International Conference on the Impact of Environmental Pollutants on Mental and Neurological Health

Universitat Jaume I – Castellón, Spain

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Presentation

One of the challenges of today's society is to minimize the impact of anthropogenic environmental pollutants on the environment in general and on human health in particular. This fact has been reflected in the European Research and Innovation Strategy 2020-2024 in two specific objectives: "General objective 1A: European Green Deal" and "General objective 5: Promoting our European Way of Life". Within the framework of the European Green Deal, the Horizon Europe missions inspire transformation in critical areas such as the health of oceans, seas, coastal and inland waters, as well as soils and food. In relation to general objective 5, the Directorate- General for Research and Innovation of the European Union points out, as a top priority, to improve and protect the health of citizens at all ages, by developing innovative solutions to prevent, diagnose, control, treat and cure diseases. As part of this strategy, the Spanish Ministry of Health also adopts the concept of "One Health", a multi-sectoral public health strategy that recognizes the interactions and interdependencies between environmental, animal and human healths. Under this view, a consortium was constituted to run the project ***Psychiatric disorders and comorbidities caused by pollution in the mediterranean area (PsyCoMed)"*** financed by the EU under the Horizon-Marie Sklodowska Curie Actions-2021staff exchanges project 101086247.

It is therefore crucial that more in-depth research be carried out to understand the link between exposure to pollutants and neurological disorders, as well as to develop effective policies to address these problems. The planned congress will provide scientific evidence to assess the magnitude of the problem.

This conference aims to promote a debate on the impact of environmental pollutants on neurological and mental health. The main objective of the meeting is to promote a multidisciplinary approach that encompasses neuroscience, toxicology, public health and environmental policies.

At this conference, we will develop seven plenary lectures, two workshops, twelve oral communications and seventeen posters. Communications come from chemical analysis to molecular biology and cover a wide spectrum of approaches to the problem of pollutants on mental health.

Francisco E. Olucha-Bordonau, President of the Conference

Marc Landry Coordinator of the PsyCoMed Project

PROGRAM

Wednesday 29 October 2025	
9:30	**Official opening**
9:50	**PL1.- Plenary Lecture.** The impact of Bisphenol A on social recognition and social behavior. **Anne-Louise Ponsonby**; The Florey Institute of Neuroscience and Mental Health Chair :Marc Landry
10:30	Coffee break
11:00	**Scientific Session 1: The neuroinflammatory reaction to external compounds, including pollutants.** Chair: Jacques Noel
11:05	**O1.-** Olfa Masmoudi-Kouki UTEM Neuroprotective Mechanism of PACAP Against Prenatal and Subchronic Glyphosate Exposure: Involvement of Oxidative Stress, Epigenetic Alterations, and Gut-Brain Axis Disruption
11:30	**O2.-** Marwen Ben Fdilen UTEM-UJI Pro-Proliferative Effects of Micro-nanoplastics on Glioblastoma
11:55	**O3.-** Rubén Gil Solsona UJI Through chemical characterization of air particulate matter (PM10, PM1 and UFP) and investigation of brain tumors exposome to assess potential associations
12:30	Poster Session
13:00	Lunch Break
14:30	**Scientific Session 2: Heavy metal-induced neurotoxicity** Chair, Esther Castillo-Gómez
14:35	**O4.-** Mercè Garí

Wednesday 29 October 2025	
	Regional differences in mercury and other neurotoxic metals, dietary habits, and impacts on child neurodevelopment
15:00	**O5.-** Amira Zaki; Alexandria University Prenatal Ethanol and Cadmium Co-Exposure Induces ADHD-like Neurobehavioral Deficits in Offspring: Implications for Preventive Medicine
15:25	**O6.-** Facundo Vitelli Storelli; UJI Association of metal exposure with inflammation biomarkers in adult population
15:50	**PL2.- Plenary Lecture** Endocrine-disrupting chemicals exposure and neurodevelopment: combining toxicological and epidemiological knowledge to improve causal inference in mother-child cohorts **Vicente Mustieles**; IBS-Granada CIBEResp Chair, Anne Louise Ponsonby
16:25	Coffee break
16:55	**Workshop 1 "Characterization of Contaminants in Environmental Matrices"** Juana Maria Delgado-Saborit & Carlos Alfaro-Perez, UJI Universitat Jaume I
18:00	**Free evening**

Thursday, 30 October 2025	
9:00	**Plenary Lecture 3.** Strategies for improving human health through the One Health concept: development of the INMA cohort. **Joan O. Grimalt** IDEA-CSIC Chair: Juana Maria Delgado-Saborit

Thursday, 30 October 2025	
9:55	**Scientific Session 3. Metabolic Effects Induced by Contaminants. Nervous System-Visceral Systems Interaction.** Chair: Liana Fattore
10:00	**O7.-** Anna Maria Carrese Universite di Napoli Federico II, Italy Brief Bisphenol-A exposure impairs synaptic plasticity and glial homeostasis in human models
10:25	**O8.-** Marc Landry, Université de Bordeaux, France The Nucleus Incertus, a new crossroads for pain pathways?
10:50	**O9.-** Chman Shahzadi Persistent Nanoplastic Retention disrupts the Neuroimmune Integrity Through Cellular Organelle Stress
11:25	Coffee Break
12:05	**Plenary Lecture 4** INMA (Infancia y Medio Ambiente/Childhood and Environment) Cohort. Experience with major environmental exposures and their neuropsychological and mental health effects in childhood. **Jesús Ibarluzea UPV-EHU** Chair: María de los Ángeles-Marqués
13:00	Lunch
14:30	**Workshop 2: Methods for Analyzing the Effects of Pollution on Behavior through AI tools Deep-Lab-Cut and SIMBA** Mohamed Zahran and Monica Navarro-Sánchez, Universitat Jaume I
16:30	Social Program. Visit to Peñíscola and conference dinner

Friday, 31 October 2025	
9:00	**Plenary Lecture 5 Francisco E. Olucha-Bordonau UJI** Blood brain barrier- neurovascular unit permeability to pollutants

Friday, 31 October 2025	
	Chair: Marc Landry
9:35	**Session 4. Impact of Environmental Contaminants on Behavior with Special Emphasis on Micro- and Nanoplastics.** Chair, Olfa Masmoudi-Kouki
9:40	**O10.** Mercè Garí IDEA-CSIC Plasticizers and brain neurodevelopment during childhood
10:00	**O11.** Mónica Navarro-Sánchez UJI The alterations in normal behavior in adult rats resulting from nanoplastic intoxication of their mothers during pregnancy and/or lactation
10:20	**O12** Cinta Zapater IATS-CSIC Nuclear estrogen receptors of European sea bass (Dicentrarchus labrax) as targets of endocrine disrupting compounds: effects on reproduction and risk assessment potential
10:40	Coffee Break
11:10	**Plenary Lecture 6 Mónica Torres-Ruiz** CNSA- ISCIII Behavioral endpoints in zebrafish larvae as indicators of neurotoxicity Chair: Carole Rovere
11:40	**Plenary Lecture 7 Ana I Cañas Portilla** CNSA ISCIII The national center for environmental health: understanding the impact of environmental pollution on health Chair: Carole Rovere
12:10	**Roundtable Discussion: Pathways to tackle Environmental Pollution in Modern Societies: From Citizens to Policies.** Chair Juana María Delgado-Saborit, UJI Mónica Torres, Centro Nacional de Salud Ambiental-CNSA-ISCIII (Center of refereence in Environmental-Health Research) María Susana Fernández Rocha, Confederación Hidrográfica del Júcar (Water Quality Management Service for continental water)

Friday, 31 October 2025	
	Inmaculada Varó Vaello IATS-CSIC Jacques Noel and Carole Rovere the Neurotox and PlasticHealth projects (Scientific Community)
13:10	Closing Ceremony

PLENARY LECTURES

PL1

THE IMPACT OF BISPHENOL A AND OTHER CHEMICALS ON EARLY SOCIAL BEHAVIOR AND NEURODEVELOPMENT.

Prof Anne-Louise Ponsonby B Med Sci MBBS PhD, FAFPHM, RACP, FAAHMS

The Florey Institute of Neuroscience and Mental Health, University of Melbourne. On behalf of colleagues at the Florey Neuroepidemiology Group, Barwon Investigator Group and Minderoo Foundation.

Neurodevelopmental conditions such as autism reflect multifactorial causation with multiple genetic and environmental factors operating through molecular pathways to cause disease. DNA methylation is an epigenetic molecular process sensitive to the early environment. In our research program, we utilize a range of approaches to strengthen causal inference. These include experimental studies, molecular mediation and genetic stratification. Genetic predisposition, male sex and early life chemical exposure and have been implicated in autism. In the Barwon Infant Study birth cohort (n = 1074 infants), higher prenatal maternal bisphenol A (BPA) levels were associated with higher autism diagnosis at age nine years only in males with low aromatase genetic pathway activity scores. Molecular mediation is a modern casual inference technique that we utilize to better understand how prenatal plastic exposure may lead to neurodevelopment harm through metabolomic, lipidomic and epigenetic molecular pathways. In two birth cohorts, aromatase gene methylation mediated the link between higher prenatal BPA and brain-derived neurotrophic factor methylation. BPA suppressed aromatase expression in vitro and in vivo. Male mice exposed to mid-gestation BPA or with aromatase knockout had autism-like behaviours with structural and functional brain changes10-hydroxy-2-decenoic acid a leviated these features and reversed detrimental neurodevelopmental gene expression. We analyzed cord blood DNA methylation and demonstrated that it mediates the association between prenatal DEHP exposure and autism and ADHD symptoms. At the institute, we are also undertaking further laboratory work, extending from the work on the BPA and autism to other prenatal plastic chemicals, metals and other child outcomes such as cognition as well as conditions such anxiety and

schizophrenia. Our work to date indicates that vulnerability to the adverse neurodevelopmental effect of prenatal phthalate chemicals with be greater among individuals with higher pro-oxidant and pro-inflammatory genetic function scores, based on genes that capture related transcriptional activity. Future work on the causation of these neurodevelopmental disorders such as autism is likely to benefit from examining chemical mixtures operating in pregnancy and both environmental and genetic factors in the context of shared biological mechanisms.

PL2

Endocrine-disrupting chemicals exposure and neurodevelopment: combining toxicological and epidemiological knowledge to improve causal inference in mother-child cohorts

Vicente Mustieles [1,2]

[1] Instituto de Investigación Biosanitaria (ibs.GRANADA), Granada, Spain; Servicio de Radiodiagnóstico, Hospital Universitario Clínico San Cecilio, Granada, Spain. [2] Center for Biomedical Research (CIBM), University of Granada, Spain; CIBER de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain.

Introduction: Phenols and phthalates are common examples of prevalent endocrine-disrupting chemicals (EDCs) that can interfere with brain development. Despite continuous advances in the field, some methodological challenges remain.

Methods: We present two case studies in the Spanish Childhood and Environment (INMA) Study and the French SEPAGES cohort, illustrating how the combination of previous toxicological and epidemiological data may increase the biological plausibility, consistency and external validity of associations, while reducing concerns on multiple testing.

Results: First, we show how the use of adverse outcome pathways (AOPs) helped identify brain-derived neurotrophic factor (BDNF) as a potential underlying pathway of bisphenol A (BPA) neurotoxicity. After testing this hypothesis in the INMA-Granada cohort, we found that blood BDNF DNA methylation mediated the longitudinal association between childhood BPA exposure and altered behavior during adolescence. Second, in the SEPAGES

cohort, we show how relying on a weight-of-evidence (WoE) prioritization and meta-analyses of previous comparable studies using Bayesian models helped to improve the biological plausibility and external validity of associations. In this case, we found that third-trimester exposure to prioritized phenols/phthalates and their mixture was associated with reduced child intelligence quotient scores, whereas non-prioritized (low WoE) chemicals showed inconsistent associations.

Conclusion: In mother-child cohorts, relying on prior toxicological and epidemiological data can help identify mechanisms, and increase the biological plausibility and external validity of neurodevelopmental associations. Since these evidence-based strategies can increase the uptake of results by regulatory agencies, there is a need to continue exploring ways to synergize toxicological and epidemiological knowledge.

PL3

STRATEGIES FOR IMPROVING HUMAN HEALTH THROUGH THE ONE HEALTH CONCEPT: DEVELOPMENT OF THE INMA COHORT.

Joan O. Grimalt, Esther Marco and Mercè Garí

IDAEA-CSIC. Barcelona

The scientific approach at the origin of the first INMA cohort is described. Preliminary steps involved the identification of high concentrations of hexachlorobenzene from non-target analysis of river water and air pollution in a school located near a chlor-alkali plant (Ebro River). First collection of blood samples from volunteers from the exposed village showed high concentrations of this pollutant. Use of the county cancer registry showed high incidence of thyroid cancer in the exposed population. A cohort of adults (age > 14 years) was organized for the study of health effects (n = 600). Causal associations between thyroid hormone disorders and hexachlorobenzene exposure were assessed. No correlation with hexachlorobenzene exposure and porphyria cutanea tarda was found. Volatile organic compounds in air and water and organochlorine compounds in diet were measured. An INMA cohort in the region to assess the health effects in newborns (INMA Ribera d'Ebre) was implemented. The area of influence of the pollutants released from the chlor-alkali was determined by environmental analysis and biomonitoring. Huge amounts of mercury, heavy metals, organochlorine compounds and

radionuclides from the decomposition of ^{238}U and ^{232}Th were found in the residues dumped in the river from the chlor-alkali plant (700,000 tons). A recovery program for their extraction, treatment and disposal in a landfill was developed. The pending tasks for elimination of the released pollutants in the area are still under scrutiny. Birth-cohorts and aligned studies in other areas in Spain were also implemented. The results showed some associations with neurodevelopment delays.

PL4

INMA (Infancia y Medio Ambiente/Childhood and Environment) Cohort. Experience with major environmental exposures and their neuropsychological and mental health effects in childhood.

Jesús Ibarluzea, on behalf of of the group of Environmental epidemiology IIS BioGipuzkoa and from de subcohorts of Sabadell, Valencia, Gipuzkoa, Asturias, Granada and Menorca (INMA).

IIS BioGipuzkoa, Grupo de Investigación de Salud Ambiental, Universidad del País Vasco, Euskal Herriko Unibertsitatea, Spain

The INMA project aims to study the impact of early environmental exposures on prenatal and postnatal physical development and on neuropsychological and mental health development in childhood and adolescence. This project is based on a study of birth cohorts located in Sabadell, Valencia, Asturias, Gipuzkoa, Granada, Menorca Island, and Ribera de Ebro. The birth cohort began in 1997-2005 and has currently completed the follow-up phase of adolescents/young adults (18-25 years). The study began with 3,174 pregnant mothers (first trimester) and 3,768 live births. At 1.5 years, 2,939 babies were followed up, at 4 years (2,816 boys/girls), and at 8/9 years (2,401). During the follow-up, anthropometric and clinical tests (respiratory function) were performed, biological samples were taken (blood, urine, saliva, hair, nails, colostrum or placenta) as appropriate, and questionnaires were applied on sociodemographic variables, residence, work and disease history, diet (parents and offspring), and lifestyle habits. At the same time Air pollution, water pollution and noise were measured or measured at different times.

Neurodevelopmental tests were administered: specific functions (memory (n-Back), attention (ANT), executive functions, language (Best)), intelligence and

its domains (Dubowitz, Bayley, and McCarthy), family (Etxadi–Gangoiti), or mental health screening tests (California Preschool Social Competence Scale, SDQ, Childhood Asperger Syndrome Test, Conners, CBCL parents and teacher).

Some associations between neuropsychological functions and mental health obtained with specific chemical exposures (metals/metalloids: lead, mercury, arsenic, or manganese, or persistent organic compounds), air pollution ($PM_{2.5}$ and NO_2), drinking water contaminants (fluoride), or exposure to green/blue environments in children will be described. The results obtained in the INMA cohort will also be compared with those obtained in other cohort studies.

The INMA cohort has published a significant number of articles in this field of study (>200) in high-impact international journals in the areas of Public Health, Environmental Epidemiology, and Pediatrics.

The project has received funding from the European Community, CIBERESP, the Health Research Fund, the European Union's Framework Programmes VI and VII (Hiwate, Escape, Hitea and Contamed projects), the Ministry of Education and Science, regional, provincial and local governments, and various foundations and institutions (Fundació La Caixa; La Fundació Roger Torne), among others.

PL5

Blood brain barrier- neurovascular unit permeability to pollutants

Francisco E. Olucha-Bordonau[1], Mónica Navarro-Sánchez[1], Isis Gil-Miravet[1], Zineb Bouargane[2], Danna Rubio[1], José Hidalgo-Cortés[1], Esther Castillo-Gómez[1], Mohamed Zahran[1]

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As a new concept, the exposome represents a collection of physical, chemical, biological and psychosocial environmental elements to which an individual is exposed. In anthropogenic environments, new elements are introducing unexpected burdens that may harm internal homeostasis and compromise health. Environmental pollutants are among these anthropogenic harmful elements, which can interfere with biological processes and thus induce or worsen ongoing diseases. There is strong and consolidated evidence showing

that particulate matter (PM<2,5μm) and ultrafine particles (UFP <0.1μm) may induce cognitive impairments and have also been associated with neurodegenerative processes. Pollutants may enter the internal milieu through different pathways including respiratory system and gastrointestinal tract but entering the central nervous system requires crossing the blood brain barrier (BBB). Thus, BBB is a key element to considering when evaluating the harmful effects of pollutants. In this contribution a critical review will be presented on the integrity and development of the BBB under the new concepts of the neurovascular unit (NVU) and the glymphatic system. These new concepts provide a dynamic interplay between the blood and the neural tissue that changes under special conditions. A wide machinery of elements maintain the stability of the NVU in which signaling pathways affecting its basic cellular elements that are the endothelial cells, the pericytes and the vascular endings of the astrocytes. Although not in direct contact with the NVU, the microglia is considered a relevant element contributing to its integrity. Pollutants may affect directly the integrity of the NVU or take the opportunity of a transient disruption to enter the CNS either during the development or in adulthood and affect the homeostatic equilibrium resulting in oxidative stress, inflammatory response, mitochondrial dysfunction, and iron metabolism dysregulation thus leading to neurological dysfunction. Knowledge of the mechanisms that stabilize and maintain the dynamics of the NVU may provide ways to overcome damage produced by pollutants.

Supported by Spanish Ministry of Science, Innovation, and Universities (Grant: PID2023-153074OB-I00), Generalitat Valenciana (CIAICO/2023/244), and European Commission (Grant MSCA-SE101086247)

PL6

Behavioral endpoints in zebrafish larvae as indicators of neurotoxicity

Mónica Torres-Ruiz [1], María Muñoz-Palencia [1], Ana Isabel Cañas Portilla [1]

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Neurodevelopmental toxicity caused by environmental pollutants represents an increasing concern due to the growing evidence linking early-life exposure with long-term neurological disorders. Zebrafish (*Danio rerio*) embryos and larvae are a widely accepted model for developmental neurotoxicity (DNT)

studies due to their transparency, genetic homology with humans, and suitability for behavioral testing. In this work, we evaluated the neurobehavioral effects of different environmental toxicants—methylmercury (MeHg), nanoplastics (NPs), and non-ionizing radiofrequencies (RF)—through a comprehensive battery of behavioral assays in zebrafish larvae. Endpoints included early spontaneous activity (tail coiling), response to visual and auditory stimuli, habituation, general locomotor activity, and thigmotaxis (anxiety-related behavior). Our results show that all compounds tested induce distinctive behavioral alterations compatible with neurotoxic effects, highlighting the sensitivity of these endpoints to detect subtle functional disturbances in the developing nervous system. Furthermore, we present the development and validation of a new automated thigmotaxis assay optimized for high-throughput screening (HTS) applications. This approach allows quantitative assessment of anxiety-like behavior in early larvae and represents a robust addition to DNT testing batteries. Overall, our work contributes to the refinement of alternative methods for neurotoxicity evaluation and supports the inclusion of behavioral assays in regulatory frameworks for environmental and chemical safety assessment.

Supported by the European Partnership for the Assessment of Risks from Chemicals (PARC), which has received funding from the European Union's Horizon Europe Research and Innovation Programme under Grant Agreement No. 101057014, by the Spanish Government grant Number PID2021-125948OB-I00 from MCIN/AEI/10.13039/501100011033/FEDER (UE) and by the Instituto de Salud Carlos III (ISCIII) grant number PI24CIII/00054.

PL7

The national center for environmental health: understanding the impact of environmental pollution on health

Ana Isabel Cañas Portilla

National Center for Environmental Health. Instituto de Salud Carlos III, Madrid, Spain

The effects of environmental pollution on health is a major global concern, as pollution is one of the leading risk factors for disease and premature death. It affects nearly every aspect of human health, from respiratory and

cardiovascular systems to neurological development and even mental well-being.
Within the Instituto de Salud Carlos III, the National Center for Environmental Health (CNSA-ISCIII) has been working for years in the study of the exposure of population to environmental pollutants, performing key studies in Human Biomonitoring (HBM), as well as in hazard and risk assessment. As national scientific reference in environmental health, the CNSA is alert about emerging chemicals and how the exposure to them affects the health. CNSA has leaded HBM studies at European and national level (COPHES, DEMOCOPHES, HBM4EU, PARC, Bioambient.es, BEA, etc..), collaborating with WHO in this topic. During the last 20 years, this work has generated the first picture of European´s exposure levels to prioritized chemicals, the definition of exposure determinants and biomarkers of effects, allowing the establishment of guidance values for the support to policy and regulation.
In addition to HBM projects, our interests have been focused on projects promoting urban health, wellbeing and liveability on the basis of evidence on environmental health determinants through strategies based on citizen participation and the development of tools that support political decision-making (URBANOME and ENVESOME).
Regarding hazard properties of emerging chemicals, several research lines in our Center assess the impact of pollutants (nanoplastics, PFAS, methylmercury, bisphenols alternatives, etc..) on neurodevelopment and endocrine disruption, mainly focused on New Approach Methodologies (NAMs) using in vitro (cell lines) and in vivo (Zebrafish embryos) approaches.

Finally, the whole spectrum of exposure and hazard results generated are integrated on several risk assessment methodologies, transforming data into information relevant to Public Health.

Supported by Instituto de Salud Carlos III (Ministry of Science, Innovation and Universities), and several European and national ongoing projects (PARC, Grant Agreement No. 101057014, ENVESOME, Grant Agreement ID: 101157269, Grant Number PID2021-125948OB-I00 from MCIN/AEI/10.13039/501100011033/FEDER).

ORAL COMMUNICATIONS

O1

Neuroprotective mechanism of PACAP against prenatal and subchronic glyphosate exposure: involvement of oxidative stress, epigenetic alterations, and gut–brain axis disruption

Boukhawiye Sidahmed Hannani[1], Amine Cherif[1], Zeineb Fridhi[1], Youssef Torjeman[1], Tasnim Chograni[1], Maha Mezghani Khemakhem[2], Chahnez Naccaache[2], Sami Zekri[3], Taoufik Ghrairi[1], Olfa Masmoudi-Kouki[1]

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Glyphosate-based herbicides are widely used agrochemicals associated with environmental persistence and neurodevelopmental toxicity. Prenatal exposure to glyphosate disrupts redox balance, induces neuronal apoptosis, and alters behavior. Pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide with neuroprotective, anti-inflammatory, and microbiota-modulating effects, was evaluated for its ability to counteract glyphosate-induced neurotoxicity. Pregnant mice were assigned to four groups: control, GBH (250 mg/kg/day), PACAP (1 µg/10 µl), and GBH-PACAP. Offspring were evaluated for behavioral, molecular, histological, and microbial alterations. GBH exposure reduced body weight, neuronal survival, and antioxidant enzyme activities (SOD, CAT), while increasing ROS, NO, and lipid peroxidation. Brain histology revealed hippocampal disorganization and cerebellar thinning. GBH-exposed offspring exhibited reduced locomotion, exploration, and increased anxiety. Intranasal PACAP administration during gestation significantly restored antioxidant enzyme activity, reduced ROS/NO levels, and improved astrocyte viability. PACAP upregulated neuroprotective genes (mTOR, Bcl-2, BDNF) and downregulated pro-apoptotic and inflammatory markers (caspase-3, IL-6). Notably, PACAP prevented global DNA hypomethylation in the prefrontal cortex and hippocampus. PACAP

reversed behavioral deficits in both juvenile and adult offspring. Subchronic GBH exposure altered gut microbiota composition, significantly affecting the abundance of key microbial taxa. Differential analysis identified 11 ASVs with altered abundance. PACAP restored the balance of beneficial bacteria while reducing opportunistic pathogens, highlighting its role in maintaining gut-brain axis integrity. In conclusion, PACAP exerts a long-lasting protective effect against glyphosate-induced neurotoxicity by targeting oxidative stress, apoptosis, epigenetic disruption, and gut microbiota dysbiosis. These findings support PACAP as a promising candidate for mitigating neurodevelopmental damage linked to environmental pollutants.

O2

Pro-proliferative effects of micro-nanoplastics on glioblastoma

Marwen Ben Fdilen[1], Mohamed Abdulkader [2], Sami Zekri [1], Fathia Labidi [1], Gallego-Porcar[4], Elvira María Martínez [4], María de los Angeles Marqués-Torrejón [3], Olfa Masmoudi-Kouki [1], Amira Zaky [2]

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Introduction: Micro-nanoplastics are emerging environmental pollutants with potential health risks, yet their impact on brain tumor progression remains unclear. Given the high proliferative capacity of glioblastoma, understanding MNP tumor interactions is critical for assessing environmental contributions to cancer biology. Cellular models such as glioblastoma cell lines and neural and glioblastoma stem cells, which drive tumor initiation, growth, and therapy resistance, are particularly relevant for evaluating MNP effects. Methods: Polyvinyl chloride and polyamide microplastics were characterized by scanning electron microscopy and Nile Red staining to determine size and morphology. Human U87-MG glioblastoma cells were exposed to different concentrations of PVC and PA, whereas murine adult neural stem cells (ANS4) and glioblastoma stem cells (NPE) received varying concentrations of polystyrene nanoplastics for 24–72 h. Cell viability was assessed by MTT assay,

and fluorescent microscopy was used to examine morphological changes. Intracellular calcium was quantified using Fluo-3 probe, while ROS/NO levels were measured as oxidative/nitrosative stress markers. In U87 cells, expression of P53, Cyclin D1, and APE-1 was analyzed by RT-PCR. In ANS4 and NPE-GFP cells, Ki67, H2AX, and active Casp3 localization were visualized by immunofluorescence with confocal microscopy. Results: PVC and PA microplastics caused dose and time-dependent changes in cell proliferation and membrane integrity. MNP exposure increased calcium and ROS/NO levels, indicating oxidative stress. Cyclin D1 and APE-1 were upregulated and p53 downregulated, while immunofluorescence showed mild P53 nuclear translocation. Conclusions: MNPs exert pro-proliferative effects on glioblastoma cells and may activate signaling pathways that favor tumor growth and survival.

O3

Thorough chemical characterization of air particulate matter (pm10, pm1 and ufp) and investigation of brain tumors exposome to assess potential associations

Daniel Gutierrez-Martin [1], Maria Christina Nika [1], Sharon Ridolfo [1], Noemi Vidal [2], Carles Majos [3], Albert Pons-Escoda [3], Fulvio Amato [1], Ruben Gil-Solsona [1], Pablo Gago-Ferrero [1]

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Chemicals are essential to modern life, improving health, quality of life, and advancing food production, industry, and agriculture. However, their release into the environment leads to widespread exposure that poses significant health risks, including chronic diseases and different types of cancer. Research on exposure-disease relationships is limited, especially regarding brain tumors such as glioblastoma, despite familial links account for <5% of

cases. Identifying environmental factors in glioblastoma development is thus essential for understanding its causes.

Air pollution is a major global health concern, with complex and varied effects on human health. Particulate matter (PM) pollution is linked to hypertension, cardiovascular disease, and mortality, and has documented associations with cancers, including lung, liver, and breast cancer. Moreover, recent studies suggest links between air pollution and brain cancer. Ultrafine particles (UFPs, ≤100 nm), mainly from vehicle emissions, are particularly concerning, as they can carry carcinogens across biological barriers, being able to be translocated from the lungs to the bloodstream and also crossing the BBB.

This study aims to (i) assess the exposure of Barcelona residents to contaminants through particulate matter analysis (UFPs, PM1 and PM10) from different sampling locations by LC-HRMS tecniques, (ii) characterize the chemical exposome of >100 brain tumor tissues from a unique diffuse glioma cohort, and (iii) explore their ability to cross BBB and affect Cholinesterase activity, with in-silico trained model. Our results indicate the presence of several organic pollutants, including tire additives, PFAS or industrial chemicals, in both PM and brain tissues, with some demonstrating capacity to cross BBB.

O4

Regional differences in mercury and other neurotoxic metals, dietary habits, and impacts on child neurodevelopment

Mercè Garí [1], Martí Nogués [1], Kinga Polanska [2], Agnieszka Jankowska [2], Maja Scieszko [2], Joanna Jerzynska [3], Joanna Jurewicz [3], Matias Torrent [4], Joan Grimalt [1], David Izquierdo-Sandoval [5], Juan V. Sancho [5], Ana Gonzalez-Ruiz [6], Monica Ballesteros [6], Ana Megia [6], Pablo Gago-Ferrero [7], Montse Marqués [7], Tania Portolés [5], Rubén Gil-Solsona [7]

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Exposure to mercury and other neurotoxic metals such as cadmium and lead during critical developmental periods may have long-lasting consequences on offspring health, particularly neurodevelopment. Regional dietary patterns, especially fish consumption, play a major role in mercury accumulation within human populations. This study aimed to examine the dietary determinants of mercury exposure across European populations and to evaluate the impact of prenatal and postnatal exposure to neurotoxic metals on children's neurodevelopmental outcomes.

Mercury levels were measured in hair samples from pregnant women (n=435) and teenagers (n=80) in Poland, 4-year-old children in Menorca, Spain (n=300), and adults in Barcelona, Spain (n=45). In addition, lead, cadmium, and essential micronutrients (selenium, zinc, and copper) were quantified in cord blood (n=435) and in urine samples from children and teenagers of the REPRO_PL birth cohort (n=315). Neurodevelopment was assessed in school-age children using validated tools: the Strengths and Difficulties Questionnaire (SDQ) and the Intelligence and Development Scales (IDS) in Poland (n=435), and the McCarthy Scales of Children's Abilities (MSCA) in Menorca (n=300).

Spanish participants exhibited higher mercury concentrations compared to Polish participants. Notably, 20% of children in Menorca and 50% of adults in Barcelona exceeded the World Health Organization (WHO) threshold of 2 µg/g, whereas only a few individuals in Poland surpassed this level. Across cohorts, mercury levels were strongly associated with frequent fish and seafood consumption. Moreover, our findings suggest that combined exposures to neurotoxic metals and interactions with micronutrients can influence neuropsychological outcomes in children, even at low exposure levels.

O5

Prenatal ethanol and cadmium co-exposure induces adhd-like neurobehavioral deficits in offspring: implications for preventive medicine

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Attention-deficit/hyperactivity disorder (ADHD) is a complex, polygenic neurodevelopmental disorder characterized by inattention, hyperactivity, and impulsivity. Recent molecular studies reveal that ADHD risk arises from gene expression programs within dopaminergic and glutamatergic circuits, modulated by epigenetic mechanisms that integrate genetic and environmental influences. While genetic susceptibility is well established, environmental neurotoxicants are increasingly recognized as major contributors to neurodevelopmental disruption. Among these, ethanol (EtOH) and cadmium (Cd) are widespread environmental agents with established neurotoxic potential, yet their combined prenatal effects remain poorly defined.

This study explored the individual and synergistic impacts of prenatal EtOH and Cd exposure on ADHD-like neurobehavioral outcomes in rat offspring. Pregnant Sprague–Dawley rats were assigned to four groups (n=5/group): control, EtOH (20% v/v; 6 g/kg/day), Cd (50 ppm), and EtOH–Cd co-exposure (20% EtOH + 50 ppm Cd twice weekly) from gestational day 8 to 21. Offspring were later assessed for locomotor activity, working memory, exploratory drive, anxiety-like behavior, impulsivity, and pain sensitivity.

Both EtOH and Cd individually impaired cognitive performance and emotional regulation, while co-exposure induced pronounced additive and synergistic deficits, particularly in cognitive flexibility, recognition memory, and impulsive behavior. Female offspring showed heightened vulnerability.

These findings reveal that prenatal EtOH–Cd co-exposure disrupts dopaminergic circuitry and amplifies ADHD-like behaviors, providing mechanistic insight into environmentally mediated neurodevelopmental risk. The results highlight the urgent need for preventive maternal health strategies aimed at minimizing exposure to neurotoxicants during pregnancy to safeguard early brain development.

O6

Association of metal exposure with inflammation biomarkers in adult population

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Introduction. Exposure to heavy metals is prevalent in many environments and can modulate the inflammatory response, with potential implications for neurological and mental health. However, the associations between urinary metal levels and interleukin profiles in blood are not fully established in populations with mixed exposures.

Objective. To evaluate the association between urinary heavy metal concentrations and interleukins in individuals participating as healthy controls in the study Long COVID UJI in Spain.

Methods. Cross-sectional analysis of levels of urinary metals and blood inflammation biomarkers in 33 adults participating as healthy controls in the study Long COVID UJI in Spain. Urinary metal concentrations were measured for Mg, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Hg, Pb using ICP-MS analysis. Inflammation biomarker levels in blood (GM-CSF, IFN-a, IFN-gamma, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-17A, and TNF-a) were characterised using multiplex ELISA. Socioeconomic variables were collected via questionnaires. Multivariate linear regression models tested the associations

between urinary metal exposure as predictors and inflammation biomarker levels in blood adjusting for sex, age, and education.

Results. V and Pb were negatively associated with Interleukin-9 (IL-9) (p-value <0.05), a cytokine supporting the anti-inflammatory function.

Conclusion. In this exploratory analysis, V and Pb exposures were associated with a reduction of IL-9, a cytokine involved in the anti-inflammatory function. Exposure to heavy metals leading to disruption of the anti-inflammatory process could have potential neurological implications related with increased systemic and neurological inflammation.

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O7

Brief bisphenol-A exposure impairs synaptic plasticity and glial homeostasis in human models

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Introduction: Bisphenol-A (BPA), a synthetic compound widely used in plastics, has been linked to cognitive dysfunctions. While its chronic effects are well documented, the impact of short-term exposure remains unclear, particularly regarding synaptic function and glial activity. Cholinergic neurons are central to learning and memory, whereas microglia contribute to synaptic homeostasis. We investigated the effects of brief BPA exposure on cholinergic neurons and HMC3 microglial cells and evaluated whether antioxidant-enriched plant extracts could mitigate these effects.

Methods: Neurite length and branching were analyzed by optical microscopy. Synaptic responsiveness was assessed through qPCR analysis of immediate-early genes (IEGs) and long non-coding RNAs (lncRNAs) after depolarization. Tangerine extracts, obtained from fruits stored under red–blue light, were

applied during neuronal differentiation. Microglial morphology was examined microscopically, and flow cytometry was used to assess phagocytic activity.

Results: BPA-treated neurons exhibited reduced neuritic outgrowth, decreased synaptic density, and impaired protein expression. They also failed to activate IEGs and lncRNAs upon depolarization, indicating disrupted synaptic responsiveness. Tangerine extracts prevented these alterations, preserving neuronal architecture. Preliminary studies in microglia suggested that BPA triggered morphological changes consistent with a reactive phenotype, along with altered phagocytic activity.

Conclusions: Even short BPA exposure compromises neuronal development and glial homeostasis. The protective effect of antioxidant-rich tangerine extracts highlights their potential to counteract environmentally induced cognitive dysfunction.

O8

The nucleus incertus, a new crossroads for pain pathways?

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Affecting around 10% of the world's population, chronic pain and its psychiatric co-morbidities are major health problems. The involvement of neuropeptides in the modulation of pain in the brain remains poorly described. The neuropeptide relaxin-3 (RLN3) has antidepressant and anxiolytic effects, and our preliminary results indicate an analgesic role in rodents. RLN3 is expressed almost exclusively in the nucleus incertus (NI) that project to cortical (e.g. anterior cingulate cortex (ACC)) and subcortical (e.g. amygdala) areas of the pain matrix.

We aim to investigate the modulatory effects of the RLN3/RXFP3 system on pain using pharmacological, behavioural and anatomical approaches in a mouse model of persistent inflammatory pain.

Persistent inflammation was induced by injection of Freund's complete adjuvant into the animal's paw. Injection of RXFP3 agonists into the ACC

attenuated mechanical but not thermal pain. The effect of chronic release of another RXFP3 agonist (R3/I5) using an adenoviral strategy confirmed these effects in the ACC. Light-sheet microscopy of RLN3 neurons after transparentization showed a dense but heterogeneous network. Quantitative in situ hybridisation demonstrated RXFP3 mRNA expression in somatostatin interneurons of the ACC.

Optogenetic stimulation of RLN3 neurons in the NI-ACC pathway underlines the anti-nociceptive role of endogenous RLN3, independently of co-released GABA.

Our data highlight the plasticity of the RLN3/RXFP3 system and a new antinociceptive role for this family of peptides, suggesting its therapeutic potential in persistent pain conditions.

O9

Persistent nanoplastic retention disrupts the neuroimmune integrity through cellular organelle stress

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Micro-nano-plastics (MNPs) have emerged as pervasive environmental contaminants that enter the human body through ingestion and inhalation. Their nanoscale dimensions enable cellular uptake, raising concerns about potential cytotoxic effects.

We investigated the effects of Polystyrene-NPs exposure on SH-SY5Y, THP-1, and macrophage cells focusing on internalization, stress responses, and morphological alterations. We used flow cytometry for NP uptake, confocal imaging (with LC3, LAMP1, iL-1β, TNFα, MAP2 and phalloidin staining) to examine stress responses, GLuc-reporter SH-SY5Y cells for ER stress, Seahorse assay for mitochondrial damage and MTS, LDH, and DAN assays for cytotoxicity.

NP internalization was markedly increased under LPS-induced inflammatory conditions in both neuronal and immune cells. After 24h of exposure, NPs

disrupted autophagy and lysosomal activity, accompanied by cytoskeletal remodeling. Inflammatory cytokine release was induced in immune cells, and enhanced ER stress was detected through UPR activation in GLuc-SH-SY5Y cells. Both SH-SY5Y cells and macrophages exhibited exacerbated mitochondrial stress. NP exposure for 24–48h resulted in a 2–17% reduction in cell viability, with a concomitant increase in LDH release. Oxidative stress was most pronounced in macrophages compared to neuronal cells. Long-term fluorescence tracking of NP clearance dynamics demonstrated persistent NP retention in macrophages, with no evidence of active excretion over a 10-day period.

In conclusion, NPs do not directly induce acute cell death but persist within cells, where their accumulation disrupts normal cellular processes and imposes sustained stress on multiple organelles. This persistent intracellular burden may compromise neuroimmune homeostasis and contribute to long-term cellular dysfunction.

O10

Plasticizers and brain neurodevelopment during childhood

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Exposures to phthalates, non-phthalate plasticizers and bisphenols may play a role in the development of child behavioral and psychomotor problems. The aim of the study was to evaluate the associations between childhood exposures to these compounds and neurodevelopmental outcomes in the REPRO_PL birth cohort (Poland).

Urine samples were collected at the time of children's neurodevelopmental assessment (7 years). Online-SPE-LC-MS/MS was used for the determination of bisphenol A (BPA) and 21 phthalate metabolites (n=400) and their replacement alternatives BPF, BPS, three metabolites of diethylhexyl-terephthalate (DEHTP) and three metabolites of 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH) (n=150). Child's neurodevelopment was assessed by the Strengths and Difficulties Questionnaire (SDQ) and the

Intelligence and Development Scales (IDS). Multivariate regression models accounting for sex-specific effects were applied, including the use of stratification and interactive approaches. Mixture models adjusted for chemical groups were also applied.

BPA, BPF and DEHTP metabolites were found to be positively associated with several SDQ scores in boys (emotional symptoms, peer-relationship and conduct problems, anti-social behavior). Conversely, metabolites of monomethyl phthalate (MMP), di-n-butyl phthalate (DnBP) and di-iso-butyl phthalate (DiBP) were associated with poorer behavioral scores in girls. For IDS, higher exposures to BPF were associated to lower scores in Fluid IQ and Cognition in boys, and DINCH and MMP with mathematical scores in girls.

This study indicates that children's exposure to several replacement compounds of BPA and phthalates, such as BPF and DEHTP, are associated with adverse effects on behavioural and cognitive development of school age children, with divergent sex-specific effects.

O11

The alterations in normal behavior in adult rats resulting from nanoplastic intoxication of their mothers during pregnancy and/or lactation

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Exposure to nanoplastics represents a growing health concern, particularly regarding their potential impact on neurological function. While previous studies have shown accumulation of nanoplastics in various organs, their effects on neural activity remain unclear due to the protective role of the blood-brain barrier (BBB). However, during development or in specific brain regions, the BBB can be permeable. This study examined whether maternal exposure to nanoplastics during pregnancy, lactation, or both periods alters offspring behavior. Pregnant rats were exposed to nanoplastics or saline during these windows, and their offspring underwent behavioral testing in adulthood,

including the open field, elevated plus maze, 3-chamber test, and contextual fear conditioning.

Nanoplastic exposure produced significant behavioral alterations with sex-dependent effects. Male offspring exposed during pregnancy or lactation showed increased locomotor activity, an effect absent in combined exposure. Anxiety-like behaviors increased in both sexes in the pregnancy group. Social assessments revealed impaired sociability: offspring exposed during lactation or both periods failed to discriminate between social and non-social stimuli, while those exposed during pregnancy or both periods did not recognize novel social targets. Contextual fear conditioning further showed disrupted memory processes. Male offspring from pregnancy-exposed mothers failed to acquire context-fear associations, whereas those exposed during lactation could learn but not extinguish fear responses. Females exhibited comparable impairments with subtle differences.

Overall, maternal exposure to nanoplastics during critical developmental stages induced long-lasting behavioral changes in offspring, underscoring potential neurodevelopmental risks associated with early-life nanoplastic exposure.

O12

Nuclear estrogen receptors of European sea bass (Dicentrarchus labrax) as targets of endocrine disrupting compounds: effects on reproduction and risk assessment potential.

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Introduction

Estrogens regulate multiple reproductive processes in teleosts through specific receptors. Two main classes exist in vertebrates: membrane-associated G- protein-coupled estrogen receptors, mediating rapid nongenomic actions, and nuclear estrogen receptors, transcription factors that bind estrogen response elements (EREs) to control gene expression.

Endocrine-disrupting compounds (EDCs) can mimic or block steroid receptor activity and affect receptor production, targeting various points along the brain-pituitary-gonad (BPG) axis.

This study investigated the role of the three nuclear estrogen receptor (Esr) subtypes in European sea bass, evaluated the effects of EDCs on receptor function using transactivation assays, generated stable in vitro clones, and assessed their potential as bioassay tools for EDC risk evaluation.

Material and Methods

Expression of Esr genes was quantified by qPCR in testis, ovary, and pituitary throughout the reproductive cycle. Immunohistochemistry localized receptor subtypes along the BPG axis. Functional characterization was performed via transient transfection of HEK293 cells with an ERE-luciferase reporter, testing natural ligand responses and two EDCs: genistein (a phytoestrogen) and fluoxetine (an antidepressant). Stable HEK293 clones expressing each Esr subtype were generated using antibiotic selection and validated by dose-response assays to determine EC50 and Emax values. These clones were subsequently tested with EDCs and environmental samples to evaluate their utility as in vitro bioassays.

Results and Conclusion

All Esr subtypes showed high, stage-specific expression and localization in reproductive tissues. Distinct ligand affinities and differential EDCs responses were observed. Stable Esr-expressing clones reliably detected EDCs activity, demonstrating their potential as screening tools for environmental monitoring and aquaculture risk assessment.

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POSTER COMMUNICATIONS

P1

Polystyrene nanoplastics and energy homeostasis: exploring an emerging hidden contributor to metabolic disorders and related comorbidities

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Chronic exposure to environmental pollutants, such as Polystyrene Nanoplastics PS-NPs, represents a potentially hidden risk factor for metabolic dysregulation, yet the underlying mechanisms remain poorly understood. Nanoplastics, due to their nanoscale dimensions, are readily ingested via food, water, and air and can accumulate in multiple tissues, including the brain, liver, pancreas, adipose tissue, and skeletal muscle.

This study aims to investigate the impact of chronic PS-NP exposure on energy homeostasis and the regulation of key metabolic and neuroendocrine systems, with particular emphasis on the hypothalamus, hippocampus, liver, pancreas, adipose tissue, and skeletal muscle, while exploring potential sex-specific effects.

Pregnant Wistar rats were exposed to 200 nm Polystyrene Nanoplastics (PS-NPs) at a dose of 50 µM, administered daily via oral gavage five times per week, from gestational days 8–10 through lactation. Two groups were established: (i) the dams, exposed during gestation and lactation until sacrifice, and (ii) the offspring, which were indirectly exposed during gestation through placental transfer and during lactation via maternal milk, and subsequently received direct oral gavage of PS-NPs after weaning until 8 weeks of age, when they were sacrificed.

Offspring were assessed by sex, and tissues were collected for histological, biochemical, and molecular analyses. Serum markers, including ASAT, ALAT, triglycerides, and LDH, were evaluated.

Functional genomics analyses focused on key regulators of energy homeostasis and stress responses, including NPY, AgRP, and Insr in the hypothalamus; BDNF in the hippocampus; Glut2, Pparα, and TNF-α/Il-6 in the liver; Ins1 and Bax/Bcl2 markers in the pancreas; Pparγ, UCP1, and TNF-α/Il-6 in adipose tissue; and mTOR, Akt, and TNF-α/Il-6 in the skeletal muscle. Histological analyses were performed to detect structural alterations linked to chronic PS-NPs exposure.

This study is designed to elucidate how chronic exposure to PS-NPs may disrupt energy homeostasis, impair metabolic and neuroendocrine functions, and contribute to the onset of metabolic disorders. By integrating functional genomics, biochemical, and histological analyses across multiple organs and both sexes, the findings are expected to provide novel insights into the systemic and neuroendocrine consequences of PS-NPs exposure, highlighting its role as an insidious environmental risk factor.

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P2

Olive oil attenuates oxidative stress and behavioral alterations induced by subacute glyphosate exposure in rat

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Glyphosate, a widely used organophosphorus herbicide, acts by inhibiting the shikimate pathway through the suppression of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Although this mechanism is absent in mammals, increasing evidence points to glyphosate-induced neurotoxicity. Dietary antioxidants, particularly vitamin E and phenolic compounds abundant in olive oil, may counteract these toxic effects.

Adults rats (200g) were divided into three groups, control (n=8), Glyphosate (800 mg/kg/day; n=8) and Glyphosate plus olive oil (10%) (n=8). Control group received water by oral gavage, glyphosate group received by oral gavage 800 mg/kg for six consecutive days and the third group treated with glyphosate under the same conditions but supplemented with an olive oil–enriched diet. Anxiety-related behavior was assessed using the Open Field test. At the end of treatment, rats were euthanized, and brain tissues were collected for cell viability and oxidative stress evaluation.

Open Field test results showed that glyphosate-treated rats displayed anxiety-like behaviours indicated by reduced exploration of the central zone. In parallel, neuronal cell cultures derived from the brains of glyphosate-exposed animals showed elevated LDH release, a reduced proportion of viable cells, and excessive ROS production, indicating oxidative stress and cell toxicity. Conversely, rats supplemented with olive oil exhibited enhanced exploratory behavior and reduced anxiety-like indicators, while neuronal cultures showed a marked reduction in oxidative stress indicated by decreased LDH leakage and the level of ROS.

Our findings demonstrate that even short-term glyphosate exposure induces anxiety-like behavior and oxidative stress–mediated neuronal cytotoxicity in rats. Notably, an olive oil–enriched diet exerts a protective effect, highlighting its potential role as a nutritional intervention against herbicide-induced neurotoxicity.

This work was supported by LR18ES03, and Horizon Europe Research and innovation programme RISE-MSCA projectPsyCoMedNo 01086247

P3

Assessing environmental contaminants in alternative aquaculture feeds: implications for food safety and human health

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Introduction: Aquaculture plays a crucial role in addressing the increasing global demand for fish, requiring the development of sustainable feed

formulations. Conventional diets are no longer sustainable and new nutritional strategies must be explored. However, incorporating alternative ingredients in feeds may introduce pollutants not typically associated to fish farming, which can produce unknown effects in both animal welfare and human health. Understanding the transfer of these pollutants through the marine food chain, from raw ingredients to commercial feeds and fish fillets, is therefore essential to assess their health risks.

Materials and methods: Eighteen raw ingredients, three formulated diets, and 48 Sparus aurata fillet samples were analysed using advanced and complementary analytical platforms: liquid chromatography (LC) and gas chromatography (GC), both coupled to high-resolution mass spectrometry (HRMS), including ion mobility-quadrupole time of flight (IMS-QTOF) and GC-Orbitrap MS.

Results: A screening of different families of contaminants (pesticides, pharmaceuticals, etc.) was carried out. The results revealed the presence of typical plant-associated ingredients, such as pesticides and PAHs, in ingredients and feeds. Residues of veterinary pharmaceuticals were also detected in animal by-products ingredients, along with emerging contaminants like UV filters, musks, and phthalates. Interestingly, these contaminants did not show correlation with diet composition, suggesting contamination may originate from processing or storage. Notably, diets incorporating alternative ingredients exhibited lower contaminant loads compared to previous studies, with minimal transfer of pollutants to fish fillets.

Conclusions: The study highlights that adopting innovative, sustainable feed formulations can reduce the introduction of contaminants within the aquaculture food chain. These results underline the critical link between environmental quality, aquaculture practices and public health, supporting safer fish consumption and advancing the sustainability of food production systems.

P4

Pesticide exposure in eight-year-old children from general population (inma-asturias)

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Pesticides and fungicides are synthetic chemicals widely applied in agriculture and domestic environments to control pests. Their toxicological activity is not highly selective, affecting non-target species, including humans. Exposure to these compounds may pose neurotoxic risks, particularly in children, due to their vulnerability during development. Although most pesticides are metabolized and excreted via urine within 4–48 hours, monitoring urinary metabolites provides a reliable tool to assess exposure levels and potential health risks in populations.

Urinary biomarkers of pesticide and fungicide exposure were measured in 310 eight-year-old children from Asturias, Spain. Isotope dilution solid-phase extraction combined with ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) was employed. The analysis included 6 organophosphate metabolites, 2 pyrethroid biomarkers, 11 neonicotinoids, and 5 fungicides. Hazard quotient (HQ) and hazard index (HI) calculations were performed to evaluate the potential health risks of detected compounds.

The fungicide metabolite of tebuconazole (OH-TEB) was found in all samples, with a median concentration of 8.1 ng/mL. Other frequently detected metabolites were from pyrethroids, 3-PBA (94%, 0.74 ng/mL), chlorpyriphos, TCPY (93%, 0.38 ng/mL), and parathion, PNP (88%, 0.30 ng/mL). HQ analysis showed that a subset of children exceeded the safety threshold (HQ > 1) for five compounds: pirimiphos (0.3%), PNP (1.3%), 3-PBA (2.3%) and OH-TEB (0.6%).

This study highlights widespread exposure to multiple pesticides and fungicides in children, with some individuals surpassing safe exposure thresholds. The findings emphasize the importance of biomonitoring for early

detection of at-risk populations and support regulatory actions aimed at reducing pesticide-related neurotoxic risks.

P5

Effects of micro- and nanoplastics on adult neurogenesis

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Recent reports have revealed the presence of microplastics in human brain tissue, raising concern about their potential effects on neuronal function and plasticity. Given the increasing environmental exposure to microplastics through ingestion, it is essential to investigate their influence on adult neurogenesis. This study aims to evaluate whether chronic oral administration of microplastics during gestation and lactation alters cell proliferation and the formation of new neurons in the subventricular zone, one of the main neurogenic niches.

For this purpose, female rats were orally administered polystyrene micro/nanoplastics (MP/NP). From their litters, four experimental groups were established: (1) exposure during gestation and lactation, (2) exposure only during gestation, (3) exposure only during lactation, and (4) a control group without exposure. Neurogenesis and cell proliferation were analyzed using immunohistochemistry. Neural stem cells were identified through GFAP (a marker of astrocytes and immature stem cells) in combination with Sox2 (a marker of undifferentiated stem cells). To distinguish between quiescent and proliferating neural stem cells, Ki67 expression was assessed, allowing identification of Ki67– and Ki67+ populations. In addition, the proportion of progenitor cells derived from stem cells was determined (Sox2+ and Ki67+ without GFAP expression). Finally, the formation of new neurons was examined by quantifying the expression of DCX and Tuj1, two markers of immature neurons.

This ongoing study is expected to provide valuable evidence on the impact of MP/NP exposure on the regulation of adult neurogenesis and the integration of new neurons, contributing to a better understanding of the risks associated with environmental microplastics.

P6

Oxidative potential of urban and rural particulate matter: potential implications for metal-driven neurotoxicity

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Introduction. Exposure to ambient particulate matter (PM) is associated not only with cardiopulmonary effects but also with neurological disorders, where oxidative stress and inflammation play central roles. Transition metals in PM are known to induce reactive oxygen species (ROS) formation, potentially affecting neurological processes through redox imbalance and neuroinflammation.

Methods. PM_{10} and $PM_{2.5}$ samples were collected on quartz filters in urban (Castellón de la Plana) and rural (Vall d'Alba) sites in eastern Spain between 2023 and 2025. PM metal composition was determined from water-soluble extracts, and oxidative potential (OP) was assessed using the acellular dithiothreitol (DTT) assay. Associations between OP and metal concentrations were evaluated using correlation and stepwise regression analyses.

Results. Urban PM showed higher OP than rural samples, with PM_{10} exhibiting greater oxidative activity than $PM_{2.5}$ in urban locations. OP was strongly correlated with PM mass ($p<0.001$) and positive correlations were found with the concentration of several transition metals, including Cd, Sb, Hg, and Pb ($p<0.05$). Moreover, stepwise regression identified Mg, Ni, Se, and Hg as significant predictors of oxidative potential.

Conclusion. These findings indicate that PM oxidative potential is strongly influenced by PM mass and metal composition, with clear differences between urban and rural environments. While acellular assays cannot directly measure neurotoxicity, results suggest potential mechanistic links between metal-rich PM and oxidative stress, which is implicated in neurodegenerative processes. These observations highlight the importance of considering PM metal composition when assessing public health risks, including possible neurological outcomes.

This study is funded by Generalitat Valenciana—Conselleria d'Educació, Universitats i Ocupació (CIDEGENT/2019/064, CIACIF/2021/234).

P7

Human chemical exposome in serum of pregnant women from the insulin cohort using gc-hrms

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Chemicals are extensively used in industrial, agricultural, medical, and daily-life applications. However, exposure to chemical contaminants, even at low concentrations, has been associated with adverse health effects. Polycyclic aromatic hydrocarbons (PAHs) and their low studied alkylated derivatives are of particular concern due to their toxicity and widespread occurrence. These compounds may enter the human body mainly through inhalation or ingestion, potentially leading to neurotoxicity, memory impairment, cognitive dysfunction, and behavioral problems, since they are largely generated by incomplete combustion processes and are present in particulate matter <2.5 μm (PM2.5), tobacco smoke, and oil refinery emissions, among others.

Pregnant women represent a particularly vulnerable population, as chemical exposure may affect both maternal and fetal health. Prenatal exposure to PAHs has been linked to reduced IQ and cognitive impairment in children, with

potential consequences for later neurodevelopment and school performance. In our study, 183 serum samples from the INSULIN cohort of pregnant women were analyzed using a validated method for 198 analytes (including pesticides, PAHs, alkyl-PAHs, PCBs, and organophosphate esters—OPEs), also allowing to extend the chemical universe analysed to chemicals not included in the method validation. The protocol allowed the detection of 16 lipophilic compounds included in the target list, as well as additionally revealed ones, including personal care products (PCPs), anesthetics, and alkyl-PAHs.

This study highlights the relevance of advanced HRMS methodologies to characterize the human exposome, representing an essential first step in detecting and quantifying neurotoxicity-related compounds in vulnerable populations, preparing the way for future interdisciplinary research.

P8

Impact of microplastics on brain function and structure

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Microplastics (MPs) have emerged as pervasive environmental contaminants with increasing evidence of adverse effects on brain health. While individual MP polymers have shown toxic effects on honey bees, the impacts of mixed polymers on brain structure, function, and resulting behaviour remain unclear. We first examined how two-days oral exposure to MPs affects cognitive performance and brain accumulation in Apis mellifera. We assessed sucrose responsiveness, appetitive olfactory learning, and memory. Polistyrene exposure reduced sucrose responsiveness, but the mixed polymers caused an even stronger effect. All treatments impaired learning and memory, with PS showing the greatest impact. Then, we used fluorescent MPs to test whether MPs could cross the blood–brain barrier, using two-photon fluorescence microscopy and an optimized DISCO clearing method. Imaging revealed that MPs penetrated and accumulated in the brain within three days. These findings indicate that MPs can disrupt bee cognition and breach the brain barrier, raising concerns about potential neural damage from environmental MPs. Next, we will test how brain function is altered by MP exposure by using two-

photon calcium imaging. By monitoring the alterations in spontaneous and task-related neuronal activity induced by exposure to MPs, this research will dissect the functional basis of MP-induced neurotoxicity. This interdisciplinary approach, combining neurobiology, molecular biology, and cognition, will enable the identification of exposure biomarkers and potential preventive measures to limit the harmful effects of plastics on health, including human health.

P9

Neurotoxic effects of glyphosate by products: insights from hplc-based detection and neuroprotection approaches

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Background: Glyphosate (N-phosphonomethyl glycine), one of the most widely used herbicides, produces persistent degradation byproducts such as AMPA, which may exert neurotoxic effects. Their detection and the evaluation of protective strategies remain limited. This study combined analytical methods for glyphosate byproduct detection using HPLC with cellular assays assessing neurotoxicity and the potential neuroprotective role of the ionic liquid [Choline][H_2PO_4].

Methods: Glyphosate degradation products were derivatized with p-toluenesulfonyl chloride (PTSC) to enhance spectrophotometric and HPLC-based detection. Neurotoxicity was evaluated in murine neuroblastoma (N2A) cells via fluorescein diacetate (FDA) viability assay, lactate dehydrogenase (LDH) release for membrane integrity, and reactive oxygen species (ROS) measurement for oxidative stress. The neuroprotective capacity of [Chol][H_2PO_4] was also tested.

Results: HPLC monitoring revealed progressive formation of glyphosate byproducts during enzymatic degradation, correlating with decreased cell viability, increased LDH release, and ROS accumulation—indicating oxidative stress-mediated neurotoxicity. Treatment with [Chol][H_2PO_4] significantly mitigated these effects, reducing ROS levels and preserving neuronal viability, suggesting partial neutralization of byproduct toxicity.

Conclusion: Combining analytical detection with cellular assays provides a comprehensive framework for evaluating glyphosate-derived neurotoxicity. The ionic liquid [Chol][H_2PO_4] exhibited a neuroprotective effect, reducing oxidative damage and improving neuronal survival. These findings highlight the need to better understand environmental neurotoxicants and explore innovative detoxification and neuroprotection strategies relevant to neurodegeneration and brain aging.

P10

Kinetics of social isolation-induced behavioral impairments and morphological alterations in the midcingulate cortex in mice

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Social isolation (SI) affects behavior and neural integrity, yet we do not fully understand how these effects unfold over time. Besides, we previously demonstrated that socially isolated aggressive mice exhibited notable morphological changes in the MCC. However, it remains unclear whether these structural alterations are a cause or consequence of the observed behavioral impairments. In this study, we aimed to determine which emerges first: the structural changes or the behavioral impairments. For this purpose, we subjected adult male mice to varying durations of SI (1, 2, 4, and 6 weeks). At the end of each SI period, we conducted behavioral tests to evaluate aggression, social interaction, and anxiety-like behavior. We then analyzed the MCC's morphology using Golgi-Cox staining. Our findings showed that behavioral changes-such as excessive aggression ($p < 0.01$), social withdrawal ($p < 0.01$), and anxiety ($p < 0.05$)-became apparent after two weeks of isolation,

and then escalate with the SI duration. Interestingly, structural abnormalities in the MCC were evident as early as the first week. These changes including reduced dendritic complexity ($p < 0.001$) and spine density ($p < 0.001$), then continue to intensify over time. These findings suggest that structural changes in the MCC precede the onset of behavioral symptoms. This highlights the MCC's pivotal role in translating early neural vulnerabilities into later behavioral challenges associated with chronic stress. By identifying these early structural markers, our research sheds light on the mechanisms driving stress-related neuropsychiatric disorders and opens up possibilities for early intervention strategies.

P11

CCL5: a key regulator of neuroinflammation and type 2 diabetes associated with nutritional obesity

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Despite its high prevalence and societal impact, obesity treatment remains largely ineffective, mainly due to limited understanding of its pathophysiology. A better comprehension of central mechanisms regulating eating behavior is crucial to develop novel treatment approaches.

A hallmark of obesity is the accumulation of pro-inflammatory molecules, but the role of inflammatory cytokines and chemokines in regulating feeding and body weight remains unclear. This project aims to investigate, under normal and obese/T2D conditions, the role of the inflammatory chemokine pathway CCL5/CCR5 within the hypothalamic neuropeptide network involved in the regulation of eating behavior and glucose homeostasis. We propose that CCL5 signaling is a key modulator in obesity and T2D, influencing orexigenic peptides such as MCH and ORX/26RFa, and that its dysregulation contributes to metabolic disorders.

The study has two objectives. On one side, to investigate the development of nutritional obesity and its metabolic consequences in a CCL5 knockout mouse model. Mice will be fed a high-fat diet to induce obesity, and parameters such as body weight, circulating leptin, fat/lean mass composition and inflammatory factors will be monitored. Glucose tolerance will also be evaluated to assess the development of a diabetes-like phenotype. On the other side, we are aimed at investigating the effects of acute peripheral and central injection of CCL5. We will monitor the evolution over time of weight, food intake and the expression of orexigenic peptides.

This work aims to clarify the link between neuroinflammation, hypothalamic regulation, and metabolism, offering potential new therapeutic targets for obesity and type 2 diabetes.

P12

Clinical and genetic determinants of glioblastoma multiforme survival: a retrospective analysis

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Glioblastoma (GB) is the most common and aggressive primary brain tumour in adults, with poor prognosis despite advances in therapy. We retrospectively analysed 57 patients with IDH-wildtype GB treated at Castellon General University Hospital (2022–2023) to identify clinical, tumour-related, and genetic determinants of survival. Clinical variables (age, sex, BMI, treatments), tumour features (location, volume, surgical resection, ventricular proximity), and genetic alterations (EGFR, TP53, CDKN2A, CNVs, MGMT methylation) were assessed. Median overall survival (OS) was 13.7 months and progression-free survival (PFS) 12.4 months. The most significant prognostic factor was tumor proximity to the lateral ventricles: patients with tumors in direct contact had a median OS of 3.7 months, compared to 17.5 months in the intermediate group, while median OS was not reached in the farthest group ($p < 0.001$). Extent of

resection also showed a favorable trend, with gross total resection associated with longer survival than partial resection or biopsy. No significant associations were observed for tumor volume, location, genetic alterations, or comorbidities. These results highlight ventricular proximity as the strongest predictor of survival in GB, supporting the role of the ventricular–subventricular zone as a biologically aggressive niche and suggesting that integrating spatial tumor features into prognostic models may improve patient stratification and therapeutic decision-making.

P13

Ecotoxicity of PHBV Bioplastic in the Aquatic Model Organism Artemia franciscana”

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Bioplastics have emerged as sustainable alternatives to conventional fossil-based plastics due to their biodegradability and biological origin. Among these, polyhydroxy-butyrate-co-valerate (PHBV), a co-polymer within the polyhydroxyalkanoates (PHAs) family, has gained increasing attention. However, despite its promising environmental profile, the potential impacts of PHBV on aquatic organisms remain poorly understood. Given the widespread presence of microplastics (<5 mm) in marine environments and their known ecological risks, this study assessed the ecotoxicity of PHBV microparticles in the aquatic model species Artemia franciscana. Apical endpoints including survival, growth, feeding rate, and gut histology were evaluated alongside

stress-related biomarkers—such as antioxidant defences (CAT, GST), neuronal activity (ChE), and xenobiotic biotransformation (CbE)—and changes in fatty acid profiles, and lipid peroxidation. Long-term (14 days) exposures to PHBV (up to 250mg L^{-1}) at 25 °C were conducted. Characterization of PHBV by FTIR, laser diffraction, and SEM confirmed its physicochemical properties. Physiological parameters (survival and feeding) were not affected up to 250 mg L^{-1}; however, growth increased at higher concentrations. Alterations in fatty acid composition and reduced lipid peroxidation were observed, accompanied by structural changes in the digestive epithelium. Ingestion and egestion of PHBV microparticles were confirmed, and SEM imaging revealed increased surface roughness in particles recovered from fecal pellets. Biochemical analyses showed significant variations in CAT and GST activities, but not in CbE or ChE, suggesting no neurotoxic effects. Overall, these findings provide valuable insight into PHBV ecotoxicity and support environmental risk assessments of biodegradable plastics in marine ecosystems.

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P14

Plasticizers-induced effects on human microglia: cytotoxic, inflammatory, and oxidative responses

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Plasticizers are added to plastics to improve flexibility and durability, but humans are widely exposed to them as they leach from products through ingestion, inhalation, and dermal contact. Di(2-ethylhexyl) phthalate (DEHP)

has been extensively used, but its use is restricted due to endocrine-disrupting properties and links to neurotoxicity. As a result, alternative plasticizers such as acetyl triethyl citrate (ATEC) and acetyl tributyl citrate (ATBC) have been industrially adopted, showing lower toxicity profiles. However, emerging evidence indicates these substitutes may still pose risks, including oxidative stress and neuroinflammation. In this context, thymol-menthol (TM), a natural deep eutectic solvent (NADES) derived from essential oils, is being explored as a novel plasticizer with presumed low toxicity. Yet, studies on its effects in neural cells remain scarce.

This study aimed to evaluate the cytotoxicity and neuroinflammatory/oxidative stress responses induced by the plasticizers DEHP, ATEC, ATBC, and TM in human microglial HMC3 cells, assessing viability with the MTT assay and quantifying gene expression of TGF-β, IL-6, IL-1β, and HMOX1 by RT-qPCR.

DEHP exhibited high cytotoxicity, robust neuroinflammatory and oxidative stress responses. ATBC showed a comparable profile, while TM induced lower neurotoxicity and remained more stable over time, with minimal gene expression changes. ATEC displayed the lowest neurotoxic effects but elicited a noticeable oxidative stress response.

These findings provide critical insights into the safety of traditional and emerging plasticizers, highlighting the need for continued evaluation to ensure safer applications in biomedical and consumer products.

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P15

Prevalence of depression and associated socio-demographic factors in Chilean and immigrant women

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Introduction. Depression represents a substantial burden on global public health, disproportionately affecting women due to gender inequalities and social determinants of health. In Chile, the situation of migrants has been poorly documented, although studies show that migration intersects with poverty, education, and access to services to create layers of vulnerability in mental health. The present study aims to analyze the association between depression and sociodemographic factors in immigrant and Chilean women residing in south-central Chile.

Methods. Cross-sectional analytical study on 1,062 immigrants and 1,062 Chileans women residing in south-central Chile. Sociodemographic variables and the migration process were recorded with bespoken in-house questionnaires. Differences were tested using Chi-square test.

Results. Depression prevalence was higher (25.7%) in Chilean than in immigrants (15.8%) women. Significant differences were observed according to marital status and socioeconomic status for both Chilean and immigrants. In addition, among immigrants, significant differences were observed according to age, educational level, employment status and immigration status. No differences were found based on having children or being the head of household.

Conclusions. Depression prevalence was significantly higher among Chilean women than among immigrants. Among the latter, depression was associated with multiple structural factors reflecting social inequalities that transcend the individual sphere. In both groups, the absence of a partner and belonging to low socioeconomic strata were linked to a higher frequency of depressive symptoms. The findings reinforce the need for public policies that integrate mental health, social equity, and gender and migration perspectives, promoting timely access to psychological and social support services.

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P16

Anterior cingulate cortex hyperexcitability in a mouse model of attention-deficit/hyperactivity disorder and pain comorbidity

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Attention deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder in children and adults worldwide. Epidemiological studies have shown associations with environmental pollutants, such as bisphenol-A, whose detoxification is compromised in ADHD patients. ADHD often coexists with anxiety, depression or sensitivity alterations. Patients report sensitization to pain and the prevalence of generalized pain is higher (up to 80%) compared to control population (17%). However, the mechanisms involved remain unknown. Neuroanatomical studies confirm that attentiveness and central mechanisms involved in pain transmission use identical neural networks (i.e., the anterior cingulate cortex (ACC) connection to insula). Thus, alterations in these brain areas or their connectivity may underlie both attentional deficits and nociceptive sensitization.

We previously validated a mouse model of ADHD obtained by neonatal 6-hydroxydopamine (6-OHDA) lesion. We demonstrated that 6-OHDA mice exhibit increased nociception, showing higher sensitization to thermal and mechanical stimuli compared to sham animals. By combining in vivo electrophysiology and optogenetics, we demonstrated that ACC hyperactivity alters the ACC–posterior insula circuit that underlies nociceptive sensitization. Using microendoscopic calcium imaging in freely moving mice, we monitored the neuronal population activity in ACC under ADHD-like conditions. Interestingly, when comparing both 6-OHDA and sham mice, the in vivo calcium imaging recordings showed different activity patterns under nociceptive stimulation.

We provide functional evidence that nociceptive sensitization relies on ACC hyperexcitability. Our data indicate that ADHD and persistent pain are mutually worsening comorbid disorders with reciprocal worsening of nociceptive sensitization and hyperactivity, and new treatments should target overlapping mechanisms for better efficiency.

P17

Indoor levels of $PM_{2.5}$ and ND PM_{10} in primary school classrooms: potential implications for environmental epidemiology on neurodevelopment research

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Introduction**.** Exposure to particulate matter ($PM_{2.5}$ and PM_{10}) in children has been associated with neurodevelopmental disorders. Since children spend a substantial amount of time in school classrooms, assessing $PM_{2.5}$ and PM_{10} in these indoor environments is essential. However, limited information is available regarding $PM_{2.5}$ and PM_{10} levels in school settings. The aim of this study is to determine the concentrations of $PM_{2.5}$ and PM_{10} in primary school classrooms.

Methods**.** Between April and May 2021, measurements were conducted in 18 primary school classrooms in Valencia, Spain. Sampling was performed from Monday to Friday using a Fidas® Frog optical aerosol spectrometer, which simultaneously measures the suspended particle fractions $PM_{2.5}$ and PM_{10}, among other particle fractions. Data were recorded at one-minute intervals, and an hourly average database was subsequently constructed. As the data did

not follow a parametric distribution, results were reported as median and interquartile ranges (IQR).

Results**.** The median concentration of $PM_{2.5}$ was 14.0 $\mu g \cdot m^{-3}$ (IQR: 10.5–20.2 $\mu g \cdot m^{-3}$). The median for PM_{10} was 24.8 $\mu g \cdot m^{-3}$ (IQR: 14.7–58.8 $\mu g \cdot m^{-3}$). Regarding short-term exposures (daily mean), 72% of the sampled days exceeded the WHO guideline value for $PM_{2.5}$ (15 $\mu g \cdot m^{-3}$), and 34% exceeded that for PM_{10} (45 $\mu g \cdot m^{-3}$) (WHO, 2021). These levels indicate a substantial exposure of schoolchildren to fine particulate indoors.

Conclusions**.** The observed $PM_{2.5}$ and PM_{10} concentrations in school classrooms frequently exceeded WHO guideline values, suggesting relevant exposure of children to fine particles in indoor environments. These results underscore the importance of including indoor air quality in future research on environmental factors with potential implications for neurodevelopment.

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WORKSHOPS

W1

Methods for detecting contaminants in environmental matrices relevant to neurological health

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Environmental exposures are increasingly recognised as risk factors for neurological health. Heavy metal exposures have been associated with lower cognitive function, behavioural changes, attention deficit hyperactivity disorder, autism spectrum disorders, and Alzheimer´s disease (AD), among others. Exposure to organic compounds has also been associated with AD, cognitive impairment and personality changes. Exposure to endocrine disruptors, such as pesticides (e.g. organocholorines), polychlorinated biphenyls, dioxines and plasticisers have been linked with Parkinson´s Disease, AD and cognitive impairment. Micro and nanoplastics are emerging threats potentially linked with neurological health due to its potential ability to cause oxidative stress, neuroinflammation and disrupting the blood-brain barrier.

Assessment of exposures to these pollutants requires knowledge on the main sources, occurrence in environmental matrices and their potential routes of exposure. Identification of key environmental matrices is crucial to define the optimum characterization protocol, from sampling to analysis.

The workshop will present the key principles of sampling and storage of environmental samples from relevant matrices such as soil, water and air. Key factors required to implement a reliable and reproducible protocol for sampling, storage and analysis will be discussed, including those related for quality control and quality assurance. Description of pre-analytical workflows

for sample preparation to increase sensitivity and specificity of analytical techniques may include extraction, purification and derivatization. The main analytical techniques to characterise environmental pollutants of neurological concern include AAs, ICP-OES, or ICP-MS for heavy metals; GC-MS and LC-MS for organic compounds; FTIR, Raman and Pyrolytic GC-MS for nano and microplastics. Government agencies, such as the US EPA, have established standardized protocols to characterise environmentally relevant pollutants in different matrices.

Overall, the workshop will provide information to identify and quantify environmental pollutants potentially relevant to neurological health from key environmental matrices using the most appropriate sample preparation workflows and analytical techniques.

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W2

Integrating deeplabcut and simba for automated behavioral analysis in contaminant-induced neurobehavioral disorders

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Understanding the impact of environmental contaminants on behavior requires reliable tools for detecting subtle and complex phenotypic changes. Advances in machine learning–based video analysis have opened new opportunities for high-throughput and objective behavioral assessment. Here, we present a workflow integrating DeepLabCut and SimBA (Simple Behavioral Analysis) to analyze behavioral alterations associated with contaminant exposure in animal models. DeepLabCut enables markerless pose estimation with high spatial and temporal precision, providing detailed tracking of body parts across experimental conditions. These data are then processed in SimBA, which applies supervised machine learning to classify and quantify distinct behavioral motifs. The combined pipeline offers several advantages: it reduces observer bias, improves reproducibility, and captures behaviors that

are difficult to detect through manual scoring. We demonstrate its application in studies of neurotoxic and endocrine-disrupting contaminants, where exposure has been linked to altered social interaction, locomotion, and stereotypic behaviors. Our workflow is adaptable to multiple species and experimental designs, supporting both hypothesis-driven and exploratory research. By integrating open-source tools into a standardized framework, this approach accelerates behavioral phenotyping in environmental health studies. Ultimately, the synergy between DeepLabCut and SimBA provides a powerful strategy to uncover the behavioral signatures of contaminant-induced neurotoxicity, advancing our understanding of how environmental stressors shape brain and behavior.

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