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tine have all been detected, persisting as environmental microcontaminants at concentrations ranging from ng/L to µg/L.[1,2] Their increasing presence raises concerns about potential ecotoxicological effects. To evaluate their developmental toxicity, we used *Xenopus laevis* embryos, a recognized model in environmental risk assessment. A modified FETAX protocol was employed, exposing embryos at the 4-8 cell stage better to simulate environmentally relevant exposure scenarios.[3] A range of concentrations, reflecting those found in surface and wastewater, was tested to assess potential dose-dependent effects. In this preliminary experiment, the three psychoactive substances, clonazepam, caffeine, and nicotine, were tested individually to establish baseline effects for each compound. At developmental stages 45/46, standard FETAX endpoints (mortality, growth, malformations) were evaluated, alongside heart rate as an additional parameter.[4] Observed outcomes included significant cephalic and abdominal edema, intestinal malformations, bent tail phenotype, and alterations in dorsal pigmentation. LC_{50}, EC_{50}, and the Teratogenic Index (TI) were calculated, revealing a notable teratogenic potential. Future studies will assess combined exposures to investigate possible synergistic or additive effects. Nonetheless, these preliminary results already highlight significant developmental risks linked to psychoactive contaminants. Given the absence of specific regulatory thresholds, further research is needed to guide environmental protection efforts and safeguard ecosystem and human health.

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DEVELOPMENTAL TOXICITY AND CYTOTOXIC/GENOTOXIC POTENTIAL OF PM_{10} EXTRACTS USING *XENOPUS LAEVIS* EMBRYOS AND A549 LUNG EPITHELIAL CELLS

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Particulate matter, including PM_{10}, poses significant health and environmental risks. While human studies highlight associations with adverse prenatal outcomes, such as low birth weight and preterm births, experimental animal data on PM_{10} remain limited. This study assessed the developmental toxicity and cytotoxicity/genotoxicity potential of PM_{10} extracts collected from a rural site in the Po Valley (Bertonico, northern Italy), using *Xenopus laevis* embryos (R-FETAX) and A549 lung epithelial cells. PM_{10} samples were chemically characterized, focusing on PM_{10} and key toxic components (NO_3-, SO_4-, NH_4+, OC, EC, Al, Si, Ti, V, Mn, Fe, Cu, Zn, Rb, Pb, and Sr). Embryos/cells were exposed to sample extracts diluted 1:10 to assess developmental toxicity and, in the cell model, cytotoxicity and genotoxicity. R-FETAX results showed no lethal effects or major malformations, while statistically significant developmental delays were observed in groups exposed to some extracts. Delays correlated both with PM_{10} and the considered analytes, including Zn and Cu. Minimal cytotoxic and genotoxic responses were observed in A549 exposed cells. Results suggest that, even in rural settings, PM_{10} can impair embryonic development without cytotoxic or genotoxic effects *in vitro*, demonstrating the effectiveness of the FETAX model for detecting subtle developmental toxicity induced by complex particulate mixtures.

MULTIPLE ENVIRONMENTAL STRESSORS: A THREAT TO THE REPRODUCTIVE HEALTH OF MUSSEL *MYTILUS GALLOPROVINCIALIS*?

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Ecotoxicological analyses performed on the species mussel *Mytilus galloprovincialis*, cultivated at the mussel farm S.A.Co.M. located in the Natural Reserve of the Lagoon of Capo Peloro (Messina, Italy), showed the potential action on male and female reproductive health of multiple environmental stressors due to climate change. Through a multi-biomarker approach, potential biomarkers useful for the assessment of ecosystem risk were identified. In fact, histological analysis underlined coherence between the autumn seasonality and the reproductive period, while histochemical (dPAS/PAS) and metabolomic (proton nuclear magnetic resonance, ^{1}H NMR) evaluations showed differences between the two sexes in the storage of energy reserves, such as glycogen. The Schmorl's method demonstrated the threshold of immune vigilance, not highlighting accumulation of melanin, as supported by the enzymatic results about the activity of catalase (CAT), glutathione *S*-transferase (GST) and levels of malondialdehyde (MDA). Current data remark that in the field there is a basal divergence in metabolite concentration for the target species subjected to multiple stresses, but also that sexual maturation remains synchronous for both sexes at least during the selected season in the natural environmental conditions.

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MOLECULAR INSIGHTS INTO THE GLUCOSE-LOWERING EFFECTS OF SICILIAN RED AND WHITE GRAPE SEED OILS ON HEPG2 CELLS

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Grape seed oil, a by-product of wine production, is rich in polyunsaturated fatty acids and antioxidant polyphenols with well-documented metabolic benefits. In preliminary experiments[1], HepG2 liver cells were exposed for 24 h to non-cytotoxic concentrations

of seed oil obtained from Sicilian white (WGSO) and red (RGSO) grapes. This increased intracellular glycogen content, as demonstrated by PAS staining, and decreased extracellular glucose levels, suggesting improved glucose utilization. To further investigate these effects, experiments were performed using the fluorescent glucose analog 2-NBDG, which revealed a significant enhancement in glucose uptake with both WGSO and RGSO, comparable to insulin stimulation. In parallel, Western blot analysis was conducted to examine the expression of glucose transporters GLUT-2 and GLUT-4, the transcription factor HNF1α, and key regulators of insulin-dependent (IRS-1, AKT, PKCζ) and insulin-independent (AMPK) signaling pathways. WGSO selectively upregulated GLUT-4 and activated the AKT pathway, whereas RGSO induced a broader effect by increasing both GLUT-2 and GLUT-4, upregulating HNF1α, and activating both PI3K/AKT/PKCζ and AMPK pathways, promoting GLUT-4 translocation. Our results suggest that grape seed oil may help regulate glucose metabolism, especially in cases of insulin resistance, making it relevant for managing diabetes. The repurposing of this wine by-product enhances its value and promotes practices, encouraging further research into its therapeutic potential.

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3D CELL MODELS: A VALUABLE TOOL TO STUDY AUTOPHAGY ROLE IN GLIOBLASTOMA BIOLOGY

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Glioblastoma (GBM) is the most common and aggressive adult brain tumor, with poor median survival and limited therapeutic options. One of the major limitations to the treatment and resolution of GBM is the presence within the tumor mass of a small subpopulation of cells called glioma initial glioma cells (GICs). GICs, harboring potent tumor-initiating capability and sustaining tumor-growth, are responsible for the high degree of morphological, molecular and cellular heterogeneity that characterizes GBM.[1] Autophagy is a cytoprotective mechanism that is often deregulated in human diseases, especially in cancer. The relevance of autophagy in GBM has yet to be fully clarified, although a growing body of evidence suggests that suppression of autophagy is correlated with gliomagenesis[2]. In order to overcome the weakness of 2D cell models in investigating tumor biology, we are setting protocols for generating reliable 3D *in vitro* models, enriched in GICs and that hopefully recapitulate the tumor feautures. In detail, we are generating both tumor spheres and tumoroids from GBM cell lines or primary cells from patients. We are characterizing the GBM 3D models for proliferation rate, expression of specific markers and autophagy and apoptosis occurrence. 3D models will allow us to indepth investigate the autophagy role in GBM biology and to correlate it to tumor features such as stemness/differentiation degree, proliferation proficiency and drug-response proficiency. Autophagy-defective GBM models will be also employed to assign a role to specific autophagy master regulators in GBM tumorigenesis.

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IN VIVO EVALUATION OF CYTOTOXIC AND GENOTOXIC RESPONSES INDUCED BY TITANIUM DIOXIDE NANOPARTICLES (TIO_2-NPS) IN GOLDFISH (*CARASSIUS AURATUS*)

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Titanium dioxide nanoparticles (TiO_2-NPs) are widely used in industrial and consumer products,[1] raising concerns about their ecotoxicological effects, especially in aquatic environments. With the expansion of nanotechnology, artificial particles are increasingly released into the environment and, due to their structure, often different from natural substances, they tend to persist and degrade slowly, posing risks to organisms lacking natural defense mechanisms.[2] Once released, it can be absorbed by aquatic organisms and enter the food chain. Prior studies, such as Rocco et al.[3], have shown that TiO_2-NPs exposure causes DNA damage and genomic instability in zebrafish. This study aimed to assess *in vivo* cytotoxic and genotoxic effects in goldfish (*Carassius auratus*), exposed to 10 µg/L TiO_2-NPs for 14 and 21 days. A control group (NC) was maintained without exposure. Three assays were performed: eosin Y staining for cell viability, NBT test for ROS production, and TUNEL test for DNA fragmentation. The results showed a significant reduction in cell viability, especially at the end of the longest exposure period (21 days), with a decrease of 78.6% compared to 96% observed in NC. In addition, a significant production of ROS was observed in the treated samples, with an increase of 27.98% at 14 days and 36% at 21 days. Similarly, a significant increase in DNA fragmentation was recorded, equal to 24% at 14 days and 28% at 21 days, compared to controls, which show a fragmentation of 13% and 15%, respectively. Furthermore, another aspect observed was the color change of the livery of the exposed specimens, which showed a change from a reddish-orange to blackish shades, suggesting a possible visible effect of prolonged exposure. These data indicate that exposure to TiO_2-NPs causes cytotoxic and genotoxic effects in goldfish, effects that become more evident with the prolongation of exposure time. The increase in oxidative stress, DNA damage and the granting of cell viability suggests that these nanoparticles, can have a significant biological impact. The consistency between the results of the three tests strengthens the hypothesis of a potential ecotoxicological risk and underlines the importance of a more rigorous regulatory monitoring of the release of TiO_2-NPs in environment.