



**XXIV REUNIÓN
CIENTÍFICA**
de la
SOCIEDAD ESPAÑOLA
de **MUTAGÉNESIS
AMBIENTAL**

MADRID
2018 20 al 22
junio

<http://www.mutagenesisambiental.com/reuniones/sema-2018/>

30 ANIVERSARIO



INVITACIÓN

INVITATION

El **Grupo de Biología y Toxicología Ambiental** de la Universidad Nacional de Educación a Distancia (UNED) y el **Laboratorio de Mutagénesis Ambiental** del Consejo Superior de Investigaciones Científicas (CSIC), en nombre de la Sociedad Española de Mutagénesis Ambiental (SEMA), tiene el placer de invitarte a la **XXIV Reunión Científica de la SEMA**. El evento se celebrará en Madrid en el antiguo edificio de la iglesia de las Escuelas Pías de San Fernando entre los días **20 y 22 de junio de 2018**, y conmemorará el 30 Aniversario del nacimiento de la Sociedad.

The **Group of Biology and Environmental Toxicology** of the National University for Distance Education (UNED) and the **Laboratory of Environmental Mutagenesis** of the Spanish National Research Council (CSIC), on behalf of the Spanish Society of Environmental Mutagenesis (SEMA), are pleased to invite you to the **XXIV SEMA Scientific Meeting**. The event will be held from **20-22 June 2018 in Madrid**, at the old church of Escuelas Pías de San Fernando, and will commemorate the 30th Anniversary of the Society.

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SEDE DEL CONGRESO

CONGRESS VENUE

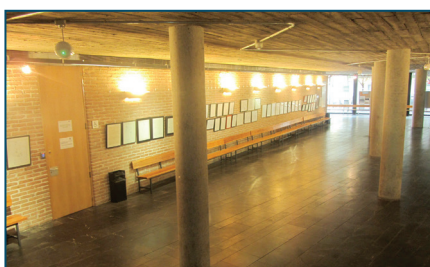
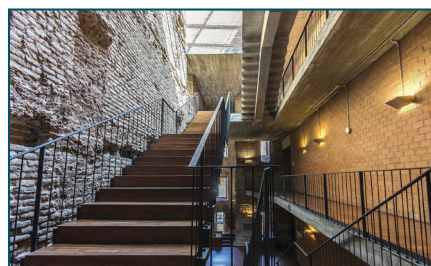
Las **Escuelas Pías de San Fernando**, que hoy se conservan parcialmente en ruinas, fueron el primer colegio que hubo en Madrid de la orden de los Escolapios. El colegio fundado en 1729, que tenía como objeto la educación de niños pobres, pronto adquirió una gran relevancia debido a la gran calidad e innovación de sus técnicas educativas. Baste decir que puso en funcionamiento la primera escuela de sordomudos del país.

Las ruinas que observamos en la actualidad pertenecen a la iglesia del colegio, construida entre 1763 y 1791 por el hermano Gabriel Escribano y destruida durante los primeros días de la Guerra Civil, en 1936. Aún hoy, podemos observar el enorme arco de medio punto rematado con el escudo de las Escuelas Pías, obra de Alfonso Vergaz, por el que se accedía a una rotonda por ocho columnas estriadas con capiteles compuestos y coronada por una suntuosa cúpula.

Posteriormente, el edificio ha sido totalmente reformado como **sede de la UNED**, dotándose de una gran biblioteca, auditorios, salones de actos, etc., y revitalizando culturalmente el castizo barrio de Lavapiés. Además, desde su reapertura es posible realizar visitas al edificio respetando los horarios establecidos por la UNED.

Escuelas Pías de San Fernando, which nowadays are partially preserved in ruins, was the first school in Madrid of the Piarists Order. The school was founded in 1729 and its main aim was the education of poor children. It acquired great relevance due to its high quality and cutting edge educational techniques. As an example, it opened the first school of deaf and dumb in Spain.

Destroyed partially during the first days of the Civil War, in 1936, the building was completely renovated as the **Associate Centre of the UNED in Madrid**, providing a large library, auditoriums, meeting halls, etc., and culturally revitalizing the district of Lavapiés, in Madrid's downtown.



info.escuelaspias@madrid.uned.es
unedmadrid.es

LOCALIZACIÓN

LOCATION

Centro Asociado de la UNED en Madrid

ESCUELAS PÍAS DE SAN FERNANDO



Calle del Tribulete, 14
28012 Madrid

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PROGRAMA CIENTÍFICO

SCIENTIFIC PROGRAMME



MIÉRCOLES 20 DE JUNIO / WEDNESDAY, JUNE 20

- 09:30 – 10:00 Entrega de documentación / Registration
- 10:00 – 10:30 Ceremonia de apertura / Opening ceremony
- 10:30 – 10:45 S0.01 *“Trigésimo Aniversario de la Reunión Científica de la Sociedad Española de Mutagénesis Ambiental (SEMA) 1988–2018”*
Eduardo de la Peña
 (Consejo Superior de Investigaciones Científicas)

- 10:45 – 11:45 **CONFERENCIA INAUGURAL / OPENING CONFERENCE**
“Plant comet assay in environmental studies: uses, limits and perspectives”
Bertrand Pourrut
 (Yncrea / ISA Lille / Lille Catholic University)

- 11:45 – 12:15 **Café / Coffee**

SESIÓN I / SESSION I **INESTABILIDAD GENÓMICA, MUTAGÉNESIS Y CARCINOGENESIS** **GENOMIC INSTABILITY, MUTAGENESIS AND CARCINOGENESIS**

Chair: **Ricard Marcos** (Universitat Autònoma de Barcelona)

- 12:15 – 12:30 S1.01 *“Follow-up to a positive genotoxicity study result: when science meets regulation criteria. Case study”*
David López Ribas
 (Innoqua Toxicology Consultants, S.L.)
- 12:30 – 12:45 S1.02 *“FRA-1 and MIR-21 involvement in arsenic-induced cell malignant transformation”*
Irene Barguilla
 (Universitat Autònoma de Barcelona)
- 12:45 – 13:00 S1.03 *“Set-up of the extraction method for the in vitro genotoxicity evaluation of deep fried meat”*
Julen Sanz Serrano
 (Universidad de Navarra)

- 13:15 – 15:15 **Comida / Lunch**

SESIÓN II / SESSION II **MÉTODOS ALTERNATIVOS A LA EXPERIMENTACIÓN ANIMAL** **ALTERNATIVE METHODS TO ANIMAL TESTING**

Chairs: **Eduardo de la Peña** (Consejo Superior de Investigaciones Científicas)
Amaya Azqueta (Universidad de Navarra)

- 15:30 – 15:45 S2.01 *“Future strategies for regulatory genotoxicity assessment”*
Guillermo Repetto Kuhn
 (Universidad Pablo de Olavide / REMA)



- 15:45 – 16:00 S2.02 *“Generation of “mini-brains” from pluripotent stem cells to study developmental neurotoxicity”*
Adela Bernabeu Zornoza
(Instituto de Salud Carlos III)
- 16:00 – 16:15 S2.03 *“New advanced tool for the computational estimation of mutagenesis”*
Sergi Gómez
(ProtoQSAR, S.L.)
- 16:15 – 16:30 S2.04 *“Pluripotent stem cells and 3D midbrain organoids as models for Dopaminergic Neurons and the study of environmental toxins associated to Parkinson’s Disease”*
Charlotte Palmer
(Instituto de Salud Carlos III)
- 16:30 – 16:45 S2.05 *“Ex vivo genotoxicity testing as an alternative approach to evaluate waterborne contaminants threat – an assay with crayfish gill cells”*
Párastu Oskoei
(Universidade de Aveiro)
- 16:45 – 17:00 S2.06 *“Development of a bioassay with *Drosophila melanogaster* as an alternative model for studying the effect of various pollutants exposure”*
Laëtitia Frat
(Université Pierre et Marie Curie)

17:00 – 17:30 **Café / Coffee**

SESIÓN III / SESSION III

EPIGENÉTICA Y TECNOLOGÍAS ÓMICAS

EPIGENETICS AND OMICS TECHNOLOGIES

Chairs: **Amadeu Creus** (Universitat Autònoma de Barcelona)
Óscar Herrero (Universidad Nacional de Educación a Distancia)

- 17:30 – 17:45 S3.01 *“DNA damage-binding protein 2 (DDB2) plays a key role in DNA methylation dynamics”*
Dolores Córdoba Cañero
(Universidad de Córdoba)
- 17:45 – 18:00 S3.02 *“Modification of the epigenome in human cancer cells by expression of a DNA demethylase from plants”*
María Victoria García Ortiz
(Universidad de Córdoba)
- 18:00 – 18:15 S3.03 *“*Prodiamesa olivacea* as a novel non-model organism for ecotoxicity studies in natural scenarios”*
Lola Llorente Ortega
(Universidad Nacional de Educación a Distancia)
- 18:15 – 18:30 S3.04 *“Toxic effects of 2-dodecanone in the insect model species *Chironomus riparius*: potential alternatives of secondary metabolites to synthetic insecticides”*
Carmen Pla Bru
(Universidad Nacional de Educación a Distancia)



- 18:30 – 18:45 S3.05 *“Rainbow trout (*Oncorhynchus mykiss*) hepatocyte 3D-spheroidal aggregates as a valuable tool for studying expression profile after long term exposure: β -naphthoflavone as a case-study”*
Azucena Bermejo Nogales
 (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria)

19:30 – 21:00 Visita Guiada / City Tour

JUEVES 21 DE JUNIO / THURSDAY, JUNE 21

SESIÓN IV / SESSION IV

RESPUESTAS GENOTÓXICAS: ALTERACIONES FISIOLÓGICAS Y DE COMPORTAMIENTO GENOTOXIC RESPONSES: PHYSIOLOGICAL AND BEHAVIOURAL ALTERATIONS

Chairs: **Luisa María Sierra** (Universidad de Oviedo)
Rosario Planelló (Universidad Nacional de Educación a Distancia)

- 10:00 – 10:15 S4.01 *“Ecotoxicological monitoring around a hazardous waste incineration plant situated in a polycontaminated area in North-West Spain, using rodent as sentinels species, and biomarkers”*
María Teresa Gómez Mora
 (Parc Científic de Barcelona)
- 10:15 – 10:30 S4.02 *“May the contamination history and gender influence the genotoxic responses of the crayfish *Procambarus clarkii* to the herbicide Viper®?”*
Sofia Guilherme
 (Universidade de Aveiro)
- 10:30 – 10:45 S4.03 *“DNA of crayfish spermatozoa as a target of waterborne pesticides – an ex vivo screening to predict the impact on progeny fitness”*
Raquel Marçal
 (Universidade de Aveiro)
- 10:45 – 11:00 S4.04 *“Influence of oncometabolites in the response to DNA damage”*
Enol Álvarez González
 (Universidad de Oviedo)
- 11:00 – 11:15 S4.05 *“A possible role of the hERG K⁺ channel on DNA damage response”*
Luisa María Sierra Zapico
 (Universidad de Oviedo)
- 11:15 – 11:30 S4.06 *“Complex responses in complex scenarios: the use of biomarkers in *Chironomus riparius* larvae in a polluted river”*
María José Servia
 (Universidade da Coruña)

11:30 – 12:00 Café / Coffee

- 12:00 – 13:00 **CONFERENCIA INVITADA / INVITED CONFERENCE**
“Greenhouse effect, chemical contamination of water, and microalgae”
Angeles Cid
 (Universidade da Coruña / Centro de Investigaciones Científicas Avanzadas)



13:15 – 15:15 Comida / Lunch

SESIÓN V / SESSION V
DAÑO, REPARACIÓN Y PROTECCIÓN DEL ADN
DNA DAMAGE, REPAIR AND PROTECTION

Chairs: **Adela López de Cerain** (Universidad de Navarra)
Antonio Guzmán (Esteve)

- 15:30 – 15:45 S5.01 *"Radiobiological characterization of primary neuroblastoma cell lines"*
Carlos Huertas Castaño
(Universidad de Sevilla)
- 15:45 – 16:00 S5.02 *"Anticancer effects of Olive Leaf Extract (OLE) on lung cell lines A549 and MRC5 and its possible modulating effect on cisplatin toxicity"*
Verónica Prados Maniviesa
(Universidad de Sevilla)
- 16:00 – 16:15 S5.03 *"The macroalga Ulva rigida affords genome protection to Drosophila melanogaster via dietary supplementation"*
Ana Margarida Marques
(Universidade de Aveiro)
- 16:15 – 16:30 S5.04 *"MTH1 a new anticancer target for neuroblastoma treatment"*
Laura Martínez López
(Universidad de Sevilla)

16:30 – 17:00 Café / Coffee

SESIÓN V (CONTINUACIÓN) / SESSION V (CONTINUATION)
DAÑO, REPARACIÓN Y PROTECCIÓN DEL ADN
DNA DAMAGE, REPAIR AND PROTECTION

Chairs: **Adela López de Cerain** (Universidad de Navarra)
Antonio Guzmán (Esteve)

- 17:00 – 17:15 S5.05 *"Antigenotoxic and longevity-promoting potential of red seaweeds Porphyra umbilicalis and Grateloupia turuturu in Drosophila melanogaster"*
João Ferreira
(Universidade de Trás-os-Montes e Alto Douro)
- 17:15 – 17:30 S5.06 *"The enzyme-modified comet assay: Measuring oxidized and alkylated bases using the 12-gels format"*
Damián Muruzábal Gambarte
(Universidad de Navarra)
- 17:30 – 17:45 S5.07 *"DNA damage induced by acute exposures to the plasticizers DEHP, BBP, and DBP, in the model species Chironomus riparius (Diptera)"*
Mónica Aquilino Amez
(Universidad Nacional de Educación a Distancia)

17:45 – 18:30 Asamblea SEMA / SEMA Assembly

21:30 Cena del Congreso / Congress Dinner



VIERNES 22 DE JUNIO / FRIDAY, JUNE 22

SESIÓN VI / SESSION VI CONTAMINANTES EMERGENTES EMERGING POLLUTANTS

Chairs: **Sofia Guilherme** (Universidade de Aveiro)
Paloma Fernández (Universidad Autónoma de Madrid)

- 10:00 – 10:15 S6.01 *"Effects of Methylmercury on cell death, proliferation and cellular phenotypic specification of human neural stem cells"*
Jordi Lli Beltrán
(Instituto de Salud Carlos III)
- 10:15 – 10:30 S6.02 *"Changes in microRNAs expression associated to long-term exposure to nanomaterials"*
Sandra Ballesteros
(Universitat Autònoma de Barcelona)
- 10:30 – 10:45 S6.03 *"miR-21 involvement in cobalt and zinc oxide nanoparticles-induced cell malignant transformation"*
Adrián Corchero
(Universitat Autònoma de Barcelona)
- 10:45 – 11:00 S6.04 *"Effects of differently shaped TiO₂NPs (nano-spheres, nano-rods and nano-wires) on the in vitro model (Caco-2/HT29) of intestinal barrier"*
Constanza Cortés
(Universitat Autònoma de Barcelona)
- 11:00 – 11:15 S6.05 *"Effects of graphene oxide and graphene nanoplatelets on the in vitro model of intestinal barrier (Caco2/HT29)"*
Josefa Domenech
(Universitat Autònoma de Barcelona)
- 11:15 – 11:30 S6.06 *"Involvement of Mth1 in the toxic and carcinogenic effects of cobalt and zinc oxide nanoparticles"*
Alba Hernández
(Universitat Autònoma de Barcelona)
- 11:30 – 11:45 S6.07 *"Time-lapse studies on the effects of silver nanoparticles in the Caco-2/HT29 model of intestinal barrier"*
Miriam Sáez
(Universitat Autònoma de Barcelona)

11:45 – 12:15 **Café / Coffee**

12:15 – 13:15 **CONFERENCIA DE CLAUSURA / CLOSING CONFERENCE**
"Impact of pollutants on terrestrial insects: from behaviour disruption to molecular mechanisms"
David Siauxsat
(Univ. Pierre et Marie Curie / Inst. of Ecology and Environ. Sciences IEES-Paris)

13:15 – 13:45 Ceremonia de clausura / Closing ceremony



Trigésimo Aniversario de la Reunión Científica de la Sociedad Española de Mutagénesis Ambiental (SEMA) 1988 –2018

E. de la Peña de Torres

*Consejo Superior de Investigaciones Científicas, c/ Serrano 115 dpdo. 28006 Madrid
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Se hace una revisión de los aniversarios celebrados por la Sociedad Española de Mutagénesis Ambiental (SEMA) desde su constitución en 1988. El décimo aniversario se celebró durante la reunión celebrada en Murcia en 1998 y que se denominó MRCIA98. No hubo ninguna mención específica al vigésimo aniversario en el décimo séptimo congreso, celebrado en Sevilla en el año 2008. En esta ocasión, celebramos en Madrid el trigésimo aniversario de la Sociedad, coincidiendo con su XXIV Reunión Científica.

Los últimos congresos celebrados han tenido lugar en la Universidad de Navarra (Pamplona, 2014), en el Parque Científico de Barcelona (2016) y en la Universidad de Oviedo (2017). Se muestran detalles de algunos de los congresos celebrados a lo largo de estos 30 años.

Se dan a conocer los resultados de la encuesta realizada por la Red Iberoamericana de Toxicología y Seguridad Química (RITSQ), acerca de las líneas en investigación y docencia relacionadas con el campo de la mutagénesis ambiental en toda Iberoamérica. Asimismo, se presentan el centenar de carteles que la RITSQ ha presentado en congresos nacionales e internacionales durante la década 2007–2017.



Recursos Docentes y de Investigación en Toxicología en Iberoamérica, promovidos por la Red Iberoamericana de Toxicología y Seguridad Química

E. de la Peña de Torres

*Consejo Superior de Investigaciones Científicas, c/ Serrano 115 dpdo. 28006 Madrid
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Las actividades de la Red Iberoamericana de Toxicología y Seguridad Química (RITSQ) se centran en la promoción y difusión de la información relativa a congresos, reuniones científicas, cursos y eventos relacionados con el ámbito de la toxicología, tanto desde la perspectiva docente como investigadora. Para cumplir con este objetivo, en el año 2008 la RITSQ puso en marcha su página web (<http://www.ritsq.org>), en la que se han registrado cerca de 2000 personas de 140 países y se han contabilizado 189.694 visitas procedentes de 61.281 usuarios.

A lo largo de su historia, la RITSQ ha celebrado tres reuniones en diferentes congresos de IUTOX, concretamente en los celebrados en Montreal (Canadá, 2007), Barcelona (España, 2010) y Mérida (México, 2016).

En mayo del año 2016 la RITSQ realizó una encuesta pública acerca de las actividades docentes e investigadoras en toxicología en toda Iberoamérica. Los resultados de la misma pueden consultarse a través de la web, en la dirección:

<http://www.ritsq.org/2017/01/03/resultados-de-la-encuesta-ritsq-sobre-oportunidades-de-formacion-e-investigacion-en-toxicologia-en-iberoamerica/>

Se presentan los 100 carteles con los que la RITSQ ha participado en congresos nacionales e internacionales a lo largo de la década 2007-2017. En todos ellos se han puesto de manifiesto las actividades de la Red, las visitas que recibe su página web y los países que más demandan la información ofrecida.

La RITSQ viene dando difusión a los congresos y reuniones de la Asociación Española de Toxicología (AETOX), la Sociedad Española de Sanidad Ambiental (SESA), la Sociedad Española de Mutagénesis Ambiental (SEMA) y la Red Española para el Desarrollo de Métodos Alternativos a la Experimentación Animal (REMA). Asimismo, sus coordinadores animan a todos los toxicólogos iberoamericanos a facilitar toda aquella información que consideren relevantes en este campo, de manera que pueda darse la difusión adecuada a través de la página web.





<http://ritsq.org>
epena@ica.csic.es

DIFUSION Y MANIFIESTO DE LAS POSIBILIDADES Y ACTIVIDADES QUE SE REALIZAN EN DOCENCIA E INVESTIGACIÓN TOXICOLOGICA EN IBEROAMERICA

XXX
Aniversario
SEMA
Cartel N° 102



**XXIV Reunión
Científica
de la SEMA**

La encuesta de la RITSQ * (publicado en <http://RITSQ>) se realizó el pasado 21 de mayo de 2016, ello permitió el conocimiento del estado actual del desarrollo de la actividad toxicológica en docencia e investigación en Iberoamérica (1 y 2). En marzo de 2008 se puso en marcha la página web de la RITSQ, y se han registrado por internet, más de 1.900 personas de 119 países, se han contabilizado un total de 90.599 sesiones, que se distribuyen anualmente en 61. usuarios y 189.633 el n° de visitas a páginas. Las actividades de la RITSQ se centra en la información de diferentes Congresos, Reuniones, y este Cartel muestra los 100 carteles presentados durante los años 2007 a 2017.

Universidades Organismos con Docencia

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Universidad de Antioquia. Colombia
Especialización en Toxicología Clínica (solo médicos)
Universidad Autónoma de México
Diplomado en Toxicología Clínica Hospital Juárez de México
Alta Especialidad en Toxicología Clínica HJM
Universidad de Buenos Aires
Doctorado en Ciencias Químicas
Universidad Colegio Mayor Nuestra Señora del Rosario. Colombia
Especialización médico-quirúrgica en Toxicología Clínica post médico
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Diplomado en Toxicología
CINVESTAV CDMX, México
Maestría en Toxicología
Ilustre Colegio Oficial de Químicos. Sevilla
Experto Internacional en Toxicología
Toxicología de Postgrado **MASTERTOX / EXPERTOX**

1

Investigación Toxicológica

1. Toxicología laboral
2. Inmunotoxicología molecular
3. Toxicología de plaguicidas
4. Toxicología Genética – Mutagénesis
5. Toxicología- Salud Pública
6. Toxicogenética
7. Toxicología y Evaluación del Riesgo.
8. Toxicidad *in vitro* de nanomateriales
9. Evaluación *in vitro*/mecanismos de acción
10. Estudios de respuesta al daño al DNA
11. Carcinogénesis química
12. Análisis de polimorfismo de genes

2

Las Tablas 1, 2 y 3, muestran: el n° de páginas visitadas, el n° de visitas y el conjunto de actividades realizadas por la RITSQ, desde su creación hasta 30 de diciembre del año 2017.

Tabla 1. Visitas / Registrados

2018	189.633	> 1.950
2017	186.733	> 1.900
2016	175.979	1.600
2015	162.650	1.400
2014	135.581	1.133
2013	114.482	904
2012	101.529	842
2011	78.143	673
2010	51.908	556
2009	35.281	486
2008	13.159	379

Tabla 2. País / N° de Visitas

México -----	10.283	- CDMX ----	2.889
Colombia --	7.707	- Bogotá ---	3.775
Perú -----	5.061	- Lima -----	2.927
Argentina -	4.912	- Bs. As. ----	1.493
Venezuela -	3.923	- Caracas --	1.619
USA -----	2.942	- N York ----	1.593
Chile -----	2.884	- Santiago --	1.160
Brasil -----	1.742	- S. Paulo --	647
Ecuador ---	1.624	- Pichincha -	671
Bolivia -----	1.074	- La Paz ----	1.071
España	36.640	- Madrid --	20.095

Figura 1. Muestra el centenar de carteles de la RITSQ en Congresos, Reuniones y Conferencias durante el periodo 2007 a 2017 (<http://ritsq.org/Historia>)

Tabla 3. Actividades de RITSQ

Año	Congresos	Carteles	N° Visitas
2018	5	-	90.599
2017	43	11	89.024
2016	30	12	83.820
2015	37	15	76.269
2014	31	9	68.670
2013	38	8	62.453
2012	31	13	54.913
2011	39	14	40.870
2010	39	8	27.367
2009	43	3	18.162
2008	20	1	5.395
2007	5	4	-----

La RITSQ creó la página Web, en marzo de 2018; durante este tiempo se han presentado los 100 carteles de la figura 1. Deseamos ofrecer en la citada página información sobre las actividades de interés toxicológico, si contamos para ello con la ayuda y colaboración de todos los seguidores de la web (<http://ritsq.org>)



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<http://ritsq.org>



CONFERENCIAS INVITADAS

INVITED CONFERENCES



Plant comet assay in environmental studies: uses, limits and perspectives

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The comet assay is a versatile technique for measuring DNA damage in eukaryotic cells and organisms, and is increasingly used to assess DNA repair. Its main applications are in genotoxicity testing, and in human monitoring, with a so far rather neglected potential in ecogenotoxicology. This is particularly true in plant research.

Comet assay was used for the first time on plants in 1993. For a decade, the comet assay remained restricted to some toxicological studies and to few model species including garlic (*Allium cepa*), tobacco (*Nicotiana tabacum*), broad bean (*Vicia faba*), and arabidopsis (*Arabidopsis thaliana*). Since 2010, the technique was exponentially applied to evaluate diverse stressors (organic compounds, radiations, nanoparticles...), and to monitor environmental pollutions *in situ*.

However, despite its increasing applications, the comparison between the number of papers using comet assay on plants (almost 300 during the last 25 years) and on humans (more than 10 000 during the same period), highlights the gap in its uses between genotoxicology and ecogenotoxicology fields. This huge difference can be explained by (i) the difficulty to isolate numerous intact nuclei in plants compared to animal systems, (ii) the lack of a standardize protocol and guideline in plants, (iii) and the lack of a high throughput comet assay scoring method.

During the last 5 years, intensive efforts have been done to develop a robust and effective new protocol to extract plant nuclei, as well as an automated high-throughput scoring of plant nuclei. Meanwhile, several authors have used enzyme-modified protocols to detect specific base damage or DNA methylation. This opens new perspectives for the development of this technique in plant studies.

During this presentation, we will review the uses of comet assay on plants, and the main bottlenecks of this technique. We will also discuss the current developments and new perspectives.



Greenhouse effect, chemical contamination of water and microalgae

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Human activities have strengthened the greenhouse effect and caused global warming. CO₂ is one of the major gases contributing to this greenhouse effect, but also is the source of carbon for the photosynthetic organisms. In a global climate change scenario with a dramatic increase in CO₂ levels, we need to know the behavior of carbon fixing organisms.

Microalgae are critical to global biogeochemistry since they produce the bulk of oxygen on Earth through photosynthesis, whereas transform atmospheric CO₂ into organic matter; however, chemical contamination of freshwater and marine environments provokes dramatic effects on microalgae, being the photosynthetic process one of the most altered.

Alterations provoked by a traditional aquatic pollutant (the herbicide atrazine, ATZ) and an emergent pollutant (the disinfectant triclosan, TCS) were studied in the model microalga *Chlamydomonas reinhardtii*.

Under near *in vivo* conditions, it has been observed that sublethal ATZ concentration provoked the decrease of cellular activity and chlorophyll *a* fluorescence, plasma membrane depolarization and hyperpolarization of mitochondrial membrane. These alterations, combined with the most significant changes in gene expression (amino acids catabolism and respiratory cellular process), suggest that photosynthesis inhibition leads cells to get energy through a heterotrophic metabolism to maintain their viability.

Furthermore, the same ATZ concentration provoked a premature senescence of microalgal cells, based on increase of caspase activity, number of cells with autophagosomes and alterations in the nuclei morphology. ROS-mediated DNA oxidation contributes to premature senescence in microalgal cells.

TCS also induced ROS overproduction which ultimately leads to oxidative stress with loss of membrane integrity, membrane depolarization, photosynthesis inhibition and mitochondrial membrane depolarization. An increase in caspase activity and altered expression of metacaspase genes, which are indicative of apoptosis, were also induced by TCS.

Stress responses to pollutants include changes in gene expression, alterations in photosynthesis, and a putative apoptotic response in *Chlamydomonas reinhardtii*. Despite these alterations, the cells were able to overcome stress and maintain cellular viability; however, aquatic pollution could lead to a dramatic decrease in CO₂ fixation at a global level, being the worst consequence in a global climate change scenario.

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The effects of sublethal doses of pollutants on crop pest *Spodoptera littoralis*: from behavior to gene expressions

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Pesticides have long been used as the main solution to limit agricultural pests but their widespread use resulted in chronic or diffuse environmental pollutions, development of insect resistances and biodiversity reduction. The effects of low residual doses of these chemical products on organisms that affect both targeted species (crop pests) but also beneficial insects became a major concern, particularly because low doses of pesticides can induce various effects. In addition to the negative effects, some studies highlighted unexpected positive - also called hormetic - effects on insects, leading to surges in pest population growth at greater rate than what would have been observed without pesticide application. The present study aimed to examine the effects of sublethal doses of various representative products of large pesticide families used against a major pest insect, the cotton leafworm *Spodoptera littoralis*, and known to present a residual activity and persistence in the environment. Using an integrated approach from genes to behavior, we studied the impact on the peripheral olfactory system and the sexual or feeding behavior of our crop pest model following application of sublethal doses of deltamethrin, methomyl and chlorpyrifos. Whereas sublethal doses of methomyl appeared to disrupt the feeding behavior of larvae, we demonstrated a hormetic response of males to sublethal dose of deltamethrin. We completed our study by molecular (qPCR), biochemical (proteomic and metabolic) and electrophysiological approaches in order to decrypt the involved mechanism in pesticide response as well as in the behavioral disruption.



SESIÓN I: Inestabilidad genómica, mutagénesis y carcinogénesis

SESSION I: Genomic instability, mutagenesis and carcinogenesis



Follow-up to a positive genotoxicity study result: when science meets regulation criteria. Case study

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Genotoxicity assessment is a key point in the development of new substances in within the pharmaceutical industry. There are currently international regulations which establish how and when this assessment has to be done. Almost in all cases, positive results in a genotoxicity assays mean a red flag and “stop the engine”. ICH S2(R1) *Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use* defines several follow-up scenarios after a positive result in order to rule out (or confirm) its relevance to humans.

This case study describes the strategy followed after obtaining a positive result in an *in vitro* Mouse Lymphoma Assay which showed an increase in mutation frequencies at several concentrations after 24 hours of exposure in the absence of S-9 mix. Other genotoxic studies (*in vivo* and *in vitro*) performed with this pharmaceutical within the standard battery defined by ICHS2(R1) were negative.

Similar positive findings in *in vitro* studies and positive results in an *in vivo* comet assay performed as part of a 2-week repeated dose toxicity study are described in the literature for other members of this chemical/pharmacological family. This family is also a known group of substances which can induce reactive oxygen species (ROS), known to responsible for damaging DNA and inducing cytotoxicity.

The information from the literature and the regulatory requirements were combined to define a customized *in vivo* comet assay where 1) toxicokinetic samples were also collected to state an exposure threshold of action, 2) the assessment for oxidative damage to DNA was incorporated by measuring 8-OH-dG adducts, and 3) genotoxicity assessment was performed on liver and GI.

Fortunately this study showed negative results, the “drug development engine” was restarted and this pharmaceutical has been allowed to progress in clinics with no comments on the genotoxicity from FDA or EMA.



FRA-1 and MIR-21 involvement in arsenic-induced cell malignant transformation

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Arsenic is a widespread and well-known human carcinogen associated with skin, lung, bladder, liver and other kinds of cancer. Nonetheless, the mechanisms of action by which chronic arsenic exposure leads to tumor development are not fully known. The *fos-related antigen 1* (FRA-1) is a transcription factor frequently overexpressed in epithelial cancers with a described role in the development of an aggressive tumor phenotype. Its ability to regulate key genes related to the invasion and metastasis processes, as well as different microRNAs such as miR-21, involved in the downregulation of tumor suppressor genes, is well known.

In this work, MEF cells chronically exposed to arsenite were monitored for FRA-1 expression levels at different time-of-exposure intervals for 50 weeks, in order to assess its correlation with the progressive development of an aggressive tumor phenotype. Expression changes of upstream and downstream components of FRA-1 related signaling pathways were evaluated at equivalent time-points.

Our results show a dose-dependent overexpression of FRA-1 from the transformation point of MEF cells, as a response to ERK and p38 activation. This is correlated with an increase in miR-21 levels and a subsequent downregulation of PTEN, PDCD4 and TMP1. Ongoing FRA-1 knock-down experiments could validate its role on miR-21 regulation and its relevance in the initiation of the transformation process and the development of an aggressive tumor phenotype. Therefore, this will be the first work to show that FRA-1 is involved not only in the modulation of arsenic-induced malignant phenotype, but also on its onset through miRNAs regulation.



Set-up of the extraction method for the *in vitro* genotoxicity evaluation of deep fried meat

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Heat treatment is one of the most used procedures in cooking with several advantages for consumers such as preservation, higher digestibility and organoleptic changes of food. However, high temperature can lead to mutagen formation.

The aim of this project is to evaluate the *in vitro* genotoxicity of mutagenic compounds generated by deep frying of food cooked in mass catering companies. A baseline questionnaire was developed in order to obtain data about fryers, deep-fried food, frying oil or monitoring and cleaning habits from mass catering companies of Navarra. Data from twenty kitchens of eleven companies were obtained. Selection of kitchens is yet to be performed, before collection of samples begins.

In parallel, an extraction method for mutagens has been set up; a non-ionic polymeric adsorbent resin is used to extract the mutagenic compounds from cooked hamburgers. The *in vitro* genotoxicity evaluation of the extracts will be performed using the mini-Ames, the micronuclei assay and the comet assay.

Mutagenicity of an extract from an overcooked meat (30' of frying) was assessed by a mini-Ames test. The test was performed following the principles of the OECD guideline for the Ames test. The extract showed clear positive results in the TA98 strain in the presence of external metabolic activation. A weak response was also seen in the TA1535 strain in the absence of metabolic activation. The presence of bacteria in the extract and the necessity to filtrate the extracts will be discussed. Extracts from meat fried during different times (2', 5', 10', 20', 30') were tested in the TA98 strain in order to demonstrate that extracted mutagens were formed during cooking. Pure extracts and five ½ serial dilutions were evaluated. Extracts obtained after long frying times showed a positive dose-response dependant on S9 fraction. Experiments were performed with extracts from different extractions to observe the variation of the process.

Different dilutions of an extract obtained after 30' of frying will be evaluated using the Fpg-modified comet assay and the micronucleus test, with and without metabolic activation, in TK-6 cells. In order to perform these experiments, the toxicity of the extracts has been evaluated using the proliferation assay.

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SESIÓN II: Métodos alternativos a la experimentación animal

SESSION II: Alternative methods to animal testing



Future strategies for regulatory genotoxicity assessment

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The present qualitative regulatory framework for genotoxicity assessment was initiated in 1983 by the US EPA. It is very inefficient due to the high rate of false positives obtained in the *in vitro* mutagenicity tests. This situation forces to carry out unnecessary animal carcinogenicity assays, of debatable relevance to human safety. Now, it is an adequate moment to consider possible improvements in the process, from easy updates to the classical tests or the selection of suitable battery of tests leading to an absolute new strategy for quantitative risk assessment. The most interesting improvements applied by the OECD and other entities to increase the reproducibility and specificity of the classical mutagenicity tests include the application of good cell culture practices with cells of controlled sources, adequately characterized, and a short number of passages; the consideration of the feeding effect in bacteria due to the presence of amino acids or the false positives due to the ubiquitous flavonoids; the recommended use of p53 and DNA repair competent human cells; the reduction of the maximum concentration assayed to 10 mM or 2000 mg/mL instead of 5000 mg/mL; the control of strong variations of pH and/or osmolarity; or, the use of the comet assay with more relevant models such as human reconstructed skin.

In relation to the strategies, the main battery test should be reduced to increase specificity as the authorities propose, combining the bacterial reverse mutation assay, to detect gene mutations, and the *in vitro* micronucleus test, to detect both structural and numerical chromosome aberrations.

The next generation testing strategy for assessment of genomic damage should provide not only qualitative, but also quantitative information in relation to the relevant mode of action, and be based on high throughput assays by measuring the induction of stress pathways/proteins as endpoints. One approach uses metabolically competent cells or battery of cells with fluorescent or luminescent reporters of genetic or cytotoxic damage. Other approaches employ the gene expression profiles to determine the molecular pathways involved in the response, and the quantitative transcriptomic data to determine the benchmark dose and estimate a point of departure for human health risk assessment.

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Generation of "mini-brains" from pluripotent stem cells to study developmental neurotoxicity

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Due to the complexity of the human brain, it is difficult to study many brain disorders in model organisms. The results that we can obtain *in vitro* nowadays are all made in monolayer cell cultures (2D), and, although highly valuable, those methods are devoid of a tridimensional component necessary for normal organ development. Therefore, the ability to model human brain development *in vitro* represents an important step in our study of developmental processes and neurological disorders.

It has recently been described that pluripotent stem cells (PSCs) in a suitable environment are capable of generating three-dimensional (3D) structures called "cerebral organoids" or "mini-brains". They recapitulate different stages of human cortical development, generating a variety of regional identities organized in discrete domains able to connect with each other. Organoids can be further engineered to mimic disease-relevant genetic and epigenetic states of a patient. Human PSC-based *in vitro* models that reflect human physiology have the potential to reduce the number of drug failures in clinical trials and offer a cost-effective approach for assessing chemical safety.

We are setting up human and mouse PSCs three dimensional organoids culture systems. The differentiation in organoids (3D) is being carried out according to the protocol recently published by Lancaster and Knoblich (2014) *Nature Protocols* 9(10):2329-40. The characterization of cultures and identification of different neural structures and phenotypes are being performed by immunocytochemistry and qRT-PCR.

Together, these studies would indicate that 3D organoids can recapitulate human and mouse neurodevelopment and it can be useful to study the pathogenesis of neurological diseases and toxicity. This combined strategy demonstrates the value of human cell-based assays for predictive toxicology and should be useful for both drug and chemical safety assessment.



New advanced tool for the computational estimation of mutagenesis

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The assessment of mutagenicity is an essential component for the evaluation of the toxicological profile of chemicals. Mutagenic effects caused by chemical agents are usually detected by the Ames test, which nowadays can be considered as a paradigm for the development of current *in vitro* toxicology.

Quantitative structure-activity relationships (QSAR) models constitute a rational and well-established methodology for prediction of chemical properties with computers. QSARs involve the construction of mathematical models relating –by means of statistical tools– the chemical structure of a series of molecules with a physicochemical/biological property. Once a correlation is established, it can be used to predict the behavior of new molecules. The use of QSARs with regulatory purposes is recognized by international agencies and institutions such as ECHA or the OECD, and for example the evaluation of pharmaceutical impurities is commonly done by companies and consultants following the requirements of the International Conference on Harmonisation (ICH) guides.

Several pieces of software are commercially available for the computational (“*in silico*”) evaluation of mutagenicity. The accuracy of Ames mutagenicity prediction by these programs is typically 70-75%, which is not far from the inter-laboratory reproducibility of the Ames test (usually estimated at 80 – 85%). These tools have different drawbacks: most of them are “black boxes” for users, in which there is no degree of certainty or reliability of their predictions, it is difficult for non-specialized personnel to understand their functioning, and only experienced staff can work with it.

We will present here a completely new QSAR model for mutagenicity estimation, based on the hugest dataset constructed until now from reliable data. This model presents outstanding advantages over the current programs, such as good statistic performances, higher applicability domain and ease of use.



Pluripotent stem cells and 3D midbrain organoids as models for Dopaminergic Neurons and the study of environmental toxins associated to Parkinson's Disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder characterized by the progressive loss of Dopaminergic neurons (DAn) in the midbrain. The loss of DAn leads to motor symptoms, autonomic dysfunction and psychological alterations. Although some genetic factors have been associated with the development of PD, this only makes up a small percentage of cases, while the majority have unknown causes. Recently, studies have found increased risk of PD associated with the exposure to environmental toxins, especially pesticides, solvents and some metals. However, since most cases of PD are sporadic, it has been difficult to create appropriate animal models for the study of the development of this disease. In this work, we suggest an alternative approach by reprogramming mouse embryonic fibroblasts (Mefs) to create a stable cell line of mouse induced pluripotent stem cells (miPSC) as an in vitro model for the development of DAn. We reprogrammed Mefs using the CoMIP minicircle plasmid and characterized the cells to demonstrate their pluripotency and ability to self-renew. This was then followed by the directed differentiation of miPSCs to DAn. We also used these miPSCs to create mini-midbrain organoids, whose 3D structure allows us to emulate the development of DAn in vivo. Once established, these same techniques could be implemented and adapted to human cell systems, which could substitute the use of animal models to study the development of PD. Both miPSCs and midbrain organoids could then be used to study the effects of environmental toxins, either individually or in combinations usually present together in the environment. This could then give us insight to how these toxins impact DAn and the development of PD.



***Ex vivo* genotoxicity testing as an alternative approach to evaluate waterborne contaminants threat – an assay with crayfish gill cells**

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Animal testing usually adopts *in vivo* and *in vitro* methods, but several disadvantages are associated to both approaches. Moreover, the implementation of the 3R's principle (reduction, refinement and replacement) has been considered a priority, reinforcing the need of finding alternative methods. Hence, the *ex vivo* method appears as a viable alternative for a fast screening of waterborne contaminants' effects, since it combines some advantages of the previously mentioned methods, while considering 3R's guidelines. This study intended (i) to evaluate the effectiveness of the *ex vivo* approach using crayfish (*Procambarus clarkii*) gill cells, while evaluating cell viability and DNA integrity and (ii) to apply this integrative approach to assess the insecticide dimethoate's genotoxic potential. Thus, crayfish gill cells viability (in PBS) was evaluated, using the MTT tetrazolium reduction test, in an *ex vivo* assay for 2, 4 and 8 h. Cell viability was above the accepted limit (>70%) for 2 and 4 h, while it was slightly compromised after 8 h (67%). Afterwards, cells were exposed to PBS (negative control; NC) and to a genotoxic model (ethyl methanesulfonate – EMS; 5 mg L⁻¹) as positive control (PC), for 2 and 4 h. Genotoxicity was evaluated using the comet assay improved with DNA lesion-specific repair enzymes, namely formamidopyrimidine DNA glycosylase (FPG) and endonuclease III (EndoIII), to assess purines and pyrimidines oxidation, respectively. Results showed that only the 2 h exposure was found to be suitable, since NC presented low levels of DNA damage, and PC demonstrated a significant increase when compared to NC, while the NC DNA integrity began to be impaired after 4 h, invalidating this and the subsequent exposure length (8 h). To accomplish the second objective, gill cells were exposed *ex vivo*, for 2 h, to two environmentally realistic concentrations of dimethoate (20 and 40 µg L⁻¹), an insecticide with unknown genotoxic potential to crayfish. The DNA integrity was once again evaluated using the comet assay. Dimethoate demonstrated to be genotoxic to crayfish gill cells, despite not inducing DNA oxidative damage. In conclusion, the *ex vivo* method, applied to crayfish gill cells, showed to be suitable for 2 h exposures, when the comet assay was used as endpoint. Moreover, this approach might be assumed as a relevant contribution towards the improvement of strategies for a rapid and effective screening of the pernicious effects of waterborne contaminants.



Development of a bioassay with *Drosophila melanogaster* as an alternative model for studying the effect of various pollutants exposure

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Contamination of environment by toxic pollutants has become commonly recognized as an environmental concern. Those compounds are often present at low concentrations making them difficult to detect. They could also cause ecological issues according to their nature (heavy metals, pesticides, endocrine disruptors...). Therefore, ecotoxicity risks are evaluated with various types of organisms, from cell-lines and microorganisms to plants, invertebrates and vertebrates. Most of them allow the detection of a phenotype, thus indicating a toxic effect and some of them can also highlight the modification of molecular actors involved in the toxic response. However, few models offer the opportunity to perform integrated study with multiple approaches, from molecular variations to physiological consequences. *Drosophila melanogaster* is a proven model organism in genetic and biology research. The fruit fly has been further considered as an emerging and suitable model in toxicology and ecotoxicology in the past few years. *Drosophila* has a short and well described development; reproduction and maintenance do not require sophisticated equipments and are rather economical. Furthermore, there are a lot of amenities available to study genetic actors involved in stress or toxic response compared with other models (i.e. open access of online molecular database, gene inhibition or over-expression, mutant production, coupling of fluorescence with gene expression...). Moreover, the fruit fly's development is under hormonal control making endocrine disruptors studies possible. To develop this "tool box" as an alternative model, we focused firstly on the impact of two heavy metals, cadmium and mercury on post embryonic development of *Drosophila melanogaster*. The first results indicate that mercury is more toxic than cadmium, and has different kind of effects on post embryonic development. We also analyzed the expression of various genes, as biomarkers, involved in different defense and detoxification mechanisms to establish a response profile that could be used in future works.



SESIÓN III: Epigenética y tecnologías ómicas

SESSION III: Epigenetics and omics technologies



DNA damage-binding protein 2 (DDB2) plays a key role in DNA methylation dynamics

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Genomic methylation patterns are the dynamic outcome of counteracting DNA methylation and demethylation mechanisms. In plants, active DNA demethylation is initiated by members of the ROS1/DME family of 5-methylcytosine DNA glycosylases and proceeds through a base excision repair mechanism. However, it is still unclear how DNA demethylation is regulated to maintain and/or reestablish DNA methylation patterns. In this work we describe a direct interplay between the DNA repair factor DNA damage-binding protein 2 (DDB2) and the ROS1-mediated active DNA demethylation pathway in *Arabidopsis thaliana*. We show that DDB2 forms a complex with ROS1 and AGO4 and that they act at the ROS1 locus to modulate levels of DNA methylation and therefore ROS1 expression. We also found that DDB2 represses the enzymatic activity of ROS1. DNA demethylation intermediates generated by ROS1 are processed by the DNA 3'-phosphatase ZDP and the apurinic/apyrimidinic endonuclease APE1L, and DDB2 interacts with both enzymes and stimulates their activities. Taken together, our results indicate that DDB2 acts as a critical regulator of ROS1-mediated active DNA demethylation.



Modification of the epigenome in human cancer cells by expression of a DNA demethylase from plants

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Patterns of DNA methylation, an important epigenetic modification involved in gene silencing and development, are disrupted in cancer cells. Understanding the functional significance of aberrant methylation in tumors remains challenging, due in part to the lack of suitable tools to actively modify methylation patterns. DNA demethylation caused by mammalian DNA methyltransferase inhibitors is transient and replication-dependent, whereas that induced by TET enzymes involves oxidized 5-meC derivatives that perform poorly understood regulatory functions. Unlike animals, plants possess enzymes that directly excise unoxidized 5-meC from DNA, allowing restoration of unmethylated C through Base Excision Repair. Here we show that expression of *Arabidopsis* 5-meC DNA glycosylase DEMETER (DME) in colon cancer cells demethylates and reactivates hypermethylated silenced loci. Interestingly, DME expression causes genome-wide changes that include both DNA methylation losses and gains, and partially restores the methylation pattern observed in normal tissue. Furthermore, such methylome reprogramming is accompanied by altered cell-cycle responses and increased sensibility to anti-tumor drugs, decreased ability to form colonospheres, and tumor growth impairment *in vivo*. Our study shows that it is possible to reprogram a human cancer DNA methylome by expression of a plant DNA demethylase.



***Prodiamesa olivacea* as a novel non-model organism for ecotoxicity studies in natural scenarios**

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Since toxicological studies on non-model organisms complement and provide powerful information in terms of natural ecosystems, these confer a breakthrough in ecotoxicology along with traditional approaches.

Chironomus (Diptera) has four OCDE standardized tests for the evaluation of water and sediment toxicity, which assess classical parameters of toxicity (survival, immobilization, reproduction and development).

Prodiamesa olivacea (Diptera) is a non-model aquatic insect species not used in toxicity tests. It frequently shares habitat with *C. riparius* but requires higher oxygen levels and lower extreme conditions. Considering this fact, it is of special interest to study the possible differences in the response of both species to pollutants.

Since information about *P. olivacea* in genomic databases is scarce, the transcriptome of this species was obtained using *de novo* RNAseq. Genes commonly used as molecular biomarkers in *C. riparius* were identified in *P. olivacea*: 1) EcR and ERR coding for hormone receptors; 2) hsp70 involved in cellular stress response 3) GPx (glutathione peroxidase) and GST (glutathione S-transferase), involved in biotransformation pathway. Quantitative real-time PCR was used to evaluate the expression of selected genes. Ribosomal gene 26S, GAPDH and actin were used as reference genes.

In the present study, the toxicity of butyl benzyl phthalate (BBP; CAS No. 85-68-7) was elucidated in both species from a polluted river (Sar) in Galicia (Spain). The effects of acute 4-h and 24-h exposures to 1 µgL⁻¹ BBP were evaluated at transcriptional level. Results revealed different responses of genes depending on the species. More severe effects were observed in most of the markers studied in *P. olivacea*, showing greater sensitivity to this compound compared to *C. riparius*. This work highlights the importance of a multi-organism study at molecular level in order to deep into the toxicity of the BBP. In addition, 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 kind of approach will allow us to have a broader view of the risk associated with the presence of pollutants in ecosystems in short, medium and long term.



Toxic effects of 2-dodecanone in the insect model species *Chironomus riparius*: potential alternatives of secondary metabolites to synthetic insecticides

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Among the mechanisms of most of plants to defend against insects, glandular trichomes are shoot epidermal hairs responsible for a significant portion of a plant's secondary metabolites. Some of these substances are weak toxins since they compromise insect survival, and may delay their growth and pupation, making insects more vulnerable. Methyl ketones are a widely-produced group of chemicals synthesized by trichomes and their insecticidal efficacy has been described against some arthropods, such as aphids or spider mites. However, information about the mode of action and molecular effects of these compounds on insects are still very scarce.

In the present study, the toxicity of 2-dodecanone (CAS 6175-49-1) was investigated in *Chironomus riparius* aquatic larvae –an insect model species in ecotoxicology studies–. The effects of acute 24-h and 96-h exposures to a range of doses (0.05 µg/L, 5 µg/L and 500 µg/L) were analysed at the molecular level by analysing changes in the transcriptional activity of genes related to the cell response to stress (hsp70, hsp40, hsp10), detoxification pathways (cyp4G, GST and GPx), endocrine system (EcR and vtg) and DNA repair and apoptosis (XRCC1, NLK, ATM, CASP3). Ribosomal gene 26S, *actin* and ribosomal protein *L13* were used as reference genes. Our results showed that 2-dodecanone caused a clear dose- and time-dependent toxicity in most of the selected biomarkers; significant effects were detected even after 24-h acute exposures. Longer treatments (96h) triggered a general repression of transcription in most of the analysed genes.

This study provides novel and interesting results on the toxic effects of an isolated secondary metabolite, naturally present in plants, in *C. riparius* and highlights the potential suitability of this organism for deep into the molecular effects of plant defences in insects. A deep knowledge of the mode of action of secondary metabolites on development and metabolism of insects, will generate new leads to tap their potential in plant resistance to pests. These findings provide new insights into insecticidal efficacy of 2-dodecanone, which might be explored under field conditions for plant protection and pest management, to reduce reliance on synthetic pesticides.

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Rainbow trout (*Oncorhynchus mykiss*) hepatocyte 3D-spheroidal aggregates as a valuable tool for studying expression profile after long term exposure: β -naphthoflavone as a case-study

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The aquatic environment is the final fate of a variety of pollutants including pesticides, personal care products, or pharmaceuticals. Rapid and sensitive screening approaches are needed to identify the potential impacts on animals. Primary culture of cells can regain tissue-specific functionalities when they are grown as three-dimensional (3D)-aggregate cultures. The aim of this work is to assess the response of a fish-hepatocyte *in vitro* model based on 3D-spheroidal aggregates culture that could respond to long-term toxicant exposures. Rainbow trout hepatocytes were extracted and cultured for 96 h to produce large 3D-aggregates in a 24-well plate. Sensitivity of the culture was determined by exposure to the inducer of detoxification enzymes (β -naphthoflavone, BNF) at three different concentrations (1.5, 12.5 and 100 μ M) for 10 days. Differential expression profile of genes related to endocrine disruption and xenobiotic metabolism, aryl hydrocarbon receptor 2 (*ahr2*), cytochrome P450 family 1 subfamily A (*cyp1A*), cytochrome P450 family 3 subfamily A (*cyp3A*), vitellogenin (*vtg*) and estrogen receptor β 2 (*er* β 2) was analysed by real-time PCR. Functionality was evidenced by baseline expression of molecular markers. Exposure to BNF caused differential dose-response functional regulation. Overall a general induction of *cyp1A*, *er* β 2 and *vtg* occurred at all concentrations tested. At the lowest concentration 3D-aggregates exhibited the highest *vtg* activation in the expression profile. At 12.5 μ M concentration there was a 3-fold induction with respect to control of *cyp1a* and *vtg* although this *vtg* up-regulation is lower than this produced by previous concentration. The 3D-aggregates exposed to 100 μ M exhibited a 7-fold induction of *cyp1a*, 2-fold of *er* β 2, and a 4-fold of *vtg* respect to controls. The results are in concordance with previous results in other fish experimental models and demonstrate that the 3D-hepatocyte aggregate culture constitutes a promising *in vitro* system to be used in medium- to long-term ecotoxicity studies.



SESIÓN IV: Respuestas genotóxicas: alteraciones fisiológicas y de comportamiento

SESSION IV: Genotoxic responses: physiological and behavioural alterations



Ecotoxicological monitoring around a hazardous waste incineration plant situated in a polycontaminated area in North-West Spain, using rodent as sentinels species, and biomarkers

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Ecotoxicological assessment was carried out around a focal source of pollution, a hazardous-waste incineration plant (HWI) situated in a polycontaminated area in North-West Spain, using mice (*Apodemus sylvaticus* and *Mus spretus*) as sentinel species, and biomarkers of exposure and effect.

Feral mice (*Apodemus sylvaticus* n = 46 and *M. spretus* n = 87) were caught by means of Shermann traps in the field in different potential polluted zones (I0, I1, I2 and I3) around a HWI and a control zone (localized 10 km from HWI) devoid of any known source of contamination and with the same climatic and ecological characteristics than the exposed ones. Haematological (WBC, LYM, MID, GRA, RBC, HGB, HCT, MCV, MCH and MCHC), genotoxicological (Comet test) and morphological parameters were evaluated as general biomarkers of toxicity.

Specimens exposed in potentially polluted areas presented some significant alterations in all the studied parameters when compared with animal from control zone. The general trend was that zone I1, placed up-wind and up-stream from the HWI, showed values quite similar to controls, while zones I0 and, especially, I3 (situated down-wind and down-stream and close to the HWI) presented significant alterations. Zone I2, near the urban and traffic-affected village of Constantí, showed some qualitatively different effects.



May the contamination history and gender influence the genotoxic responses of the crayfish *Procambarus clarkia* to the herbicide Viper®?

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Several species can inhabit aquatic environments impacted by high levels of multiple anthropogenic pollutants or/and natural toxins. Despite being well known that populations' success is related to the development of adjustment strategies, the way non-target organisms cope with the presence of pesticides is still poorly understood. These organisms' strategies are frequently related with the local contamination history as well as gender specification, which appear as factors determining physiological responses. These factors are not commonly studied, despite they are argued highly important when performing a population study. DNA integrity assumes a key role, since genetic damage may escalate into severe problems from intracellular to individual (and populational) levels. Genotoxicity can be modulated by mentioned factors, either independently or jointly. Bearing this in mind, and owing to the vital role of DNA integrity, the major aims of the present study were: (i) to understand the influence of contamination history and gender in the genotoxic responses of the crayfish *Procambarus clarkii* following exposure to a widely used penoxsulam-based herbicide - Viper® and (ii) to investigate the mechanisms involved in putative adjustments shown by *P. clarkii*. In this way, male and female specimens of two populations, one from a reference site and the other from an historically contaminated site, were exposed to environmentally relevant Viper® concentrations and standard (alkaline) comet assay was adopted to assess the genetic damage in gill cells. The results proved the genotoxicity of the penoxsulam-based herbicide to the non-target crayfish, and males from the historically exposed population displayed a higher susceptibility towards the non-specific genotoxic pressure posed by Viper®. In contrast, DNA oxidation patterns suggested an increased ability of males to deal with this particular type of damage. It is worth remarking that the influence of the exposure history on the protection/vulnerability to Viper® was only evident in males. Overall, the influence of contamination history and gender was demonstrated, highlighting the importance of considering differential physiological background in ecogenotoxicological analysis, hence favouring the elaboration of more plausible and holistic approaches integrating the environmental risk assessment of pesticides.



DNA of crayfish spermatozoa as a target of waterborne pesticides – an *ex vivo* screening to predict the impact on progeny fitness

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Germ cells have a central role in life, being the integrity of their genetic material vital to the offspring fitness. Male gametes, in particular, have lower antioxidant defences and a poor capacity of repairing DNA, wherefore they are more vulnerable to genotoxic agents, including in comparison with the oocytes. The DNA damage in spermatozoa cells might be the cause of low fertilization rate, troubles on embryonic development and a consequent decrease prolificacy, impairing the population success. It was already demonstrated that the huge amounts of pesticides released into the environment affect biota, even the non-target species which, in theory, would be protected or unaffected. The invasive crayfish, *Procambarus clarkii*, is a useful model organism for ecotoxicological studies since it can be considered a non-target specie for several pesticides. Bear this in mind, the aim of this study was to clarify if the spermatozoa DNA of *P. clarkii* are affected by waterborne pesticides at environmentally relevant concentrations. Thus, an *ex vivo* assay was performed and the genotoxic effect of six pesticides on male gametes of *P. clarkii* was assessed. The spermatozoa were exposed to (i) post-emergence herbicides: glyphosate (9 and 90 µg L⁻¹) and penoxsulam (2.3 and 23 µg L⁻¹), (ii) insecticides: dimethoate (2.4 and 24 µg L⁻¹) and imidacloprid (13.1 and 131 µg L⁻¹); (iii) fungicides: pyrimethanil (2.2 and 22 µg L⁻¹) and imazalil (16 and 160 µg L⁻¹). DNA damage was assessed using the alkaline comet assay. Genotoxicity (non-specific damage), measured by GDI (genetic damage indicator) values, was observed in the higher concentrations of glyphosate, penoxsulam, dimethoate, pyrimethanil, and imazalil. Penoxsulam and dimethoate revealed to be genotoxic even at lower concentrations, 2.3 and 2.4 µg L⁻¹ respectively.

Penoxsulam was the pesticide with higher GDI. On the other hand, imidacloprid was the only pesticide that did not cause DNA damage in crayfish spermatozoa. The improved version of comet assay, using DNA lesion-specific repair enzymes (FPG and EndoIII), showed no significant oxidative damage considering all the tested pesticides. Overall, this work demonstrates that the *ex vivo* assay is an effective tool in ecotoxicological screening assays and, moreover, that environmental concentrations of waterbone pesticides could be genotoxic to spermatozoa of non-target species.



Influence of oncometabolites in the response to DNA damage

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One of the main characteristics of tumour cells is the deregulation of the energetic metabolism, caused by accumulation of metabolites from the Krebs cycle. It may be caused by punctual mutations in genes encoding Krebs cycle enzymes, such as isocitrate dehydrogenase, succinate dehydrogenase and fumarate hydratase which result in the accumulation of oncometabolites: R-2-hydroxyglutarate, succinate and fumarate, respectively. Through inhibition of histone demethylases, that contain Jumonji domain (JmjC), and TET 5-methylcytosine hydroxylase proteins, which demethylate DNA, they are able to alter chromatin structure and, therefore, to directly modify the accessibility of DNA repair systems to DNA damage. Since alteration of DNA damage repair might be very relevant when considering cancer treatments, like chemotherapy and radiotherapy, the general aim of this work is to study the impact of these molecules on DNA damage responses, after treatment with different genotoxic agents.

As a first step, PC12 cells, from a rat pheochromocytoma of the adrenal medule, were used to study the influence of succinate in the response to hydrogen peroxide induced DNA damage, using the comet assay to quantify it. Since succinate is not capable of entering cells, its methyl derivative was used in this work. PC12 cells were pre-treated with methylsuccinate concentrations in the range 1-5 mM (much lower than the corresponding IC₅₀) for one hour, and then part of them were also treated with 200 μ M H₂O₂ for 10 min. The other part were used to detect possible genotoxic effects of methylsuccinate.

The obtained data show that 1h treatment with 1 mM methylsuccinate (physiological concentration) presented not significant effect, whereas treatment with 5 mM methylsuccinate increases around a 30% the spontaneous DNA damage, but almost a 50% the DNA damage induced by H₂O₂. In both cases the increases were statistically significant. Although these are preliminary results, if confirmed, they could suggest that alteration of chromatin structure would make cells more susceptible to the effect of genotoxic agents.



A possible role of the hERG K⁺ channel on DNA damage response

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Some voltage dependent K⁺ channels, as those of the KCNH family Kv10.1 (eag) and Kv11.1 (hERG), can play an important role on processes like cell proliferation and/or tumour progression, conferring sometimes selective advantages to tumour cells. These effects can be related to the ability of those entities to impact the electrophysiological cell properties, but may also depend on some "non-canonical" properties of the protein, unrelated to its function as a conductive ionic channel.

The human *ether-a-go-go-related gene* (hERG) channel can be located not only at the cell membrane of a variety of cells, but also on their cytoplasm in a rather high proportion, although in this case its function is not clear.

In this work we have studied if expression of hERG plays a role on DNA damage response using two human cell lines, the HEK293 that does not endogenously express this protein, and the HEK-H36, obtained by permanent transfection of the HEK293 with a plasmid containing the hERG gene, that overexpresses the channel. Both cell types were treated with the monofunctional alkylating agent methyl methanesulfonate (MMS), a genotoxic agent with a well known mechanism of action, and the induced DNA damage was subsequently quantified and compared with the comet assay.

Viability analysis showed that both cell lines were similarly sensitive to MMS. When cells were treated for 3 h with 50, 100, 200 and 350 µM MMS (all concentrations clearly under the respective IC₅₀) to study the DNA damage response, our preliminary results showed that although there were not differences in endogenous DNA damage levels between both cell types, their response to MMS treatment was different, because more DNA strand breaks were induced in HEK293 cells than in the HEK-H36 ones that express the hERG channel. Since this channel is preferentially expressed in some tumour tissues, these results suggest that the expression of this protein may help the cells to respond to the presence of DNA damage, and that it could be used, for instance, as a tool to regulate response to chemotherapy.



Complex responses in complex scenarios: the use of biomarkers in *Chironomus riparius* larvae in a polluted river

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At present ecotoxicologists have a vast array of techniques and biomarkers available to test the effects of multiple stressors in model organisms. However, most of these laboratory-tested protocols have been scarcely used in natural scenarios, partly because of the complex mixtures of factors that might have an effect on those organisms. The objective of the present work has been to gain a deeper knowledge on the physiology of the larvae of the model organism *Chironomus riparius* by exploring cellular stress, endocrine functioning, detoxification response and energy metabolism. The novelty of our approach is that these aspects have been investigated in a complex scenario, using individuals from a chronically-polluted river.

Larvae of *Chironomus riparius* were collected at five sampling stations in a 14 km-long stretch of river Sar (NW Spain). Sampling stations were selected to obtain a diverse array of stress conditions, mainly those possibly associated to the presence of important facilities and infrastructures. Stress responses were investigated through changes in 1) expression of genes related to cryoprotection (*hsp70* and *hsc70*); hormonal signalling (*EcR*); and xenobiotic detoxification pathways (*cyp4g*), 2) growth and metabolic rates indicators (RNA:DNA ratio, total protein and glycogen content), and 3) developmental sublethal effects (mouthpart deformities). Results show that several of these biomarkers (*hsp70*, *EcR*, *GAPDH*, total protein content) present a higher basal activity in field conditions. Also, differences in some biomarkers (*hsp70*, *EcR*, glycogen content, DNA:RNA) were detected among sampling points, which arises the question of the possible influence of chemical characteristics of the sediments at those sites. Opposite, *hsc70* and *cyp4g* appeared to be stable. Interestingly, the *hsp70* gene seems to be particularly sensitive to conditions of pollutant exposure, while its constitutive counterpart *hsc70* showed invariable expression, reinforcing the *hsc70/hsp70* ratio as a potential indicator of polluted environments.

Although these differential responses should be further investigated, they open a new window to explore present conditions organisms are facing in natural ecosystems, which complexity clearly exceeds that experienced in laboratory tests.

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SESIÓN V: Daño, reparación y protección del ADN

SESSION V: DNA damage, repair and protection



Radiobiological characterization of primary neuroblastoma cell lines

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Neuroblastomas (NBs) are the most common extra-cranial solid tumor of infancy, representing 7-10 % of pediatric cancers and 15 % of all pediatric cancer deaths. They are embryonic tumors that originate from sympathoadrenal cells of the neural crest. One of the main features of these tumors is their enormous phenotypic heterogeneity: from low risk patients with spontaneous regression (stage 4S/MS) to high-risk tumors (stage 4/M), frequently metastatic and resistant to treatments. Amplification of MYCN is considered a prognostic factor for high risk NB. There is emerging evidence that cancer stem cells (CSCs) determine tumor evolution and also resistance to treatments and relapses. In fact, CSCs are resistant to chemotherapy and radiotherapy and their mechanisms of DNA damage repair are involved in this skill. In addition to that, tumor stromal cells have emerged as a key point for the biology of CSCs and their environment. There are many underlying biological mechanisms of NBs that remain unclear. Less than 40 % of patients older than 12 months with metastatic disease at diagnosis survive, despite of multimodality treatment. So we have focused on radiotherapy treatments against high risk NB, that have been changing from hypofractionated total body irradiation to local primary tumor radiation.

The main objective of our study was the radiobiological characterization of NB using different cell lines as model. We also consider extremely interesting to know the relevance of non-tumorigenic cells in NB tumors insulted by ionizing radiation (IR). Viability and survival, tumorspheres growth, repair of DNA damage and cell cycle checkpoints efficiency of irradiated NB cells have been analyzed. Our results demonstrated: (1) primary non-tumorigenic NB cell line (NB14t) shows higher viability against IR as compared to 3 tumorigenic cell lines with different genetic background and phenotypic features. (2) NB14t has an efficient clearance of double-strand breaks (DSBs), assessed by the *foci* assay, caused by IR and a correct efficiency of G1 cell cycle checkpoint. (3) IR induces G1 arrest in NB14t cell cycle before a differentiation process. (4) Tumorigenic and amplified MYCN cell line (IMR-32) shows lower viability against IR than the other cell lines tested. (5) In IMR-32, efficiency of repair of DSBs caused by IR is clearly lower. These cells have a dramatic lower capacity of tumorspheres formation after IR exposition too. (6) Co-culture of a non- tumorigenic NB cell line could have radioprotective effect on tumorigenic and amplified MYCN cell line (IMR-32).



Anticancer effects of Olive Leaf Extract (OLE) on lung cell lines A549 and MRC5 and its possible modulating effect on cisplatin toxicity

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Natural polyphenols are well-known for their healthy effects. Olive leaf extract (OLE) contains much more and different types of polyphenols than those found in extra virgin olive oil, which is the basis of traditional Mediterranean diet. The most abundant polyphenol in OLE is oleuropein, which plays an important protective role in cancer cell models, due to both anti-inflammatory properties and protection against DNA damage produced by free radicals of oxidative stress.

Cisplatin is a routine chemotherapeutic drug used against solid tumors with toxic side effects. This crosslinker agent may insert into the purine bases on the DNA, interfering with DNA repair mechanisms, causing DNA damage and apoptosis in cancer cells. Platinum based drugs are often used as standard treatment in lung cancer. Their non-specificity is a major challenge because these compounds not only kill cancer cells but also are extremely toxic for normal cells.

In this work we have studied the possible antitumor activity of OLE extract, either alone or in combination with cisplatin, on a human lung carcinoma *in vitro* model. For that, we used the A549 lung adenocarcinoma cell line and the MRC5 normal cell line. Cell viability was measured by sulphorhodamine assay, DNA damage was analyzed by γ -H2AX foci assay, and cell cycle progression was assessed by flow cytometry.

Our results reflect a great decrease in cell viability in A549 cancer cells and MRC5 normal cells exposed to OLE extract and cisplatin alone. The treatment with OLE and cisplatin together decreases the cell viability of A549 cancer cell line too. What is more, this combination of drugs produces a remarkable protective effect in MRC5 normal cells, comparing with these drugs alone. These results indicate that the combined treatment could be more selective preventing the damage in normal cells than the usual therapy only with cisplatin in lung carcinoma. The oleuropein may be responsible of this protective effect. Regarding the non-specificity and the side effects of the cisplatin treatment in human lung cancer, the addition of OLE extract could improve the protection of normal cells resulting in a less harmful chemotherapy.



The macroalga *Ulva rigida* affords genome protection to *Drosophila melanogaster* via dietary supplementation

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Currently, marine macroalgae have been defended as functional food, due to their beneficial properties (e.g. anti-inflammatory, immunomodulatory and anti-tumour). In contrast, their genome's protective potential is still poorly studied, albeit some evidences about their antioxidant, antigenotoxic and antimutagenic effects. The green alga *Ulva rigida* is an edible species, native in the Atlantic coast and easily grown in aquaculture, although it is underexplored regarding its biomedical/nutraceutical potential. Yet, some studies reported the antigenotoxicity of *U. rigida* and *U. fasciata* extracts and the antioxidant potential of *U. lactuca* extract, suggesting that species of *Ulva* genus may increase genome protection. Nevertheless, it must be pointed that those studies evaluated only the effects of algae extracts through *in vitro* trials, disclosing a gap of knowledge about *in vivo* effects of the whole algae ingestion on genome integrity maintenance. Hence, our goal was the search for beneficial effects of *U. rigida*, through an increased genome protection, aiming a functional characterization of healthy foods and human health promotion. For that, the antigenotoxic potential of *U. rigida* was assessed in *Drosophila melanogaster* following a dietary exposure, and against an exposure to streptonigrin (mutagenic agent). Thus, somatic mutation and recombination test (SMART) and comet assay were adopted, measuring somatic mutations/recombination events and DNA breaks, respectively. Two concentrations of *U. rigida* were tested and groups were distributed according to the following conditions: C (control); 2.5U/5.0U (2.5 or 5.0% of *U. rigida* supplementation); S (streptonigrin); 2.5U+S/5.0U+S (2.5 or 5.0% of *U. rigida* supplem. + streptonigrin). Regarding the antigenotoxic potential measured through SMART, both alga doses were beneficial against the streptonigrin-induced damage, though no differences were observed between them. In parallel, both levels of diet supplementation with *U. rigida* revealed their antigenotoxic ability only against DNA breaks induced by streptonigrin and, in this case, 5.0U revealed higher antigenotoxic potential than 2.5U. Overall, a diet supplemented with *U. rigida* showed to promote genome protection in *D. melanogaster*, particularly against damage induced by streptonigrin. These findings may contribute to the algaculture industry development, as well as the reinforcement of the idea of algae as functional food.



MTH1 a new anticancer target for neuroblastoma treatment

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Cancer is a highly unpredictable disease, capable of affecting different parts of the human body and usually associate with a harsh medical treatment. Even though there are a lot of blank pages in the book of cancer, there are some traits that start to be well defined. One of this is the high levels of reactive oxygen species (ROS) that drive oncogenic signaling.

Different authors suggest that ROS level can influence, even regulate, processes such as anti-tumor responses in cancer cells, but also cell death or senescence. Many cancers, show increased levels of the MutT Homolog1 (MTH1) enzyme to counteract the high levels of oxidative stress that present. This enzyme sanitizes dNTP pools eliminating 8-oxo-7,8-dihydro-2'-deoxyguanosine triphosphate (8-oxoGTP), in order to prevent its incorporation during DNA replication. MTH1 is non-essential in normal cells, but a recent study demonstrates that MTH1 function is essential in cancer cells to avoid the incorporation of oxidized dNTPs; that would lead to DNA damage and cell death. This is the reason because MTH1 has been proposed as a selective anticancer target.

In our group, we are using different MTH1 inhibitors to treat one specific type of cancer: neuroblastoma. Neuroblastoma is a pediatric cancer arising from sympathetic nervous system. One of the best known features of this cancer is the heterogeneity in outcome. Amplification of the oncogene MYCN is related with unfavorable prognosis.

In the first part of our study, we treat three neuroblastoma's cells lines with MTH1 inhibitors: TH588 and TH1579. After the treatment, we observed reduction in viability levels as well as increased DNA damage in all of them. In view of this result we can hypothesized that MTH1 has an essential role in neuroblastoma cancer cells, though, right now, we cannot conclude anything about the mechanism behind.

Due to the high rate of mortality of neuroblastoma, we are also working on developing new therapies to treat these tumors. Since we know the potential of MTH1 inhibitors, we are trying to combine them with a panel of chemotherapy drugs and DNA repair inhibitors. Of interest a promising combination showing synergistic effect in MYCN amplified neuroblastoma cells was to combine MTH1 inhibitor with a DNA-PK inhibitor (NU7026).

Although our results are quite preliminar, our data could open novel ways for the treatment of high risk neuroblastoma tumors.



Antigenotoxic and longevity-promoting potential of red seaweeds *Porphyra umbilicalis* and *Grateloupia turuturu* in *Drosophila melanogaster*

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Seaweeds have been increasingly explored for human food industry and, in particular, red seaweeds *Porphyra umbilicalis* and *Grateloupia turuturu* are often included in the diet (mainly in East Asia), displaying a rich chemical composition and beneficial effects. Nevertheless, there are knowledge gaps in studies focusing their potential to protect genome integrity, a critical premise for disease prevention and longevity-promotion. In line, *in vivo* studies assessing the effects of the full intake of these algae are lacking. Thus, *Drosophila melanogaster* diet was supplemented with *P. umbilicalis* and *G. turuturu* aiming the evaluation of their potential as genome integrity- and longevity-promoters, using somatic mutation and recombination test (SMART) and longevity assay, respectively. For longevity assay, seaweeds were incorporated in the media at 1.25, 2.5, 5, 10 and 20% (w/w). Alga supplementation occurred until eclosion. Flies were counted weekly until death. For SMART, the diet was supplemented with 5 and 10% *P. umbilicalis* and 10 and 20% *G. turuturu* until eye observation in adults. A DNA damage inducer, streptonigrin (SN), was added as genotoxic insult. Eye observation consisted in counting the total number of spots per eye, *i.e.*, white phenotype ommatidia in red eyes. Supplementations with 10% *P. umbilicalis* and 20% *G. turuturu* were able to promote an increase of the longevity of *D. melanogaster*, with *G. turuturu* reaching the highest potential; there were no significant differences between males and females longevity. The spontaneous genotoxicity (without SN-challenge) was reduced in flies supplemented with 10% *P. umbilicalis* and 10 and 20% *G. turuturu*. On the other hand, all seaweed concentrations demonstrated antigenotoxic potential against SN-induced genotoxicity. The major promoter of genome integrity was 20% *G. turuturu* and 5% *P. umbilicalis* the lowermost. Furthermore, SN demonstrated its genotoxic capacity, depicted in the increased number of spots comparatively to unchallenged flies. Considering the seaweed supplementation level of 10% for SN-challenged conditions, a higher antigenotoxic potential was displayed by *G. turuturu*. Within the framework of nutrition-toxicology, the tested seaweeds proved to be promoters of a safe and healthy nutrition as functional foods.



The enzyme-modified comet assay: Measuring oxidized and alkylated bases using the 12-gels format

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The standard alkaline comet assay detects DNA strand breaks and alkali-labile sites at single-cell level. However, many DNA-damaging agents induce other lesions (e.g. oxidized and alkylated bases). This limitation is overcome by the combination of the assay with DNA repair enzymes. Indeed, several enzymes are already being used, but their specificity to detect certain lesions has not been assessed.

The final aim of this work is to study the specificity of several enzymes in order to select the most convenient ones to detect oxidized and alkylated bases. The following commercial enzymes are being used: formamidopyrimidine DNA glycosylase (Fpg), endonuclease III (Endo III), human 8-oxoguanine DNA glycosylase (hOGG1) and human alkyladenine DNA glycosylase (hAAG). For the first time, the hAAG, is being used in combination with the comet assay. The widely used Fpg produced and distributed by NorGenoTech (Norway) (Fpg-NGT) has also been tested.

The differences between the 2 and 12 gel/slide systems were assessed by the titration of Fpg-NGT in both formats and using different times of incubation (37°C). TK-6 cells treated with potassium bromate (KBrO₃) to induce oxidized bases were used. Results showed a different pattern when using different formats.

The rest of the enzymes were titrated using the 12 gels/slide system and 1 hour of incubation. TK-6 cells treated with methyl methanesulphonate (MMS), to induce alkylated bases, were used for hAAG. On the other hand, TK-6 cells treated with KBrO₃ were used to titrate the rest of the enzymes. The same reaction buffer was used with all the enzymes which is very convenient if different enzymes are meant to be used at the same time. Results of the titrations will be presented and compared with the manufacturer instructions. Endo III was not successfully titrated; another compound to induce Endo III-sensitive lesions will be tested.

The specificity of the enzymes was also studied on Glyco-SPOT biochips by LXRepair, obtaining the expected results. To complete this work, all enzymes will be tested in TK-6 cells treated with non-cytotoxic concentrations of different genotoxic compounds including MMS and KBrO₃. Data showing the ability of hAAG to detect MMS-induced lesion but not KBrO₃-induced lesions will be presented.

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DNA damage induced by acute exposures to the plasticizers DEHP, BBP, and DBP, in the model species *Chironomus riparius* (Diptera)

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Plasticizers are additives that increase the plasticity or viscosity of plastic materials, especially polyvinyl chloride (PVC), making them soft and flexible. Other uses outside the PVC industry include their presence in fragrances and personal care products, adhesives, paints, household cleaning products, printing inks, textiles, and many more. Among the many substances used for these purposes, phthalates are a widely-used group of chemicals with a very high production worldwide. Given that phthalates are not chemically bound to the polymeric matrix and due to their high production worldwide, they are considered as ubiquitous contaminants that can be found in almost any environmental compartment.

Three of the most common phthalates used in the manufacture of plastics are: bis(2-ethylhexyl) phthalate (DEHP), benzyl butyl phthalate (BBP), and di-n-butyl phthalate (DBP). They have been found in a diversity of environmental samples, detected in tissues of exposed animals and humans, and classified as toxic in numerous studies, especially regarding their activity as endocrine disrupting compounds (EDCs). Several international agencies have classified DEHP as carcinogenic, while there is some controversy regarding the carcinogenic potential of BBP, and no evidences of carcinogenicity have been described for DBP.

In the present study, we used the comet assay to assess the ability these three phthalates to induce DNA damage in insects. *Chironomus riparius* larvae were exposed for 24 hours to environmentally relevant concentrations (1 and 100 µg/L). Several parameters (mainly %DNA in tail) demonstrated that 100 µg/L DEHP, BBP, or DBP altered the DNA integrity (2.4-fold, 1.7-fold, and 1.8-fold, respectively), while 1 µg/L showed a genotoxic response only in the case of DEHP (1.8-fold).

These findings provide new insights into the genotoxic potential of DEHP, BBP, and DBP, particularly in invertebrates, and bring attention on potential human and environmental health risks arising from widespread and continuous exposure to these substances.

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SESIÓN VI: Contaminantes emergentes

SESSION VI: Emerging pollutants



Effects of Methylmercury on cell death, proliferation and cellular phenotypic specification of human neural stem cells

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Methylmercury (MeHg) is a long-lasting organic pollutant that is mainly found in aquatic environments. The main source of MeHg human exposure is the MeHg- contaminated seafood. MeHg accumulates in various organs, however, the brain is its main target. The developing brain is particularly affected by MeHg although adult brain may also be vulnerable.

MeHg interacts with cysteine to go through the cysteine channels of the blood brain barrier. After that, it accumulates mainly in the cerebellum and the cerebral cortex. MeHg affects the cellular cytoskeleton and mitochondria, it affects the intracellular Ca^{2+} and extracellular glutamate concentrations and induces apoptosis.

Human neural stem cells (hNSCs) are the precursors of neurons and glia, these cells can differentiate into all neural cells of the Central Nervous System (CNS). hNSCs are valuable tool in neurotoxicology assays giving the opportunity to study early stages of neural development.

In the present investigation, we are using human neural stem cells as model. The neurotoxic assays are being performed in the cellular line hNS1 that is a clonal and multipotent cell line. To observe the effects of MeHg, we have exposed the hNS1 cells to different concentrations of MeHg and analyzed cell viability, proliferation and cell fate specification under proliferation and differentiation conditions.

The objective is to identify the key targets for developmental neurotoxicity (DNT) to obtain toxicity pathways and biomarkers that could be incorporated into testing strategies in DNT chemical assessment.



Changes in microRNAs expression associated to long-term exposure to nanomaterials

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Nanotechnology is an emerging field with good perspectives in many biomedical and industrial applications. For this reason, the presence of nanoparticles in the environment is steadily rising and, potentially, affecting humans. Therefore, research on the molecular mechanisms affected by the presence of these nanoparticles is required to achieve a better understanding of their exposure associated health risks.

MicroRNAs are small non-coding single-strand RNA molecules of 20-24 nucleotides-long, which main function is posttranscriptional regulation of gene expression. They participate in numerous biological processes, such as cancer, inflammatory processes, etc. Consequently, changes in their expression can serve as biomarkers for early detection of tumoral phenotype.

The main objective of this study is to establish if there are expression changes in a battery of microRNAs related to cancer and disease after an *in vitro* long-term exposure to low-dose to nanomaterials able to induce cell transformation. For that purpose human lung epithelial BEAS 2B cells were exposed chronically to different nanomaterials, specifically, cerium dioxide NPs (CeONPs), titanium dioxide nanoparticles (TiO₂NPs), and multi walled carbon nanotubes (MWCNT). In addition, cerium dioxide was co-exposed with cigarette smoke condensate (CSC), since CeO₂ did not induce cell transformation on its own, but was found to increase the oncogenic potential of CSC. Cells were collected before and after the acquisition of the tumor-like phenotype and changes in the microRNAs expression were evaluated by qPCR.

The study revealed changes in the microRNAs expression pattern during the NM-long-term exposure which were found to be dependent of both, time-of-exposure and dose. Hence, some microRNA elicited as new candidate biomarkers of NM long-term exposure and transforming effects.



miR-21 involvement in cobalt and zinc oxide nanoparticles-induced cell malignant transformation

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The growing application of nanotechnology in industrial processes has increased the exposure to nanoparticulated material by the overall population. The few existing information on the properties of these nanoparticles, in terms of potential genotoxic risk, urge the scientific community to evaluate the adverse effects of these nanomaterials in order to correct negligence and mitigate damage to humans and environment. In this scenario, a new discipline has born: Nanotoxicology.

Recent studies, have shown dysregulation in the expression of micro RNAs resulting from exposure to nanomaterials. MicroRNA molecules (miRNAs) are non-coding RNAs with a length ranging from about 20 nucleotides in animals, and with a regulatory function. Their sequences are complementary to a target mRNA, and when hybridizes with it, it silences its translation into protein. As they contribute to the regulation of gene expression at the cellular level, their biogenesis is highly regulated both temporally and spatially. Alterations in this process of genesis and maturation triggers multiple diseases in humans, including cancer. Among them, miR-21 is particularly linked to the most common tumors in humans, such as breast, lung and prostate cancers. This fact leads us to think that miR-21 can be involved in the regulation of the expression of cellular Tumor Suppressors. That is the reason why we have focused our studies on this specific miRNA.

We cultured MEF cells long term exposed to sub-toxic doses of cobalt and zinc oxide nanoparticles. Different assays were performed weekly in order to determine the moment of transformation and tumoral phenotype acquisition. After weeks of treatment, the expression of miR-21 before and after cellular transformation was quantified reporting dysregulation. Simultaneously, we studied the relative expression of three tumor suppressors: PTEN, PDCD4 and TPM1. To confirm the trends shown in the qPCR analysis and establish cause-effect relationships, we studied the expression of miR-21 and Tumor suppressors after exposing the cells to mimics and inhibitors of their expression.



Effects of differently shaped TiO₂NPs (nano-spheres, nano-rods and nano-wires) on the *in vitro* model (Caco-2/HT29) of intestinal barrier

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The biological effects of nanoparticles depend on several characteristics, such as size and shape which must be taken into account in any type of toxicological assessment. The increased use of titanium dioxide nanoparticles (TiO₂NPs) for industrial applications, and specifically as a food additive, demands a deep assessment of their potential risk for humans, including their abilities to cross biological barriers.

In this study we have investigated the interaction of three differently shaped TiO₂NPs (nano-spheres, nano-rods and nano-wires) in an *in vitro* model of the intestinal barrier, where the co-culture of Caco-2 and HT29 cells confers inherent intestinal epithelium characteristics to the model (i.e. mucus secretion, brush border, tight junctions, etc.).

According to the obtained results, adverse effects in the intestinal epithelium were detected by studying the barrier's integrity (TEER, trans-epithelial electrical resistance), permeability (LY, Lucifer yellow translocation) and changes in the gene expression of selected specific markers. In addition, using Laser Scanning Confocal Microscopy, we detected a different behaviour in the bio-adhesion and bio-distribution of each one of the TiO₂NPs. Moreover, we were able to specifically localize each type of TiO₂NPs inside the cells. Interestingly, general DNA damage, but not oxidative DNA damage effects, were detected by using the FPG version of the comet assay.

As a conclusion, our results indicate different interactions and cellular responses related to differently shaped TiO₂NPs, nano-wires showing the most harmful effects.



Effects of graphene oxide and graphene nanoplatelets on the *in vitro* model of intestinal barrier (Caco2/HT29)

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Nanotechnology has become a booming discipline due to the wide range of applications of new nanomaterials (NMs). Among these, Graphene stands out as an attractive carbon-based NM due to its physicochemical properties. Its optical and electric properties make it a perfect candidate for applications in a variety of fields such as physics, healthcare, material science, and, recently, biomedicine. Moreover, the possibility to customize these properties through chemical modifications has generated a wide range of graphene materials, among which are graphene oxide (GO) and graphene nanoplatelets (GNPs).

Given the usefulness of graphene NMs in innovative approaches such as drug delivery systems and biosensors for health monitoring, it is important to define their interaction with potentially-exposed human tissues. Considering oral intake as an exposure route for graphene NMs, we aim to evaluate the interaction, distribution, and toxic effects of GO and GNPs in the intestinal barrier. For this purpose, we used a co-culture *in vitro* model composed of differentiated Caco2 and HT29 cells, which respectively mimic the phenotype of the enterocytes and goblet cells.

After the characterization of GO and GNPs by DLS and TEM, cell viability assays showed that concentrations ranging 5-100 µg/mL GO and GNPs were non-cytotoxic in our model. To detect the potential adverse effects of these NMs, Caco2/HT29 barriers will be treated with 5-50 µg/mL of GO and GNPs. TEER and LY experiments will elucidate the effects of GO and GNPs on the barrier's integrity and permeability, while Laser Scanning Confocal Microscopy assays will show their distribution in our intestinal barrier model. In addition, genotoxic and oxidative damage assessment by comet assay and changes in the expression of *HO1* and *SOD2*, two ROS-scavenging involved genes will give us information on the oxidation state of the cells and DNA damage. Finally, as it has been suggested that the relationship between autophagy and nanoparticles could be related with their mechanism of toxicity, we will also study the expression of autophagy markers by Western Blot in response to GO and GNPs treatments.



Involvement of *Mth1* in the toxic and carcinogenic effects of cobalt and zinc oxide nanoparticles

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We have previously shown that short-term exposure to low doses (between 0.05 and 1 µg/mL) of cobalt and zinc oxide nanoparticles (CoNPs, ZnONPs) are able to induce reactive oxygen species (ROS) and oxidative DNA damage (ODD) in mouse embryonic fibroblast cells (MEFs). This ODD was found also to have a prominent role in the transforming effects of the NPs found under chronic exposure scenarios, and characterized by morphological cell changes, significant increases in the secretion of metalloproteinases (MMPs) and anchorage-independent cell growth ability; all cancer-like phenotypic hallmarks.

The MutT homolog 1 (*Mth1*), is a pyrophosphorylase that effectively sanitizes oxidized dNTP pools to prevent incorporation of damaged bases into DNA. In this work, we sought to assess the role of *Mth1* in the toxic and carcinogenic effects of chronic Co and ZnONPs exposure. Thus, *Mth1* knock-down was carried out in MEF cells chronically exposed to the NPs for 12 weeks by using MISSION® constructs carrying shRNA sequences targeting mouse *Mth1*. Knock-down cells were then exposed to the NPs for 2 more weeks, and cytotoxic and carcinogenic end-points were evaluated and compared to the empty vector controls.

Results demonstrate a *Mth1* expression knock down of about 90% efficiency, and *Mth1* was found to be involved in the toxic and carcinogenic effects of Co and ZnONPs, as the absence of expression is associated with a phenotype with higher sensitivity to both NPs and oxidant agents, and with a reduced aggressiveness of the transformed phenotype.



Time-lapse studies on the effects of silver nanoparticles in the Caco-2/HT29 model of intestinal barrier

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The use of nanoparticles (NPs) has increased in the last years, particularly in food-grade products, where NPs are used to improve texture, flavor, microbiological control, food processing and packaging. In this context, silver nanoparticles (AgNPs) are highly used by the food-packaging and pharmacology industries due to their antimicrobial properties. Since the health effects of this constant exposure are not well-known, the assessment of potential risks for humans related to AgNPs intake became crucial.

The objective of this study is to analyze the effects of the exposure to AgNPs over the gastrointestinal epithelium (morphologically and functionally) over a period of 96 h. To this aim, we used an *in vitro* co-culture monolayer model composed of differentiated Caco-2 and HT29 cells, which respectively mimic enterocytes and goblet cells. We have evaluated the effects of two different sub-cytotoxic concentrations of AgNPs: the estimated daily intake of Ag of about 80 µg (2.58 µg/mL), and the highest non-cytotoxic concentration tested in our system (100 µg/mL). The evaluation of parameters such as the trans-epithelial electrical resistance (TEER) and paracellular permeability (LY), will give insight on the effects of AgNPs over the barrier's integrity. Confocal microscopy images will give us information on the uptake and cellular localization of the nanoparticles. Changes in the expression of intestinal epithelial markers by RT-PCR and a Western Blot, such as brush border and tight-junctions proteins, will give us further information on the possible changes regarding the monolayer's digestive and barrier functions. Finally, the quantification the translocation through will measure the ability of AgNPs to cross the intestinal barrier.



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