants, arouse considerable interest in the scientific community. It is now established from various studies that they do pose a possible human health risk when we come into contact with them. One of the potential routes of exposure to humans could come from inhalation, due to their widespread presence in the air. Our present study aimed to understand the toxic effects of two types of MNPLs, such as nanosized polyethylene terephthalate (nPET) and nanopolystyrene particles (PS 80 and 430 nm), in human primary nasal epithelial cells (HNEpC), the first line of cells acting as a barrier to MNPLs in the respiratory system. For this, we chose several endpoints such as estimation of cell viability, generation of iROS, and intracellular localization of these MNPLs. Besides, we evaluated the role of the autophagy pathway which is a normal cellular process involved in the recycling of damaged organelles, aged proteins, and oligosaccharides into simpler forms for cell survival, as a potential toxic mechanism of the selected MNPLs.

Our data revealed there was no significant decrease in cell viability due to different concentrations of PET and PSs (from 0.5 to 100 µg/mL) in comparison to untreated control after 24 h. Furthermore, there were no significant increases in the induction of ROS when cells were treated with both PET and PSs (100 µg/mL) as compared to the control after 24h. However, we observed an increase in cellular internalization of both PET and PSs as compared to untreated control using flow cytometry and confocal microscopy. Nevertheless, there seemed to be an increase in the autophagy markers LC3II and P62 in western blotting after the treatment of the cells with both PET and PSs at exposures lasting for 24 h, suggesting the possible induction and blockage of the autophagy pathway by the tested MNPLs. This seemed to indicate a ROS independent effect induction, as well as insufficient autophagy in the treated cells.

Furthermore, this study envisages the potential of present MNPLs to cause mitochondrial damage due to loss of mitochondrial membrane potential and induction of mitophagy as subcellular effects.

FUNDING. This work was funded by the EU Horizon 2020 (965196, PlasticHeal) and the Spanish Ministry of Science and Innovation [PID2020-116789, RB-C43].

Polystyrene nanoplastics: Surface- and Size-dependent effects on human primary endothelial cells

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As of 2015, humans had produced 8.3 billion metric tons of plastics, 6.3 billion tons of which had already become waste. Although plastic wastes are considered long-lasting and stable in the environment, under the influence of different physical and chemical factors plastics undergo fragmentation into micro- and nanometer-level particles, named micro- (100 nm – 5 mm) and nanoplastics (≤ 100 nm) (MNPLs), respectively. During this weathering process, plastics suffer from changes in their physicochemical surface properties that can influence its toxicological potential. Nowadays, there is increasing evidence suggesting that environmental MNPLs can reach the human body through different pathways: ingestion, inhalation and dermal contact. Indeed, Leslie et al., (2021) have been able to detect plastics ≥700 nm, including polystyrene, in whole blood samples representative of the general population. However, the potential effects of MNPLs on the health of exposed individuals are still unknown and require further research.

In the present study, polystyrene nanoparticles (nPS) and Human Umbilical Vein Endothelial Cells (HUVECs) were used to better understand what are the toxicokinetic and toxicodynamic interactions of MNPLs with the vascular system.

To explore the influence of physicochemical properties on the observed effects, representative nPS of different sizes (PS-COOH 30 nm, 50 nm, and 100 nm) and surface characteristics (Pristine PS, carboxyl (-COOH) and amino (-NH_2)) were included in the study.

Our results suggest that although all nPS are internalized by HUVECs, the internalization dynamics are modulated based on the functionalization and the size of the particle. Interestingly, our flow cytometry data also shows that both PS-COOH 50 nm and PS-COOH 100 nm are able to modify the morphology of the cell and increase its inner complexity/granularity. When analyzing possible toxic effects by treating the cells with a concentration of 100 µg/mL we observe that only PS-NH_2 50 nm is able to reduce cell viability (- 40% vs control; 12 h treatment). Finally, our first approach to study ROS generation when treating the cells with 100 µg/mL of PS-COOH 50 and PS-COOH 100 nm for 24 h shows an increase in ROS production with both types of nPS particle (+ 70% in both cases vs control).

Overall, our results indicate a surface- and size- dependent effect of nPS on HUVEC cells. Further experiments are needed to fully understand the impact of MNPLs on the vascular system, and how the particle's properties influence the effects.

FUNDING. This work was funded by the EU Horizon 2020 (965196, PlasticHeal) and the Spanish Ministry of Science and Innovation [PID2020-116789, RB-C43].

The Mechanistic Effects of Human Digestion on Magnesium Oxide Nanoparticles: Implications on Probiotics *Lactobacillus rhamnosus GG* and *Bifidobacterium bifidum VPI 1124*

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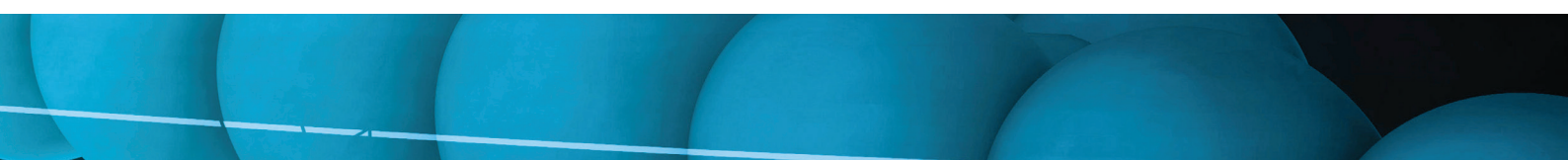
The gastrointestinal (GI) microbiota is linked to intestinal homeostasis and crucial for overall host health. When the microbiota is impaired or altered, common inflammatory and metabolic disorders are more likely to be developed. Exogenous factors such as diet have been reported to highly impact microbial dysbiosis. Because of their unique physicochemical properties, metal oxide nanoparticles (NPs) are widely used as food additives. MgO NPs are an EU-approved food additive (E 530) present in milk, canned food, and dietary supplements, and can be used as an anti-acid and laxative. In high doses, MgO act as antimicrobial and antibiofilm. The relationship between MgO NPs and the human intestinal microbiota, however, has not been thoroughly investigated.

The aim of this study is to investigate the impact of physiologically relevant concentrations (267 mg/day) of food additive MgO NPs (65 nm) on the viability, growth, and biofilm development of two human gut-derived commensal bacteria: the Gram-positives *Lactobacillus rhamnosus GG* and *Bifidobacterium bifidum VPI 1124*. Considering the biological implications of human digestion, MgO NPs were subjected to an *in vitro* digestion (with gastric enzymes; inorganic salts; changing pH and temperature), then diluted in bacterial growth medium at low (L-MgO; 4.3×10^{-5} mg/mL), medium (M-MgO; 4.3×10^{-4} mg/mL) and high (H-MgO; 4.3×10^{-3} mg/mL) physiologically-relevant concentrations to treat both planktonic (free cells) and biofilms (sessile bacterial communities) of *L. rhamnosus* and *B. Bifidum*.

The obtained results indicate that after *in vitro* digestion and due to pH oscillations, MgO NPs partially dissociate into Mg^{2+}, but some particles remain as crystal structures in the nm range. The presence of M- and H-MgO significantly increased cell growth of both *L. rhamnosus* and *B. bifidum* when growing as biofilms but not as planktonic cells. Although no differences in biofilm morphology were observed by confocal imaging, COMSTAT2® analysis detected a 1.5-fold increase in biomass of H-MgO samples compared to control. Interestingly, M- and H-MgO maximized the biofilm development of *L. rhamnosus,* but reduced *B. bifidum*. Hence, we suggest that the presence of released Mg^{2+} could be selectively used as a nutrient playing a major role in the described results. Moreover, no detrimental effects were detected after short-term exposures as interactions of MgO NPs-bacteria are not favored since both structures are negatively charged.

SESIÓN III: Educación en mutagénesis y genómica
SESSION III: Mutagenesis & genomics in education



Use of cell division graphics as a tool for teaching Environmental Toxicology

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The teaching of Toxicology in general, and Environmental Toxicology in particular, seems to be reserved for higher educational levels. Nevertheless, there are more studies that support an education in context and applied to everyday situations of the learner. However, the teaching of contents on cell divisions in high school are reiterated and extremely theoretical and abstract, far from being put into practice in order to solve problems.

Our team has been working for some time on detecting problems in the teaching of cell division in teacher training and in high school students. During this time, we have developed didactic proposals that require the mobilisation of contents on cell division for the resolution of practical cases related to cancer treatments or reproductive problems arising from environmental pollution.

In this paper, we present some examples of activities in which cell divisions are contextualised in topics related to genotoxicology and environmental mutagenesis. All of them encourage the use of evidence and arguments based on realistic images and tables and graphs.

In general, the activities present a problem image and a thought-provoking question. From it, they mobilise their previous knowledge, their mental models, which are captured and shared. Group work leads to discussions to build a common explanatory model (formulation of hypotheses). Moreover, we also sometimes provide them with graphs and tables to assess how they extract information, compare it and use it to formulate arguments and complement the hypothesis. With all this, we can analyse what scientific-mathematical knowledge and skills (mainly research) they bring into play when solving problems associated with images, graphs and tables, and detect the most frequent limitations they show when interpreting data of scientific-mathematical origin.

Overall, this action is a didactic resource of interest that brings Toxicology closer to the classroom for different educational levels, from secondary school to university, and promotes an integrated strategy of scientific training in the field of human and environmental health and mathematics within the framework of STEAM.

Iberoamerican Network of Toxicology and Chemical Safety. Activities carried out from its creation until 2022

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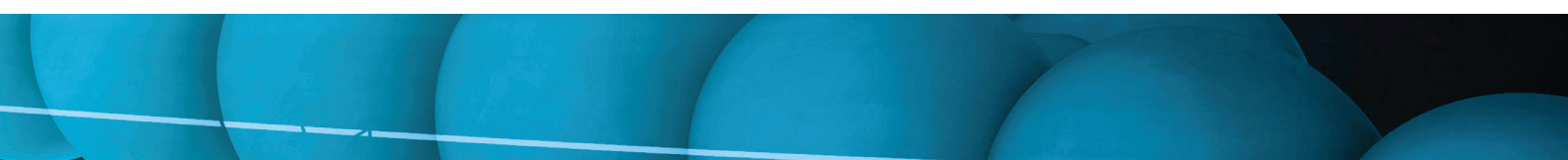
A summary of the activities of the RITSQ is made, highlighting the 125 posters that we have presented in different scientific events held, since the preparatory meeting in August 2006, in Santiago de Chile, in the Congress of Toxicology and Chemical Safety organized by ALATOX, to the present. The objectives of the RITSQ are: a) to coordinate the participation of research groups; b) strengthen collaboration and academic exchange; c) favor the realization of projects; d) delve into short and long-term test methods; e) develop and standardize analytical methods; f) disseminate and promote the use of alternative methods; g) encourage the exchange of toxicology professionals; and h) publication of all information on meetings of toxicological interest. A constant annual review is made of reports, RITSQ activities, organization of conferences and courses, number of posters, and the growing number of 73,623 users, 107,323 sessions and 217,783 number of page views; To the organizers of congresses and meetings, we ask that they kindly inform you and send us the pertinent information on any activities of toxicological interest and chemical safety; The network wishes to maintain constant information on all events of toxicological interest that are organized in Latin America, Portugal and Spain (http://ritsq.org).

SESIÓN IV: Daño, reparación y protección del ADN
SESSION IV: DNA damage, repair and protection



Assessing DNA repair ability in salivary leucocytes with the challenge-comet assay

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The challenge-comet assay provides a quantitative and functional determination of DNA repair ability, by combining a challenge treatment with a genotoxic agent and estimation of the remaining DNA damage with the comet assay after a certain period for repair. In addition, kinetics of the repair process can be monitored. Peripheral blood mononuclear cells (PBMC) are the cells most frequently employed in biomonitoring studies using the challenge-comet assay. However, a validated alternative of non-invasive biomatrix would be highly convenient for certain population groups and circumstances. Thus, the objective of this study was to validate the use of salivary leucocytes in the challenge-comet assay. Saliva samples were collected from 10 healthy volunteers, and leucocytes were isolated and challenged (either in fresh or after cryopreservation) with three genotoxic agents acting by different action mechanisms: bleomycin, methyl methanesulfonate, and ultraviolet radiation. DNA damage induced and remaining was evaluated by the comet assay just after treatment, and also at other three additional time points. The results obtained demonstrated that saliva leucocytes were as suitable as PBMC for assessing DNA damage of different nature that was efficiently repaired over the evaluated time points, even after 5 months of cryopreservation (after a 24 h stimulation with PHA). Furthermore, recommendations to perform the challenge-comet assay with salivary leucocytes depending on the type of DNA repair to be assessed are given. Validation studies are needed to verify whether the method is reproducible and results reliable and comparable among laboratories and studies.

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Resistance to temozolomide in glioblastoma: role of DNA repair mechanisms

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Glioblastoma (GBM) is an aggressive form of brain cancer with a low survival rate, due in large part to resistance to temozolomide (TMZ), a DNA methylating agent used in combination with radiotherapy as first-line treatment after surgery. The resistance to therapy is a consequence of the action of DNA repair mechanisms that repair the damage caused by an antitumor agent. A role in TMZ resistance has been proposed for high expression of DNA repair proteins such as MGMT, (O6-meG DNA methyltransferase), which directly demethylates O6-meG lesions, or MPG (N-Methylpurine DNA Glycosylase), which repairs N7-meG and N3-meA via Base Excision Repair (BER). However, increased levels of MGMT or MPG only explain a small percentage of cases of tumours resistant to TMZ. Importantly, the most abundant lesion induced by TMZ is N7-meG, which is frequently lost from DNA generating toxic abasic (apurinic/apyrimidinic, AP) sites. Such AP sites are repaired through a pathway initiated either by AP endonucleases or by AP lyases. It is generally assumed that AP endonucleases play a major role, but the contribution of AP lyases to TMZ-resistance in GBM remains largely unknown. Here, we have characterized a panel of GBM cell lines to analyse sensitivity to TMZ and to study the expression of different DNA repair genes. We have found significant differences between TMZ-resistant and TMZ-sensitive GBM cells in the expression levels of some genes involved in AP endonuclease-independent AP site repair. Our results point towards a role of an AP lyase-dependent pathway in the repair of TMZ-dependent damage and may help to identify novel predictive biomarkers and/or therapeutical targets in GBM treatment.

Opposite base specificity during DNA repair of abasic sites

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Abasic (apurinic/apyrimidinic, AP) sites are ubiquitous DNA lesions that arise though spontaneous base loss or by the catalytic activity of DNA glycosylases on damaged or modified bases during base excision repair. Processing of AP sites may proceed by either AP endonucleases or AP lyases, but the relative roles of these two types of enzymes are not well understood. We hypothesized that sequence flanking the AP site and/or the identity of the orphan base on the complementary DNA strand may determine the enzyme responsible for its processing. We have analyzed AP endonuclease and AP lyase activity from plant, bacteria and human on DNA substrates containing an abasic site opposite either G or C in different sequence contexts. We found that the major *Arabidopsis* AP lyase (FPG) exhibited preference for AP sites opposite C, whereas no preference for the orphan base was observed in the AP lyase activity detected in human cells extracts. In contrast, the major plant AP endonuclease (ARP), in either recombinant or native form, preferred AP sites opposite G. However, the major human AP endonuclease (APE1) did not show any significant preference for the orphan base. Interestingly, we found that bacterial AP endonucleases, such as Exo III and Endo IV behave similarly to the plant enzyme and also prefer G as the base opposite the abasic site.

Through structural and homology modelling, we searched for differentially conserved amino acids between animal, plant and bacterial AP endonucleases, focusing on those residues that may interact with the orphan base. One such residue is Met270 in human APE1, whose homologs are Arg488 in *Arabidopsis* ARP and Arg216 in *E. coli* ExoIII. Importantly, at this amino acid position most animal AP endonucleases have methionine, whereas plant and bacterial enzymes usually have arginine. To test the prediction that Arg488 has a role in the preference for the orphan base of *Arabidopsis* ARP, we generated two mutant versions in which Arg488 is changed either to glycine (R488G) or methionine (R488M). We found that both mutants proteins have lost the capacity to distinguish between cytosine and guanine on the complementary strand. Our results suggest that the preference for G opposite the abasic site is an ancestral feature of AP endonucleases, and that such specificity has been lost in the metazoan lineage.

Flash-comet and CometChip, a comparison with the standard comet assay

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The alkaline comet assay is a widely used genotoxicity technique. However, its throughput has always been an aspect for improvement and some modifications have been made in that direction, such as developing the CometChip® (Bio-Techne) (1), based on a 96 well-format, or reducing the duration of alkaline unwinding and increasing the voltage gradient using a lithium-based solution, the so-called Flash-comet assay (2). The objective of this work was to compare the results obtained with these modifications with a standard version of the assay.

TK6 cells were treated with methyl methanesulfonate (MMS) and hydrogen peroxide (H_2O_2) at different concentrations. In the standard version of the assay, 2 gels/slide format was used; cells were lysed for 1 h followed by 20 or 40 min of alkaline treatment and electrophoresed at 1.2 V/cm during 20 min. Concerning the Flash-Comet, cells were lysed for 1 h at pH 8.5 or 10, followed by 2.5 min of alkaline treatment in a lithium hydroxide solution (pH 12.5) and an electrophoresis of 1 min at 5 V/cm in the same solution. The CometChip® was used following the manufacturer's protocol. Briefly, cells were loaded in the chip, covered with agarose, subjected to an alkaline treatment of 40 min and electrophoresed at 1 V/cm for 50 min. In this case, the Fpg-modified version was also performed in $KBrO_3$-treated cells.

The sensitivity of the Flash-comet was lower compared to the standard version of the assay, being unable to detect the MMS-induced damage. Dose response curves, with slight differences, were obtained with both compounds using the CometChip® and standard version. Concerning the Fpg-modified version, almost no DNA in tail (%) was obtained using the CometChip®.

FUNDING. This work was funded by Spanish Ministry of Science and Innovation (BIOGENSA2, PID2020-115348RB-I00).

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The role of succinate and fumarate in the response to cisplatin induced DNA damage

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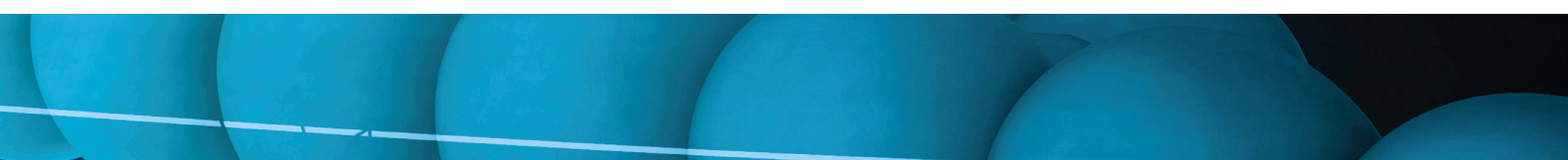
When the genes encoding Krebs cycle enzymes succinate dehydrogenase (SDH) and fumarate hydratase (FH) are mutated, cells accumulate succinate and fumarate, respectively. This increase in metabolites results in dysregulation of energy metabolism, but also in the development of a tumor phenotype, because these oncometabolites inhibit α-ketoglutarate dependent dioxygenases (α-KGDD), like histone and DNA demethylases. Moreover, this inhibition modifies chromatin structure and, consequently, DNA repair activities, and thus might influence cancer treatments such as chemotherapy and radiotherapy.

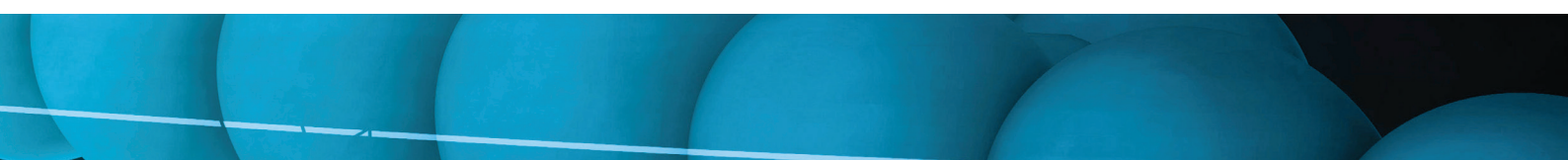
To study the role of the oncometabolites succinate and fumarate in the response to DNA damage induced by a 3h treatment with 20 μM cisplatin, we worked with ovarian carcinoma A2780 cells, sensitive to cisplatin, and with GM04312 human fibroblasts, mutant for the XPA gene and deficient in the nucleotide excision repair (NER) system. We used succinate and methyl-succinate, to check a possible effect of succinate receptor. To analyze this DNA damage response (DDR), we analysed apoptosis, cell cycle progression, viability, clonogenic activity and genomic instability (using the comet assay); cisplatin-induced DNA adducts were determined with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) as DNA-bound platinum. Furthermore, the presence of SDHB and FH proteins was determined by immunofluorescence in both cell lines, and in a third one, SDHB knockout, from a kidney cancer tumor (RCC4).

In A2780 cells, the results show that the oncometabolites, under the conditions and concentrations tested, did not induce relevant mortality, did not modify cell cycle progression or apoptosis, and did not influence clonogenic activity, except in the case of methyl-succinate, but cisplatin did. The % of tail DNA (used as a measure of DNA damage) shows that fumarate and succinate, regardless of concentration, generated DNA damage.

In GM04312 cells, both in pretreatments and co-treatments with these metabolites, the percentage of cisplatin-induced % of tail DNA increased with the highest oncometabolite concentrations. Moreover, these concentrations also increased the cisplatin DNA adducts, suggesting that oncometabolite accumulation prevents the repair of these DNA damage.



SESIÓN V: Inestabilidad genómica, mutagénesis y carcinogénesis
SESSION V: Genomic instability, mutagenesis and carcinogenesis



Genotoxic activity of *Pteridium aquilinum in vivo* in *Drosophila melanogaster*

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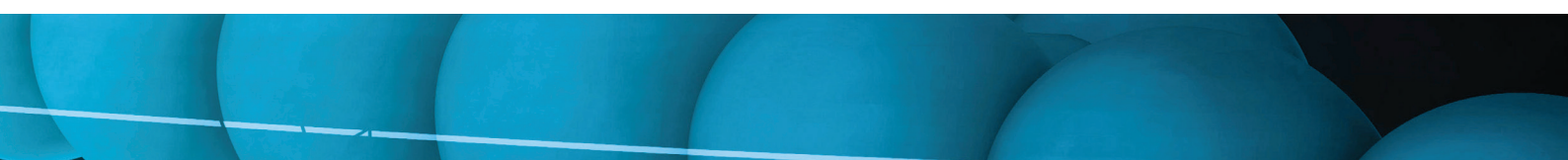
The fern *Pteridium aquilinum* is distributed worldwide since millions of years ago, belonging to open forest communities since then. In the last decades, its prevalence has expanded considerably, partly due to changes in human land-use, but mostly because of the natural aggressiveness of this plant towards competing species. Furthermore, as found many years ago, this fern is the only known carcinogenic plant, through the action of its illudane glycoside metabolites, ptaquiloside, caudatoside and ptesculentoside, and their corresponding pterosins and dienone-like secondary metabolites. This carcinogenic activity, originated by the DNA damage induced by these chemicals, was found not only on livestock animals (sheep, horses and mainly cattle), but also in humans.

Although several studies have demonstrated that plants from different countries and continents present differences in their illudane glycosides contents, there is not information about the possible relationship between the metabolite content and the genotoxic potency of the plants. And there is not information about the genotoxic activity of this plant in Spain, despite its wide distribution in the north part of the country and also in Extremadura.

In this work we have studied the genotoxic activity of aqueous extracts of *P. aquilinum*, collected at different places, *in vivo*, using the eye-SMART assay of *D. melanogaster*, analyzing the effects of time in the preparation of the extracts, and of frond age. Additionally, we have studied the possible genotoxicity of water from fountains, troughs and wells surrounded by ferns.

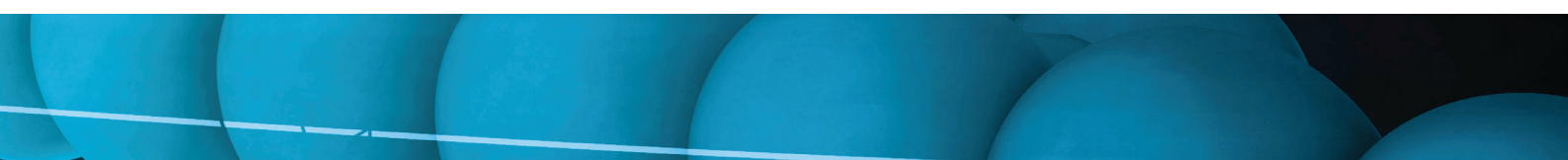
The results show that: (i) all the extracts are potent genotoxins; (ii) there are differences between plants from different places; (iii) there are differences between the genotoxic activity of young and old fronds; (iv) the tested water samples show genotoxic activity, at least when concentrated (3-fold).

While the metabolite content of the several extracts and their relationship with genotoxic activity have still to be determined, these data demonstrate the threat represented by this fern *in vivo* and the necessity to widely spread information about the risk associated to its exposure.



SESIÓN VI: Respuestas genotóxicas: alteraciones fisiológicas y de comportamiento

SESSION VI: Genotoxic responses: physiological and behavioural alterations



Further information about the assimilation and activity of ultra-small, non-magnetic iron oxide nanoparticles with potential use in biomedicine

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The use of iron oxide nanoparticles as therapeutic agents has been a field of interest in biomedicine for some time. Among the wide variety of these particles, the ultra-small (< 10 nm), non-magnetic ones, composed by a ferrihydrite core covered with tartaric and adipic acids (TA-Fe-NPs), are absorbed by the enterocytes as nanoparticles, because of their low solubilization rate in the rat intestinal lumen, and are solubilized inside the cells into free iron. However, since their solubilization might produce reactive oxygen species (ROS), that could be the origin of protein, lipid, and DNA oxidative damage, the assimilation of these TA-Fe-NPs by different cells, as well as their activity, in terms of cell survival and genotoxicity, both *in vitro* and *in vivo*, should be checked.

In this work, we determined both TA-Fe-NP cellular intake and their solubilization in different cultured cells, using HPLC with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) methodology. Cell viability and ROS production in these cells were analyzed after exposure with different concentrations, and the possible induced genotoxicity was evaluated with the Comet assay. *In vivo*, using *Drosophila melanogaster* as model organism, the TA-Fe-NP effects on viability and genotoxicity were studied, using the eye SMART assay, after treating larvae from efficient repair and nucleotide excision repair (NER) deficient strains, (*OK*-NER$^+$ and *mus201*-NER$^-$, respectively), in surface and chronic treatments.

Results show that the NPs solubilize at a slow rate, enter the cells, and increase their Fe content. In addition, TA-Fe-NPs do not impair cell viability and produce low levels of ROS in the studied cell lines. Moreover, in all these cells they induce significant but low increases of DNA damage, only with the highest tested concentration after 3h treatments. These increases, after 24h treatments, were only detected on NER deficient GM04312 cells. *In vivo*, these NPs increase the Fe level in treated larvae, do not show effects on viability and present genotoxic activity only in *OK*-NER$^+$ larvae, after surface treatments.

These data demonstrate that, despite preliminary indication against it, these TA-Fe-NPs might be a good option for the treatment of anemia.

Influence of alpha-synuclein overexpression on DNA damage in neuronal cell lines

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Parkinson's disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons and the presence of intracellular aggregates enriched in alpha-synuclein (alpha-syn). Its underlying cause is still unknown. Recent findings of somatic mutations in human brains might provide evidences associating environmental genotoxicity with the etiology of some neurodegenerative diseases. On the other hand, misfolded alpha-syn has been hypothesized to cause DNA damage in some neuronal cells and to increase the sensitivity of cells to oxidative stress.

Mycotoxins are naturally occurring food contaminants produced as secondary metabolites by filamentous fungi. Among them, ochratoxin A (OTA) is one of the most relevant ones due to its genotoxic and carcinogenic potential as well as to its ability to induce oxidative stress. Recently, our group has demonstrated *in vitro* and *in vivo* that OTA replicates several PD features.

In order to study whether the over-expression of alpha-syn affects the response of cells to DNA damage and oxidative stress, SH-SY5Y neuronal cells and a clone of the same cell line over-expressing full-length human alpha-syn were exposed to the oxidant agent potassium bromate ($KBrO_3$) and OTA. Both cell types were exposed to $KBrO_3$ (0.15 - 2.5 mM) for 3 h or to OTA (0.2 - 25 µM) for 1, 3 and 6 h. The standard and the Fpg-modified comet assays were used to detect DNA strand breaks and oxidized bases, respectively. The proliferation assay was carried out at the same time to study cell viability. $KBrO_3$ showed a dose-dependent increase of oxidized bases, whereas OTA did not produce significant levels of DNA strand breaks or oxidative damage at any concentration or time tested. In general, the response in both cell types was similar. In conclusion, results suggest that over-expression of alpha-synuclein does not change the response to oxidative stress of the cell line and that OTA does not induce DNA damage (i.e, DNA strand breaks, alkali labile sites or oxidized bases) in SH-SY5Y cells.

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Study of the effects of plasmatic concentrations of sertraline, duloxetine and fluoxetine on THP-1 cells using the comet assay

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Antidepressants are widely prescribed for the long-term treatment of major depressive disorder (MDD) and other psychiatric conditions, being selective serotonine reuptake inhibitors and serotonine and noradrenaline reuptake inhibitors first-line drugs. According to the neuroinflammatory hyphotesis, increased inflammatory events in the brain and at the periphery of depressed patients may play a key role in the pathogenesis of MDD. Moreover, a long-term increase of proinflammatory markers is linked to the production of oxygen reactive species (ROS), a major inducer of oxidized bases in the DNA.

Because of the difficulty to recruit drug-free depressed patients, and with the final purpose of studying the effect of this disease on the level of oxidized bases, in this work we aim to study the effect of the three most prescribed antidepressant drugs, at plasmatic concentrations, on this biomarker. We evaluated the potential of duloxetine, sertraline and fluoxetine to induce DNA stand breaks (SBs) and oxidized bases on THP-1 cells after 6 and 24 hours of treatment. For this purpose, plasmatic concentrations of fluoxetine (1 and 10 μM), duloxetine (0.43 and 4.30 μM) and sertraline (0.18 and 1.8 μM) were used and the standard and formamidopyrimidine DNA glycosylase (Fpg)-modified comet assays were applied. Moreover, the vulnerability or resilience of antidepressant-treated cells to $KBrO_3$, an oxidant agent, was also studied.

Results indicate that none of the antidepressants produce SBs or oxidized bases. Moreover, none of the antidepressants alter the level of oxidized bases induced by $KBrO_3$.

Wolbachia alters gene expression related to immunity and energy metabolism in *Chorthippus parallelus* (Orthoptera: Acrididae)

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Wolbachia pipientis is a mainly maternally transmitted obligate endosymbiotic bacterium, widely distributed in insects, with which it establishes complex symbiotic relationships. The continuity of the symbioses may rely on the physiological advantages that *Wolbachia* may confer to their host. Two subspecies of the grasshopper *Chorthippus parallelus* (Orthoptera: Acrididae), the Iberian endemism *C. p. erythropus* and *C. p. parallelus,* which is widely distributed throughout the rest of Europe, differ in morphological, behavioural, mitochondrial, nuclear and chromosomal characters, but also in the strains of the maternally transmitted bacterial endosymbiont *Wolbachia* infecting them. The distribution of both subspecies overlaps in the Pyrenees where they form a stable hybrid zone (HZ), so representing an appropriate system to identify 'key genes' that actually maintain genetic boundaries between emerging species. In fact, *Wolbachia* contributes to the reproductive barrier between both subspecies inducing in them uni- and bidirectional cytoplasmic incompatibilities.

In this work, we *de novo* characterised relevant genes in *C. parallelus*, as potential molecular biomarkers that show the physiological responses in individuals infected by *Wolbachia*, with particular attention to energy metabolism and immunity. *Wolbachia* induces the expression of carbohydrates and lipids metabolic genes as well as some others related to the immune system. This research explores the expression of reporter genes in the gonads of infected and uninfected adults of both sexes performed by means of quantitative real-time PCR. Reproductive organs were chosen since they are the main target of *Wolbachia* infection. Significant *Wolbachia* -and sex- dependent transcriptional effects were observed for most of the analysed biomarkers in infected and non-infected adults. Our data show how *Wolbachia* interferes with essential systems of *C. parallelus*, providing more information about its symbiotic relationship. Our initial, promising results show new sensitive biomarkers suitable for the study of the reproductive barrier that *Wolbachia* induces in the hybrid zone.

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Genotoxicity and DNA methylation patterns associated with Electronic Cigarettes

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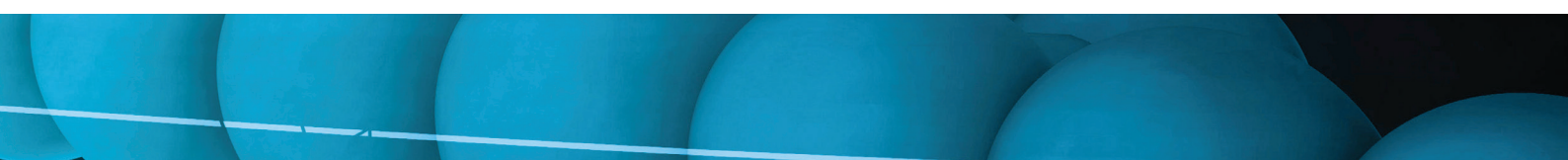
Cigarette consumption is the leading cause of preventable death worldwide. In recent years, it has been claimed that electronic cigarettes (EC) present a safer alternative since they don't use tobacco directly but rather derivatives of it, such as nicotine. Under this premise, indiscriminate consumption was opened and without normative regulation, which has favored a significant increase in the use of EC. Given this background is important to determine the impact of EC use on human health. The aim of this work was to evaluate levels of genotoxicity and LINE-1 methylation levels associated with exposure to EC.

For these purpose, 64 whole blood samples from vaping individuals (n=32) and controls (n=32) were analyzed, in which genotoxicity frequency was determined through cytokinesis blockade micronucleus assay (CBMN). LINE-1 methylation levels were evaluated by quantitative methylation specific assay (qMSP) coupled to quantitative PCR. Additionally, LINE-1 expressions were analyzed by quantitative PCR. Finally, logistic regression analyzes were performed between demographic variables, consumption, serum cotinine biomarker and genotoxicity levels, methylation patterns and transcriptional expressions.

Significant increases in genotoxicity levels associated with use of electronic devices were identified. Additionally, epigenetic alterations related to loss of methylation of LINE-1 elements were detected as a result of exposure to EC aerosol, which in turn was consistent with transcriptional expression increases.

This work highlights the biological impact of vaping and provides a first approach to the scientific evidence that shows that these devices are not completely innocuous and that their use has an impact at the genetic and epigenetic level.



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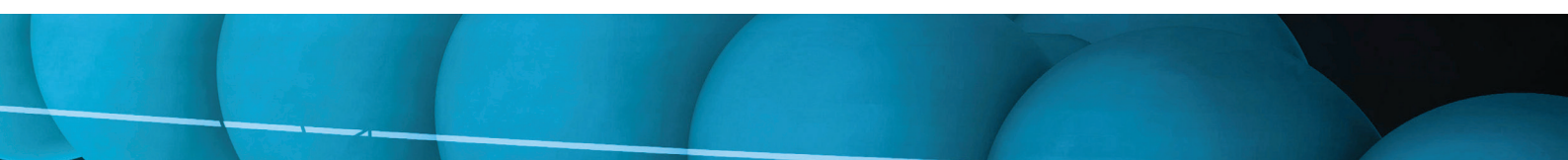

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