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SOCIEDAD ESPAÑOLA DE MUTAGENESIS AMBIENTAL

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XXV Reunión Científica

22 junio 2021
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 **XXV Reunión Científica de la SEMA**. El evento se celebrará online el día **22 de junio de 2021**.

The Spanish Society of Environmental Mutagenesis and Genomics (SEMA), are pleased to invite you to the **XXV SEMA Scientific Meeting**. The event will be held online on **June 22, 2021**.

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SESIÓN I: Contaminantes emergentes
SESSION I: Emerging pollutants

HUMAN WHITE BLOOD CELLS AFTER *EX VIVO* EXPOSURE TO POLYSTYRENE NANOPLASTICS

***Sandra Ballesteros*[1], *Josefa Domenech*[1], *Irene Barguilla*[1], *Constanza Cortés*[1], *Ricard Marcos*[1], *Alba Hernández*[1]**

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Most of the produced plastic ends in the environment as a waste after use, causing an important ecological problem. Once in the environmental matrices, plastic degrades to micro and nanosizes (MNPLs) forming a non-visible source of environmental pollutants. At these sizes, MNPLs can be easily intake by organisms, including humans, supposing a potential health concern. Independently of the exposure route, the uptaken environmental MNPLs end into the body general compartment (blood), potentially interacting with blood cells. In this study, we propose a novel approach to understand the risk of polystyrene nanoparticles (PSNPs) exposure for humans, as a model of MNPLs. Thus, *ex vivo* whole blood samples from different donors were exposed to different doses of PSNPs in exposures lasting for 24, 48, or 72 h. The evaluated effects were determined in different subsets of white peripheral blood cells (WBCs), namely lymphocytes, monocytes, and polymorphonuclear (PMN) cells, to determine specific cellular sensitivity. Our results show no relevant toxicity of PSNPs when evaluated on the overall WBCs population. Interestingly, the different cell lineages manifested sharp differences in PSNPLs uptake with very limited uptake in lymphocytes, and very high uptake in monocytes. Furthermore, significant increases in the levels of DNA damage were observed in monocytes and polymorphonuclear cells, but not in lymphocytes. Furthermore, our results showed that PSNPLs exposure-induced changes in the whole blood secretome. These findings were further confirmed when the expression of different cytokines was analysed, revealing a significant increase in the expression of different cytokines related to the inflammatory, immune, and stress response, as well as cell proliferation. Summarizing, our results support that the *ex vivo* model is a powerful strategy to study the nanoparticles effects on the human blood system. Moreover, they confirm that exposure to PSNPs can negatively affect the WBCs, showing clear differences between the different cell subtypes.

Polystyrene nanoparticles as carriers of environmental pollutants and its toxicological assessment on *in vitro* human intestinal Caco-2 cells

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Micro- and nanoplastics contamination has become a big issue placed in the foreground of mass media, political agendas and public due to its ubiquity and persistence. Thus, humans are exposed to these environmental contaminants through airborne inhalation or dermal contact, but ingestion through food chain contamination is considered the main route of exposure to MNPLs. Although previous studies have depicted its inert behavior on human-derived *in vitro* intestinal models, MNPLs have been described as carriers of other coexisting environmental contaminant which are toxic to humans. Owing to this so-called *Trojan horse* behavior, evaluating the effects of the co-exposures MNPLs/other environmental contaminants is urgent.

Aiming to address this concern, we studied the physical interaction between polystyrene nanoparticles (PSNPs) and silver materials, namely silver nanoparticles (AgNPs) and silver ions (Ag^+ derived from $AgNO_3$) as models of trace metal contaminants. Further experimental approaches were carried out to evaluate potential harmful endpoints in the human intestinal cell line Caco-2.

Firstly, the characterization of the silver/PSNPs interaction was visualized using transmission electron microscopy (TEM), and TEM coupled with energy dispersive X-ray (EDX) was used to confirm the elemental composition of the adsorbed silver on PSNPs' surface. On the other hand, toxic and genotoxic effects of silver materials, nanoplastics and silver/nanoplastic complexes were evaluated to determine whether additive, synergistic or antagonistic effects were induced by the co-exposure. Although cytotoxicity, oxidative stress induction or genotoxicity of AgNPs or Ag^+ on Caco-2 cells were not altered with the addition of PSNPs, a slight increase of silver uptake was detected with increasing concentrations of PSNPs. Nevertheless, taking all the data together, PSNPs do not seem to exacerbate AgNPs or Ag^+ harmful effects, so further investigation is required to elucidate the interaction's implications.

Oncogenic effects caused by *in vitro* long-term co-exposure to polystyrene nanoparticles and arsenic

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The widespread and persistent presence of micro- and nanoplastics (MNPLs) in the environment calls for urgent evaluation of their potential risks. These particles have been found to enter the human body, translocate through physiological barriers, and exert a mild yet relevant impact at different levels: cytotoxicity, ROS generation, DNA damage, and secretome and pro-inflammatory response alterations. However, many questions remain to be answered regarding the potential hazard that MNPLs pose to human health, specially at the long-term. Moreover, increasing interest is being directed towards the likely role of MNPLs as carriers for other more hazardous contaminants, resulting in the so-called *Trojan horse* effect; that is, MNPLs may enhance the bioaccumulation and impact of other pollutants of concern.

Hence, in this work we have aimed to examine the interaction and the long-term joint effects of polystyrene nanoplarticles (PSNPs) and arsenic (As^{III}), both being prevailing water pollutants. Interestingly, we could demonstrate a physical interaction between both pollutants using transmission electron microscopy (TEM) coupled with energy dispersive X-ray (EDX). Regarding the effects of the PSNPs and As^{III} mixture, we established a new model in which cells previously transformed by chronic arsenic exposure were further exposed to PSNPs, As^{III}, and the combination PSNPs/As^{III} for 12 weeks. Our results indicate that the continuous co-exposure enhances the DNA damage and the aggressive features of the initial transformed phenotype. Remarkably, when compared to cells exposed to arsenic or PSNPs alone, the co-exposed cells present a higher proportion of spindle-like cells within the culture population, an increased capacity to grow independently of anchorage, as well as enhanced migrating and invading potential.

Therefore, our work reveals the MNPLs' potential to exacerbate arsenic-transforming effects. Besides, this study highlights the need to further explore the long-term effects of MNPLs and the importance of considering their role as carriers for other pollutants to effectively perform risk assessment.

In vivo and *in vitro* genotoxicity of new ultra-small non-magnetic iron oxide nanoparticles with potential use in Biomedicine.

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Ultra-small (<10 nm) non-magnetic iron oxide nanoparticles, with a core similar to ferrihydrite and coated by tartaric and adipic acids (TA-Fe NPs), were designed a few years ago with a high potential in Biomedicine, as an anemia treatment and/or a drug nanocarrier. They showed a high cellular uptake, low or null toxicity and low solubilization rate. Moreover, in rat models, they reach the small intestine and iron was absorbed at high levels (79%), without effects on cell viability, DNA damage (*in vitro* on cultured cells) or lipid peroxidation, and with rather low levels of reactive oxygen species (ROS) production. Despite these promising data, to assure their biosafety for clinical uses, more complete genotoxicity assays must be performed. Because of that, in this work we have analyzed the possible genotoxicity of these TA-Fe NPs both *in vivo*, using *Drosophila melanogaster* as model organism, and the eye SMART assay to detect induction of mutation and/or recombination in somatic cells, and *in vitro*, using several human cell lines, and the Comet assay to detect induction of DNA strand breaks.

The SMART assay was performed in efficient and deficient nucleotide excision repair conditions (NER^+ and NER^-, respectively), and with chronic and surface treatments, with NP concentrations between 0.1 and 5 mM. Methyl methanesulfonate (MMS; 2.5 mM) was used as positive control. The alkaline Comet assay was performed in A2780, Caco-2 and Hep92 cell lines with concentrations of NPs between 0.25 and 2 mM, in 3 h treatments; 0.25 mM MMS was used as positive control.

Results showed that TA-Fe NPs are genotoxic *in vivo*, with surface treatments and doses over 2 mM, without detected toxicity, in NER^+ conditions. However, no genotoxicity was detected in chronic treatments in the same repair conditions, nor in NER^- conditions, independently of the treatment. *In vitro*, these NPs showed genotoxicity in all the analyzed cells, but only with the highest (no toxic) concentrations. These results demonstrated that these NPs might not be safe enough to be used for anemia treatment. However, they might still be employed as nanocarriers for antitumour drugs, such as cisplatin, because in this case their genotoxicity could represent an added value.

Evaluación de los efectos del tetrabromobisfenol A y nanoplásticos de poliestireno en células de trucha arcoíris RTgill-W1

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La preocupación sobre los contaminantes emergentes, como los retardantes de llama bromados (BFRs) y nanoplásticos, está aumentando debido a su persistencia y presencia ubicua en el medio ambiente. La mayoría de estos compuestos pueden bioacumularse en la cadena trófica, causando efectos negativos en la salud humana y medioambiental. Se cree que la interacción de los nanoplásticos con otros contaminantes podría modificar los efectos tóxicos en los organismos acuáticos, aumentando el interés en este campo de estudio.

Este trabajo evalúa los efectos adversos del tetrabromobisfenol A (TBBPA), uno de los BFR más prevalentes, y nanopartículas de poliestireno (NPs, CML Latex Beads, 40 nm) en una línea celular epitelial de branquias de trucha arcoíris (RTgill-W1) después de la exposición individual y combinada de los compuestos durante 24 horas. Se han utilizado diferentes medios de cultivo para comparar su influencia en la citotoxicidad: Leibovitz L15 (L-15) con un 10% de suero, L-15 con un 2% de suero y L-15/ex. Se emplearon tres ensayos complementarios para evaluar la actividad metabólica (Alamar Blue, AB), la integridad de membrana (5-Carboxyfluoresceina Diacetato Acetoximetil Éster, CFDA-AM) y el compartimento endosomal (Captura del Rojo Neutro, NRU). Después de evaluar exposiciones individuales en rangos de 0,27 µg/mL a 108,8 µg/mL de TBBPA y 0,1 µg/mL a 200 µg/mL para los NPs, se seleccionaron condiciones relevantes para las coexposiciones. Además de la evaluación citotóxica se realizaron ensayos complementarios, incluyendo cambios en la resistencia transepitelial (TEER), evaluación morfológica de los cultivos celulares y estudios de genotoxicidad mediante el ensayo de cometa alcalino.

Nuestros resultados sugieren que ambos, TBBPA y NPs, tienen efectos negativos en la viabilidad de las células RTgill-W1 bajo nuestras condiciones experimentales, tanto de forma individual como combinados. El daño es dependiente de la composición del medio y la actividad metabólica es la prueba más sensible. La presencia de suero en el medio de cultivo influye en el comportamiento de los NPs, induciendo agregación y tamaños mayores de las partículas, lo que podría explicar los efectos observados. Los resultados del estudio de genotoxicidad indican que las muestras tratadas con las concentraciones seleccionadas de TBBPA y NPs presentan un incremento en los sitios de sensibilidad con la enzima FPG (formamidopiridina DNA glicosilasa).

Effects of the *in vitro* digestive process on the toxicological profile of polystyrene nanoplastics in different human hematopoietic cell lines

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In the past few years, growing production/consumption of plastic have turned plastics into the world's largest polluter. Plastics released into the environment reduce their size range into micro-nano plastics (MNPLs) by the effects of many environmental agents. Human exposure to MNPLs can be through ingestion, airborne inhalation, or dermal exposure. However, oral ingestion is considered the major exposure route. Once MNPLs enter the human body via the oral route they must pass through different compartments of the gastrointestinal tract that may affect physicochemical properties and surface features. Probably one of the most important parameters that digestion could affect is the formation of the protein corona, affecting biological interactions and intestinal uptake of particles. To effectively analyze the toxicity of MNPLs, the influence of the digestive tract environment should be considered. Polystyrene micro-nano plastics (PS-MNPLs) are one of the most frequently observed MNPLs in the environment. PS-MNPLs have demonstrated their ability to translocate from the intestinal mucosal tissues and access the blood and lymphatic circulation. For this reason, the aim of this study is to determine the influence of the digestive process on the toxicity of PS-NPLs in three different human leukocytic cell lines: Raji-B (B-lymphocytes), TK6 (lymphoblasts) and THP-1 (monocytes).

An *in vitro* digestion assay was performed on pristine PS-NPLs (50 nm). Using transmission electron microscopy (TEM-EDX), scanning electron microscopy (SEM- TDX), and Z-sizer, PS-NPLs were characterized. The three cell lines were exposed to different concentrations of *in vitro* digested PS-NPLs (dPS-NPLs). Cytotoxicity, reactive oxygen species (ROS) production, and genotoxicity were assessed at different time points. Preliminary results show no significant cytotoxicity effects in the cell lines. Only moderate effects were observed at the highest concentration of dPSNPs in TK6 at 48 h exposure. Regarding intracellular ROS production measured by flow cytometry with DHE, a slight increase of ROS production was observed in TK6 and Raji-B cell lines at 24 h of exposure, but without reaching statistically significant. Additionally, the comet assay is going to be assessed to measure the levels of genotoxic and oxidative DNA damage.

Interactions of in-house made polyethylene terephthalate nanoparticles with human hematopoietic cell lines as an improved model for assessing the health risk of nanoplastics

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Despite the increasing literature in the field of exposure or detection of environmental micro- and nano-plastics, few data has been reported at the nanoscale level. This is considering a nanoscale that is under 1 µm not under 100 nm, as the definition adopted by the European Union on 2011. Moreover, the reported literature on their biological effects is mainly focused in the use of commercial pristine perfectly round engineered material, which is not difficult to conceive to be different to the polydispersity in terms of size and shape that can be found in nature or more accurately in the environment. To solve this gap, hereby we propose the use of polyethylene terephthalate (PET) samples intentionally degraded to nanoscale size starting from environmental PET bottles, which can mimic more accurately nanopollutant (nanoPET). To evaluate the potential effects of such samples we use human hematopoietic cell lines such as THP-1 (monocytes) and TK6 (lymphoblast). The NanoPET size and shape were analyzed by transmission electron microscopy (TEM), while the size measurement was compared with diameter measurements by dynamic light scattering on zetasizer ultra, confirming the nanoscale of the particles in the preparations. Particle concentration was also measured by these means. Fourier transform infrared spectroscopy (FTIR) analysis confirmed the presence of PET in the preparations and scanning electron microscopy coupled with EDX confirm the chemical composition of the NanoPET suspension.

By staining the NanoPET suspension with fluorescent Nile red we success to demonstrate cell uptake, as observed by flow cytometry. In spite of the observed internalization, our preliminary results show no significant biological effects on cell viability at exposures lasting for 24 and 48 h. Furthermore, no induction of intracellular reactive oxygen species (ROS) was detected after 3 or 24 h exposures, using the dihydroethidium (DHE) methodology. Further effects on other biomarkers are under development.

SESIÓN II: Daño al ADN e inestabilidad genómica
SESSION II: DNA damage and genomic instability

Novel insights into biodegradation, interaction, internalization, and impacts of high-aspect-ratio TiO_2 nanomaterials: A systematic *in vivo* study using *Drosophila melanogaster*

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The elongated nature of the high-aspect-ratio nanomaterials (NMs) can help us to obtain valuable information on its biodegradation, physical interaction with target-cells, and internalization. Three different length nano-titanium has been studied using *Drosophila*, TEM, and different biological markers. Nano-titanium regardless of their shape was eroded and degraded just entering the gut lumen of the larvae.

Results showed that the distinguished shape of nanowires helps to understand the interactions of NMs with the intestinal barrier. The peritrophic membrane, as the first defense line of the intestinal barrier, succeeded in the reservation of NMs, though the perpendicular particles of nanowires stabbing it, making pores, permitting their translocation into intestinal cells. On the other side, the exposure to TiO_2NPs did not decrease egg-to-adult viability, but all its different shapes, especially nanowires, mediated a wide molecular response including changes of expression in genes involved in stress, antioxidant, repair, and physical interaction responses. All these changes concerning their ability to elevating ROS level ultimately led to potential genotoxicity.

So, the high aspect ratio NMs are efficient in understanding the outstanding issues of NMs exposure, but at the same time could induce genotoxic impact rather than the low aspect ones.

TOWARDS A HIGH-THROUGHPUT COMET ASSAY

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Introduction: Comet assay is a sensitive, versatile and inexpensive technique used in genotoxicity testing in fields such as pharmaceuticals for human use and food additives. The objective of this study was to increase comet assay throughput decreasing the time of electrophoresis through the application of a higher voltage than the one used in the standard protocol.

Materials and methods: A standard comet assay protocol, applying 20 minutes electrophoresis at 1.1 V/cm, was followed in untreated and methyl methanesulfonate (MMS)-treated TK6 cells. Afterwards, different protocols using different times of electrophoresis (2.5 minutes, 5 minutes, 10 minutes, 15 minutes and 20 minutes) and the maximum voltage that the power supply could reach were tested (~ 3 V/cm). In all experiments, temperature and voltage, from power supply as well as from an external voltmeter, were recorded. Finally, another electrophoresis at the selected timepoint was run in order to compare results with the standard protocol.

Results: An electrophoresis time-dependent increase in the % DNA in tail was observed in MMS-treated cells subjected to a high electric field. This increase was also observed in untreated cells after 10 minutes of electrophoresis. Some difficulties were found in comet scoring after 10, 15 and 20 minutes of electrophoresis. Voltage and temperature varied during the electrophoresis. Results similar to the ones obtained with the standard protocol were observed between 5 and 10 minutes of electrophoresis so, a 7-minute electrophoresis at high voltage was the selected timepoint to compare with the standard comet assay. In 300 µM MMS-treated cells, a 35.3 % DNA in tail was obtained after 7 minutes at high voltage in comparison with 41.2 % DNA in tail for the standard protocol. Both negative controls had low % DNA in tail values.

Conclusions: A short and strong electrophoresis can be use as a strategy to shorten the comet assay protocol.

Keywords: Comet Assay; Electrophoresis; DNA damage; genotoxicity

Salivary leucocytes: a suitable non-invasive alternative for the comet assay in human biomonitoring studies

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Traditionally, studies that explore DNA damage in populations by the comet assay employ leucocytes isolated from peripheral blood (PBL). However, validation of alternative non-invasive biomatrices would suppose an extension and potential advantage in the enforcement of this assay in human biomonitoring. The aims of this work were to test the validity of salivary leucocytes (SL) as a suitable sample for the comet assay, to evaluate the ability of this approach to detect different types of primary and oxidative DNA damage, and to determine whether frozen SL are still adequate to show these types of DNA damage. Fresh and frozen leucocytes isolated from saliva samples (six healthy non-smoking volunteers) were exposed to different kind of genotoxic agents inducing both primary DNA damage (methyl methanesulfonate, actinomycin-D, ultraviolet radiation) and oxidative damage (potassium bromate), and standard or enzyme-modified comet assay was carried out. Results were compared with those obtained from PBL. Dose-dependent increases of primary and oxidative DNA damage were found in cells exposed to all genotoxic agents, demonstrating the adequacy of these samples to detect genetic damage from different nature. Comparing basal DNA damage, only a mild significant increase in primary DNA damage in frozen SL relative to the other biomatrices was obtained, but similar outcomes were found regarding sensitivity to induction of DNA damage by all agents tested. This work demonstrates that SL can be used in comet assay as an alternative or complement to blood samples. Frozen SL were proved to be a very suitable sample in wide biomonitoring studies.

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Complementary function of AP endonucleases and AP lyases during Base Excision Repair: an unsolved question

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Abasic (apurinic/apyrimidinic, AP) sites arise frequently in DNA by cleavage of the N-glycosylic bond between the nitrogenous base and deoxyribose. Such cleavage may occur by spontaneous hydrolysis or by the catalytic activity of DNA glycosylases on damaged or modified bases. AP sites may be processed either by AP endonucleases or AP lyases, but the relative roles of these two classes of enzymes are not well understood. An unresolved question is whether the sequence flanking the AP site and/or the orphan base on the opposite DNA strand influence the probability of processing either by an AP endonuclease or an AP lyase. AP sites opposite G are common intermediates during repair of deaminated cytosines, whereas AP sites opposite C arise during repair of oxidized guanines. We have analyzed the activity of plant and human AP endonucleases and AP lyases on DNA substrates containing an abasic site opposite either G or C in different sequence contexts. In all contexts the major *Arabidopsis* AP endonuclease (ARP) exhibited a significantly higher activity on AP sites opposite G. In contrast, the main plant AP lyase (FPG) showed a greater preference for AP sites opposite C. The major human AP endonuclease (APE1) preferred G as the orphan base, but only in some sequence contexts. No preference for the orphan base was observed in the AP lyase activity detected in human cells extracts. We propose that plant AP endonucleases and AP lyases play complementary repair functions on abasic sites arising at C:G pairs, neutralizing the potential mutagenic consequences of C deamination and G oxidation, respectively.

A new component of the cellular response to TOP1-induced DNA damage: the ALS protein FUS

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FUS (Fused in Sarcoma) is a nuclear RNA/DNA binding protein that plays a key role in multiple steps of RNA metabolism and the DNA Damage Response. Autosomal dominant mutations in FUS have been associated with both familial and sporadic cases of ALS (Amyotrophic lateral sclerosis), the most common adult-onset motor neuron disease, characterized by progressive degeneration of motor neurons. Of particular threat to neural maintenance and function is DNA damage induced by topoisomerases, a class of enzymes that remove torsional stress from DNA by creation of transient DNA strand break. It has been proposed that ALS mutations cause pathological changes in FUS-regulated gene expression and RNA processing, due either to loss of normal FUS function, toxic gain of function, or both. However, the nature of the endogenous sources of DNA damage that might trigger a requirement for FUS and/or other RNA-processing factors is unknown. Here, using a variety of different cell types, including human spinal motor neurons, we showed that FUS is a component of the cellular response to topoisomerase I (TOP1)-induced DNA breakage. FUS relocalised from nucleoplasm to sites of nucleolar rRNA synthesis in response to RNA polymerase II transcriptional stress induced by abortive TOP1 DNA breakage. This relocalisation was rapid and dynamic, reversing following the removal of TOP1-induced breaks and coinciding with the recovery of global transcription. The molecular role of this response is unclear, but we propose that FUS moves from sites of stalled RNA polymerase II to sites of RNA polymerase I activity either to regulate pre-mRNA synthesis and/or processing during transcriptional stress, or to modulate some yet unidentified aspect of rRNA biogenesis. Finally, we found that HeLa cells and ALS patient fibroblasts expressing mutant FUS are hypersensitive to TOP1-induced DNA breakage, highlighting the possible relevance of our findings to ALS disease pathology.

Particulate Matter (PM_{10}) alters Nucleotide Excision Repair pathway in lung epithelial cells

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Particulate matter with an aerodynamic diameter ≤10 μM (PM_{10}) is a major air pollutant and is classified as a carcinogen, primarily to the lung. PM_{10} is a heterogeneous mixture of metals, endotoxins and polycyclic aromatic hydrocarbons (PAH), which induces genotoxic damage, including PAH-DNA adducts formation; however, the mechanisms of carcinogenicity associated with exposure to PM_{10} need to be more precisely defined. The nucleotide excision repair pathway (NER) is responsible for repairing bulky DNA lesions, including adducts. This pathway includes more than 30 proteins, among which XPC, RAD23, XPD, XPA and ERCC1 act during the recognition, verification and repair of damage by binding to the DNA strand and forming protein complexes. Specifically, XPA requires posttranslational modifications for proper DNA repair. If DNA damaged is not correctly repaired, genomic instability and mutations might occur. DNA repair pathways can be inhibited by multiple compounds that are present in PM_{10}, nevertheless, the effect of PM_{10} exposure on the functionality of the NER pathway has not been described. In this study, it was evaluated whether PM_{10} (10 μg/cm^2) modified the levels, posttranslational marks and complex formation of the main NER proteins and whether these changes have an impact on repair function in A549 human lung cells. It was found that exposure to PM_{10} induce the formation and accumulation of benzo[a]pyrene diol epoxide-DNA adducts with an inhibition of NER pathway activity. PM_{10} exposure increased the levels of RAD23 and XPD, responsible for the recognition of damage and opening of the DNA strand, respectively and increased the levels of H4K20me2, that acts as a recruitment signal for XPA, the principal scaffold protein of this pathway. However, a decrease in the levels and phosphorylation of XPA at serine 196 was found (pXPA^{S196}) associated with the increase of phosphatase WIP1 levels, besides, the formation of the protein complex between XPA and RPA is inhibited. We conclude that PM_{10} deregulates the NER pathway and consequently induce the accumulation of DNA adducts in A549 human lung cells. These findings help to understand how PM_{10} exposure is a risk factor for lung carcinogenesis.

In vitro genotoxicity assessment of an antiseptic formulation containing silver nanoparticles

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The increase of antimicrobial resistance is one of the most concerning public health problems worldwide. A material composed of kaolin containing silver nanoparticles on its surface (AgNPs-kaolin), is being evaluated to be applied in animal feed as an alternative to antibiotics. AgNPs-kaolin safety is being assessed following the EFSA "Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health".

An *in vitro* genotoxicity evaluation has been carried out following the strategy recommended in the EFSA guidance, under good laboratory practices (GLPs). Given the nature of the AgNPs-kaolin, the assessment has included three *in vitro* assays: i) the mouse lymphoma assay (MLA) to evaluate the induction of gene mutations, ii) the micronucleus (MN) test to evaluate the induction of structural and numerical chromosomal aberrations, and iii) the standard and formamidopyrimidine glycosylase (Fpg)-modified comet assay, as a complementary assay to evaluate the induction of pre-mutagenic lesions, in particular strand breaks and oxidized bases.

The MLA was performed following the OECD TG 490. Both AgNPs-kaolin and Ag/AgNPs released from the antiseptic formulation after agitation in cell culture media, were tested on L5178Y $Tk^{(+/-)}$ cells. Five concentrations in the range of 0.37-10 mg/mL, determined by previous cytotoxicity assays, were tested. The MN test was performed following the OECD TG 487. Both AgNPs-kaolin and released Ag/AgNPs were tested on TK6 cells, at four concentrations in the range of 0.0185-0.5 mg/mL, determined by previous cytotoxicity assays. For the comet assay, both AgNPs-kaolin and released Ag/AgNPs were tested on TK6 cells, at five concentrations in the range of 0.0067-0.5 mg/mL, determined by previous proliferation assays. Given the nature of the testing material, metabolic activation was not used in any of the assays. Negative results were obtained from every *in vitro* assay in every condition. Due to the complexity of the formulation, an *in vivo* evaluation is being carried out, following EFSA recommendations.

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Influence of oncometabolites in the response to DNA damage

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Mutations in genes encoding Krebs cycle enzymes, such as succinate dehydrogenase (SDH) and fumarate hydratase (FH), result in the acucumulation of their respective metabolites, succinate and fumarate. This accumulation produces a deregulation of the energetic metabolism, and leads to the development of a tumoural phenotype. Besides, through the alteration of chromatin structure, by inhibiting histone and DNA demethylases, these metabolites might modify DNA repair and, then, influence cancer treatments, like chemotherapy and radiotherapy. With this in mind, the general aim of this work is to study the impact of these molecules on DNA damage responses (DDR), after treatment with different genotoxic agents.

PC12 cells, from a rat pheochromocytoma of the adrenal medule, were used to study the influence of succinate and fumarate in the response to hydrogen peroxide, and human GM04312 cells (*XPA* mutant) to cisplatin induced DNA damage. Presence of the proteins SDHA, SDHB and FH were determined by immunofluorescence in PC12 cells. The influence of metabolites was studied with 1 h pretreatments, using 1-5 mM concentrations (lower than the respective IC50), followed by treatments with 200 μM H_2O_2 for 10 min, or with 20 μM cisplatin for 3 h. Methylated and/or unmethylated metabolites were used to check the effect of the cell-entering pathway. DDR were studied analysing cell viability, cell cycle progression, apoptosis, and DNA damage.

Results show that these metabolites, at the analyzed conditions and concentrations, were not toxic, and did not modify cell cycle progression nor apoptosis; however, they slightly increased the detected DNA damage, probably due to an impaired repair of spontaneous DNA damage. In response to the genotoxic agents, although no effects on viability or cell cycle progression were detected, influences of these metabolites were detected on apoptosis and induced DNA damage. Furthermore, differences were found between the methylated and unmethylated metabolites, both for DNA damage and apoptosis. In general, fumarate and methylfumarate increased the genotoxin-induced DNA damage, whereas succinate and methylsuccinate did not. Moreover, succinate and methylsuccinate showed different effects, problably related with their cell-entering pathway.

SESIÓN III: Cambio climático y contaminación ambiental
SESSION III: Climate change and environmental pollution

Transcriptional response of *Diamesa zernyi* (Chironomidae) reveals metabolic alterations due to chlorpyrifos exposure in glacier-fed streams

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Over the years, pesticides have been in an imbalance between the benefits for agriculture trough control pests and their harmful power on non-target organisms. Pesticides can be transported at a medium-high distance due to the drift effect, reaching mountain regions, glaciers, and snow covers, previously consider as pristine areas. With the melting process, pesticides enter the freshwater ecosystems, polluting and becoming a threat for wildlife fauna, dominated by Diptera Chironomidae. Chlorpyrifos (CPF), an organophosphorus insecticide, is one of the most used in alpine vineyards and apple orchards and frequent in icemelt waters. Previous studies have demonstrated the toxicity of CPF on the cold stenothermal chironomid *Diamesa zernyi*, with mobility and biochemical alterations. In this study we use *D. zernyi* as a target species due to its predominant role in this ecosystem, to address a novel approach that assesses CPF alterations at the molecular level. Larvae were collected in two glacier-fed streams (Presena and Amola) in the Italian Alps. Firstly, *de novo* transcriptome was obtained, and secondly, a 48-gene array was designed to study the molecular response of a wild population of *D. zernyi* exposed to three sub-lethal CPF concentrations corresponding to 1/100 LC10 (0.011 µg/L), 1/10 LC10 (0.11 µg/L), and LC10 (1.1 µg/L), for 24h. The sub-organismal response was evaluated by Real-time PCR (RT-PCR). After 24h, the endocrine system (*E75*, *E93*, *EcR*, and *Met*), detoxification response (*GSTO3*, *GSTS1*), and stress response (*hsp75*, *hsp83*, and *HYOU1*) resulted altered. These effects of CPF could deriving in defective larval development, disrupt the cellular homeostasis (deriving in defective protein folding and mitochondria functions), and finally, cause oxidative damage (confirmed by GSTs increased expression). For the first time, molecular studies detected early alarm signals on wildlife *Diamesa* in glacier environments providing a new tool on the assessment of environmental risks and freshwater toxicology in these ecosystems. Our findings confirm the high environmental risk of CPF affecting aquatic insects' metabolism, and our results raise the level of concern about this pesticide for high altitude water bodies, considered generally pristine. We also confirm the importance of including molecular approaches in the toxicology evaluation to detect early adverse effects of pollutants before they reach higher levels of organization.

Potential health risk from Organochlorine pesticides in Tecocomulco Hidalgo, Mexico

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The objective of this research is to estimate the potential health risks due to chronic exposure to organochlorine pesticides in the Tecocomulco Hidalgo lagoon, Mexico. The identification and quantification of organochlorine pesticides from the sediment of the lagoon was carried out, the presence of six compounds (Aldrin, DDT, Endosulfan, Endrin, Dieldrin and Lindane) was verified, of which five are above the maximum permissible levels in the rule, Guidance for Sediment Quality Assessments. The dose-response relationship was evaluated through the biological effect through the analysis of effect indicators. The following indicators were calculated: risk index, daily exposure dose, potential for toxicity, potential for mutagenicity, probability of cancer, risk of cancer development, and cancer incidence. Five compounds have a higher hazard ratio than the norm (Aldrin> Endosulfan> DDT> Endrin> Dieldrin). The six compounds are toxic, mutagenic, teratogenic and carcinogenic, with DDT> lindane> Endosulfan being the pesticides with the highest incidence of cancer. Due to the agricultural activities in the study area, it was determined that there is a high vulnerability of the exposed sectors, a high index of danger due to the observed concentrations, a high exposure rate and a high capacity for toxic, mutagenic, teratogenic damage. and carcinogenic; therefore, there is a potential high health risk associated with exposure to these compounds.

Particulate matter PM10 destabilizes mitotic spindle trough downregulation of SETD2 function in A549 lung cancer cells

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Background: Air pollution represents a global problem, impacting negatively in human health. This effect is seen predominantly in major cities, where intense traffic or industry activities are common. Particulate matter of 10 micrometers or less in diameter (PM_{10}) is considered as an agent related to pulmonary diseases, including lung cancer. Chromosomal segregation is controlled by chromosome-microtubule interactions, where SETD2 plays an important role in microtubule stability through binding to alfa tubulin (α-TUB) and promoting microtubule polymerization. Also, spindle assembly checkpoint (SAC) orchestrate a mitosis-delay signal through proteins such as BUBR1, AURORA B and SURVIVIN, to ensure genomic instability. Alterations in microtubule stability as well as in SAC cause aneuploidy, a cancer hallmark. Although PM_{10} are associated to the generation of chromosomal alterations, the impact in chromosomal segregation mechanisms still being an opportunity area. The **aim** of this study is to evaluate the effect of PM_{10} in the expression of SETD2, as well as the effect in the expression of SAC and mitotic genes in the control of chromosomal segregation/mitosis, using the A549 cell line (lung cancer). **Materials and methods:** A549 cells were exposed to PM_{10} (10 μg/cm^2) for 24 h to evaluate protein levels of SETD2, α -TUB, CICLIN B, BUBR1, AURORA B and SURVIVIN by Western Blot. As controls of SAC activation, cells were exposed to taxol (100nM) and nocodazole (0.2 μg/mL). **Results:** PM_{10} decreases the levels of SETD2 (36.6%), α -TUB (27.3%) and BUBR1 (23.3%), and increases the levels of AURORA B (22.6%) and SURVIVIN (83.6%) in A549 cells, compared with non-treated cells ($p<0.05$). PM_{10} also caused a decrease in mitotic index (37.3/300 cells) when compared with control group (49/300 cells). Co-localization of SETD2/ α -TUB was lower in PM_{10}-treated cells in comparison with non-treated cells (43.6/300 cells vs 82.3/300 cells, respectively). Finally, micronuclei (MN) frequency was higher in PM10-treated cells in contrast with non-treated cells (36.3/500 cells vs 13.3/500 cells, respectively), being the presence of whole chromosomes more common in PM_{10}-treated MN than in non-treated MN (24.6/500 cells vs 9/500 cells, respectively). **Conclusion:** Particulate matter PM_{10} induce missegregation and aneuploidy, through a downregulation of SETD2 and SAC components, probably inducing survival and predisposing to the generation of aneuploid transformed cells.

Prodiamesa olivacea, a potential sentinel organism for ecotoxicity studies in natural scenarios

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Toxicological studies on non-model organisms complement and provide powerful information regarding natural ecosystems and along with traditional approaches, they confer a breakthrough in ecotoxicology. *Chironomus* (Diptera) has four OECD standardized tests that assess classical toxicity parameters for evaluating water and sediment toxicity (survival, immobilization, reproduction and development). *Prodiamesa olivacea* (Diptera) is a non-model aquatic insect species not used in toxicity tests and that frequently shares habitat with *C. riparius*, although it requires higher oxygen levels and less extreme conditions. Regarding this, it is of special interest to study the possible differences in the response of both species to pollutant exposure.

This work characterizes and tests the effectiveness of early effect biomarkers related to environmental pollution in water on natural populations of *P. olivacea* larvae under different stress conditions. This will contribute to increasing the limited knowledge about xenobiotics effects on benthic aquatic invertebrates, one of the most sensitive group. The *de novo* RNAseq transcriptome was obtained from *P. olivacea* and used to identify and characterize genes related to stress and immune system responses such as *Hsp60, Hsp70, PGRP, Toll* or *JAK/hopscotch* among others. Quantitative real-time PCR was used to evaluate the expression of selected genes. In this study, the toxicity of benzyl butyl phthalate (BBP; CAS No. 85-68-7) was elucidated in *P. olivacea* and *C. riparius* species from a polluted river (Sar) in Galicia (Spain). Transcriptional effects of acute BBP 4-h and 24-h exposures were evaluated, and the results revealed species-dependent gene responses. *P. olivacea* showed a higher sensitivity to BBP than *C. riparius*, as more severe effects were observed for all the analyzed biomarkers.

This research provides new tools for assessing and monitoring water quality and it highlights the importance of a multi-organism ecotoxicological approach to deep into BBP toxicity. It is essential to assess the tolerance / sensitivity of not only natural populations of model organisms but also non-model insect species chronically exposed to complex mixtures of pollutants. This approach will avoid drawing incomplete conclusions in the light of highly tolerant model organisms. This approach will allow us to have a broader view of the risk associated with pollutant's presence in ecosystems in the short, medium, and long term.

SESIÓN IV: Respuestas genotóxicas y toxicología computacional
SESSION IV: Genotoxic responses and computational toxicology

In silico and *in vitro* characterization of mycotoxins of genotoxic concern.

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Mycotoxins (more than 300 compounds) are food contaminants produced as secondary metabolites by filamentous fungi. The enhanced incidence of emerging mycotoxins and modified forms and the characterization of mixtures are bringing new challenges. As the number of possible combinations is very high, it is important to develop an efficient tiered strategy to prioritize the mycotoxins (and combinations) that should be evaluated from a toxicological point of view. The genotoxic potential of individual mycotoxins was characterized by two levels of evidence, i*n silico* and *in vitro,* in three phases. The *in silico* approach was shaped with two predictive tools: DEREK, a knowledge-based expert system for qualitative toxicity prediction (phase 1) and Vega, a qualitative structure-activity relationship (QSAR) model platform (phase 2). Levels of evidence “certain, probable, plausible or equivocal” were considered positive with DEREK and “inactive or nothing to report”, negative. VEGA label “mutagenic” was positive, “no mutagenic” negative and “suspect mutagenic” equivocal. *In vitro* testing was conducted with the SOS/umu test (phase 3) with three assay conditions. As usual, the assay was carried out without and with the commonly used liver metabolic activation (liver S9). For the first time, kidney metabolic activation (kidney S9) was used to assess potential renal metabolites of the mycotoxins. Indeed, in this case, it was observed that kidney S9 was able to activate the positive control 4-nitroquinoline which is not activated by liver S9.

The concordance among the *in silico* and *in vitro* tools for the well-characterized mycotoxins, aflatoxin B1 (positive) and ochratoxin A (negative), validated the strategy. Sterigmatocystin was considered genotoxic in all phases, except when kidney S9 fraction was used. Regarding *Fusarium* mycotoxins, deoxynivalenol and fusarenone-X were negative except with DEREK. Some trichothecenes type B (nivalenol and deoxynivalenol acetylated forms) showed inconclusive results: positive (DEREK), equivocal (kidney S9) and negative (VEGA and liver S9). Zearalenone was negative in all phases. The kidney S9 fraction, revealed a higher genotoxic response for some mycotoxins: i) T-2 toxin was positive except when VEGA (negative) and the liver S9 fraction (equivocal) were used, ii) HT-2 was positive in all phases except after liver bioactivation, and iii) fumonisin B1 was predicted as negative in all phases except for the kidney S9 fraction (equivocal).

Toxic effects of the methyl ketone 2-dodecanone in *Drosophila melanogaster*

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Among the plant defence mechanisms against insects, glandular trichomes are specialised hairs responsible for an important part of their secondary metabolites. Some of these compounds are toxic substances that compromise insect survival and may delay their growth and pupation. Methyl ketones are a widely-produced group of chemicals synthesised by trichomes, and their insecticidal efficacy has been described against some arthropods, such as aphids or spider mites. However, information about their mode of action and molecular effects are still very scarce. Few models offer the opportunity to perform an integrated study with multiple approaches, from molecular variations to physiological consequences. In this sense, *Drosophila melanogaster* is a proven model organism for research in genetics and biology. The fruit fly has also been considered a suitable model in ecotoxicology in recent years due to its short developmental time and its extensive and well-described genome information.

This work analysed the effect of 2-dodecanone on different developmental stages of *D. melanogaster*. Specifically, transcriptional alterations induced by sub-lethal concentrations (5 µg/L and 500 µg/L) of this methyl ketone were evaluated in third instar larvae (acute 24-hour exposures), as well as in adult males and females (chronic full life-cycle exposures). Fertility (average number of eggs) in exposed adults was also analysed. Quantitative real-time PCR (qPCR) was used to measure the expression levels of selected genes related to the endocrine system (*EcR, ERR, HR3,* and *BR-C*), the cell-stress signalling pathway (*Tl*, *def*, *p38*, *hsf*, *hsp22*, *hsp40, hsp70,* and *hsc70*), and detoxification mechanisms (*cat*, *sod*, and *phgpx*). Our results showed that 2-dodecanone caused significant alterations in the transcriptional activity of most of the genes tested even after 24-hour exposures and that these toxic effects at the molecular level ultimately translated into a dose-dependent decrease in fertility.

This study provides for the first time in *D. melanogaster* novel and interesting results on the toxic effects of an isolated secondary metabolite naturally present in plants and highlights the potential suitability of this organism to delve into the molecular effects of plant defences in insects.

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Genotoxic effect induced by arsenic and chromium in peripheral erythrocytes of zebrafish

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Arsenic and chromium are very common environmental pollutants due to their extensive industrial use and their presence in the environment naturally. These compounds are usually at concentrations that can cause adverse effects to biota, ecosystem and humans. The purpose of this study was to evaluate the genotoxic effect induced by exposure to different concentrations of arsenic and chromium salts, through the analysis of the frequency of micronuclei (nuclear abnormalities) in peripheral zebrafish (*Danio rerio*) erythrocytes. Experimental organisms were exposed under laboratory conditions to nominal solutions of arsenic salts ($NaAsO_2$) (0.0031-0.0500 mg L^{-1}) and chromium ($K_2Cr_2O_7$) (0.065-0.22 mg L^{-1}) for 48 hours, through the toxicity curve of arsenic and chromium. The probit test was used to establish the mean lethal concentration (LC_{50}), and from this, three subtoxic concentrations (LC_{25}, $LC_{12.5}$ and $LC_{6.25}$) were selected to evaluate the genotoxic effect of both metals on peripheral blood erythrocytes of zebrafish. The results showed that both sodium arsenite and potassium dichromate were statistically positive in the genotoxicity test, with an increase in the frequency of micronucleated erythrocytes compared to the test concentrations, evidence of genotoxic damage to both metals. This study shows that arsenic and chromium can induce DNA damage in peripheral blood erythrocytes of zebrafish, demonstrating the ability of the bioassay used, as well as the bioindicator to detect damage in genotoxic studies.

SESIÓN V: Toxicología regulatoria
SESSION V: Regulatory toxicology

15 years of the Iberoamerican Network of Toxicology and Chemical Safety

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Through a joint initiative of collaboration, between Dr. Barros, the Brazilian Society of Toxicology and the University of Sau Paulo, member of IUTOX, and Dr. de la Peña, then president of the Spanish Association of Toxicology (AETOX) and member the Spanish Research Council (CSIC); In that year, 2006, the activity of the Iberoamérican Network of Toxicology and Chemical Safety (RITSQ) began. The promoter meeting was held at the ALATOX congress in Santiago de Chile, we initially used its institutional infrastructure and later we carried out the aforementioned activity from a new domain (htto: //ritsq.org). We carry out the activities of the RITSQ through the following actions: 1st By publishing on the RITSQ website, the information on how many meetings of toxicological interest and chemical safety are organized, in Latin America, and in Portugal and Spain, the website is publishes in Portuguese and Spanish simultaneously; 2º By presenting ad hoc posters in the different congresses, meetings and conferences, since 2007, 113 posters have been presented, all of them published on the website (http://ritsq.org); and 3rd Accounting, the number of users 70,922, the number of sessions 103,016 and the number of page visits. 208,815. Since the creation of the RITSQ, a large number of activities have been carried out, including: news, congresses, courses, topics related to the RITSQ, dissemination, and toxicological and chemical safety dissemination. This opportunity is used to include information on voluntary organ donation, transplants save lives, 1 donation saves 8 lives, so I request, please, become Donors.

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