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P08-08

Ecotoxicological responses of Gilthead Seabream (Sparus aurata) upon chronic dietary exposure to microplastics particles from petroleum-based and alternative polymers

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In recent years, the scientific community has devoted considerable attention towards the understanding of the effects posed by marine litter on marine ecosystems. This growing concern is due to the persistence and ubiquity of small plastic particles [1, 2], i.e. microplastics that can be ingested by marine biota and cause a handful of effects, depending on their size and level of exposure [3, 4, 5]. As a result, the industry has invested many efforts on the development of alternative biodegradable and recyclable polymers derived from natural molecules/ingredients (the so called “bioplastics”), with the aim of replacing, as much as possible, the use of conventional plastics derived from fossil fuels and, in long-term, mitigate plastic pollution [6]. However, there is still a lack of empirical knowledge concerning the potential toxicological attributes of these new bioplastics in marine organisms [7, 8]. Hence, this study aimed to assess the ecotoxicological responses of an ecologically and commercially relevant fish species following chronic (28 days) dietary exposure to microplastics particles from 3 polymers, i.e the conventional petroleum-based polyethylene terephthalate (PET), and the bioplastics polybutylene succinate (PBS) and poly[propylene] fumarate (PPF).

S.aurata (~100 g) were kept in recirculation aquaculture systems (RAS) under optimal development conditions (19ºC, > 7 mg/L O2). During 28 days of feeding trials, fish were fed ~1% of their body weight with commercial (and non-intentionally contaminated) diet (CTR) and three experimental diets contaminated with ~60 particles PET, PBS, PPF g-1 of feed. Following this exposure period, fish underwent a 7-day depuration period, during which they were fed only with CTR feed. Fish (n=12) were sampled from each treatment at day 28 (upon exposure) and 35 (upon depuration), and liver, brain and gut were collected to evaluate the following physiological endpoints: oxidative stress (catalase activity [CAT], superoxide dismutase activity [SOD], lipid peroxidation [LPO] and glutathione S-transferases [GST]), metabolic enzymes activity (lactate dehydrogenase [LDH] and citrate synthase [CS]) and digestive enzymes activity (a-amylase, pepsin and trypsin). Data analysis is still ongoing. However, preliminary results showed that PPF may not be as harmful as PET and PBS. These findings show that PPF can be a sustainable and less environmentally damaging alternative to petroleum-based plastics or, even, other widely commercialized bioplastics.

Acknowledgments

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LP-44

Evaluation of Immune Responses to Combustible Cigarettes and Tobacco Heating Products in Ulcerative Colitis Patients

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The influence of nicotine-containing products on immune regulation in ulcerative colitis (UC) remains a subject of debate. Although traditional combustible cigarettes (CCs) have long been linked to changes in UC disease activity, the exact immune mechanisms involved are still not fully understood. Meanwhile, the emergence of electronic nicotine delivery systems—particularly Tobacco Heating Products (THPs)—has raised concerns, warranting further investigation into their potential adverse effects. This study aimed to assess how CCs and THPs affect systemic immune responses in UC patients, with the goal of clarifying how these exposures impact immune-mediated inflammation and disease progression. Peripheral blood samples were collected from UC patients categorized as non-smokers (UCAIR), THP users (UCTHP), and CC smokers (UCCC). Flow cytometry was employed to analyze the frequency and function of mononuclear immune cells. Patients using THPs showed a significant increase in regulatory T cells (Tregs) producing IL-10, TGF-β, and IL-35, indicating a shift toward an immunosuppressive or regulatory immune profile. In contrast, patients in the UCCC group exhibited a decrease in several inflammatory T cell subsets, including Th1 (TNF-α, IFN-γ), Th2 (IL-4), and Th17 (IL-17) cells. This widespread suppression of effector T cells may reflect a reduction in systemic inflammation among CC users. Interestingly, non-smoking UC patients (UCAIR) displayed higher levels of these inflammatory T cell populations compared to both nicotine-exposed groups. These results reveal that CCs and THPs exert distinct immunomodulatory effects in UC. Although their mechanisms differ, both forms of nicotine exposure appear to reduce inflammation and may contribute to symptom relief in UC patients. THP use was specifically associated with enhanced regulatory T cell activity, while CC use correlated with a broad suppression of effector T cell responses. This study offers new perspectives on how various nicotine delivery systems may shape the immune landscape in UC and suggests implications for tailoring disease management in nicotine-exposed individuals.

LP-65

Modulation of the effects of cholesterol-supplemented high-fat diet by aryl hydrocarbon receptor (AHR) activation and/or tryptophan reduction

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Obesity and its associated metabolic syndrome are among the most important and still increasing global health problems today. Yet many physiological aspects of obesity such as the mechanisms for the alterations occurring in energy metabolism and body weight set-point are still incompletely understood. The most often used animal model to study obesity-related changes in energy metabolism is high-fat diet (HFD)-induced dietary obesity in C57Bl/6J mice.

Recent studies have shown that aryl hydrocarbon receptor (AHR) plays a key role in energy metabolism (1). AHR is a ligand-activated nuclear receptor that regulates a wide variety of genes involved in multiple functions. Whole-body AHR deficiency was reported to protect against HFD-induced obesity, visceral fat accumulation, insulin resistance, glucose intolerance, and steatohepatitis, while not influencing body weight on a standard diet (1,2). AHR deficiency appeared to enhance energy expenditure (2). Conversely, persistent activation of AHR by a low dose of TCDD was reported to augment body weight gain by HFD.

Here, fifty male C57BL/6JRccHsd mice were assigned to one of five feeding groups, control diet (CD), high-fat diet (45% of energy from fat), HFD with only 70% of the regular TRP concentration (HFDtrp), HFD supplemented with a weakly toxic AHR agonist C2, or HFDtrp with C2. All diets contained 2% cholesterol and were fed for 18 weeks. On weeks 14–16, the mice were tested for gas exchange and locomotor activity, and on weeks 15–17 for glucose tolerance and insulin sensitivity. At termination, tissue samples were collected for biochemical and AI-assisted histological analyses. Body weight gain (BWG) was only 28–38% higher in the HFD groups than in the CD group, but the HFD-fed mice accumulated 43–61% more fat. Calorie intake was greater in the two low-TRP groups than in the two other HFD groups, while BWG remained similar. C2 induced *Cyp1a1* expression (an index of AHR activity) in all tissues examined and increased the ratio of micro-/macrosteatosis in the liver. The HFDs tended to reduce insulin sensitivity, CO_2 production, and the ability to respond appropriately to a low-temperature challenge. These findings suggest that the effects of AHR activity modulation on energy balance are strongly context-dependent. A sensitive response to long-term AHR activation appears to be elevated micro-/macrosteatosis ratio in the liver when exposed to HFD.

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LP-66

Nocebo effects in 5G exposure: dissociating physical and psychological impacts on alpha band electrical brain activity

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INTRODUCTION

The rapid deployment of 5G technology has raised public health concerns. Beyond direct radiofrequency effects, psychosocial factors can alter physiological responses through the nocebo effect, where negative expectations influence outcomes [1,2].

This study investigated the acute effects of 26 GHz (5G) exposure on brain activity (EEG) and salivary cortisol. While initial analyses found no significant differences between real and sham exposure, we hypothesize that participants' subjective beliefs about exposure could modulate these physiological responses, aligning with nocebo mechanisms.

METHOD

Data were extracted from a larger study using a validated experimental design from our laboratory [3,4,5,6]. For this analysis, 10 healthy subjects aged 18-30 years (4 women, 6 men) were studied using a randomized, double-blind, crossover protocol with 26 GHz exposure (2 V/m, 26.5 minutes) in a shielded room. EEG and salivary cortisol were collected before, during, and after exposure.

Analysis focused on cortisol and alpha EEG band (8-12 Hz) during eyes closed. Only participants who clearly expressed beliefs about whether they were exposed in both sessions were included in the analysis. Subjects were grouped based on subjective exposure perception: 10 participants believed they were exposed in one session and not in the other, 5 participants misjudged the exposure condition, while 5 correctly identified it.

RESULTS

Kruskal-Wallis tests showed no significant cortisol differences between groups.

Results revealed no significant main effects or interactions (period of exposure and electrodes) when considering only actual exposure condition ($p > .05$) for alpha band. However, when analyzing based on subjective exposure, we observed a significant main effect ($F(1,81) = 8.90$, $p = .0038$), with higher alpha activity values when participants thought they were being exposed, regardless of the actual condition. This effect was validated with Tukey-adjusted post-hoc.

CONCLUSION

This exploratory study demonstrates that participants' subjective beliefs regarding 5G exposure can have a significant effect on EEG alpha spectral power. This finding supports evidence that cognitive and psychological factors can modulate physiological responses through nocebo effects.

No significant changes were found in salivary cortisol levels across exposure conditions, consistent with previous findings [7,8,1]. Regarding EEG alpha power, our finding that belief modulates alpha activity aligns with evidence that expectation and attention can alter resting-state brain dynamics [9, 10].

Several limitations must be acknowledged: small sample size ($n = 10$) limits statistical power and generalizability. Future research should employ fully randomized protocols where each subject experiences all combinations of true vs. perceived RF exposure.

Our results highlight the need to consider subjective perception as a key confounder in 5G risk assessment and communication.

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LP-67

Deriving a Point of Departure for Assessing Skin Sensitization Risk of Chemicals Using the SENS-IS Assay

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Background and Purpose: Skin sensitization risk assessment is essential in toxicology, especially given the push toward non-animal testing methods. The SENS-IS assay, an in vitro test, offers a promising alternative for identifying and classifying the skin sensitization potential of chemicals. Previously, using a panel of 139 reference chemicals from the OECD's list, the SENS-IS assay demonstrated robust accuracy—95% in identifying sensitization hazards and 86% in classifying sensitization potency according to UN-GHS criteria. However, existing in vitro methods are limited by an inability to define No Expected Sensitization Induction Level (NESIL) values between tested concentrations. This study aims to refine the SENS-IS assay's ability to determine a continuous point of departure (PoD) for sensitization risk, potentially improving its precision and relevance for regulatory use.
Methods: We compiled a comprehensive database of 137 reference chemicals, each tested across multiple concentrations. The chemicals were assessed for expression of the 61 gene biomarkers utilized in the SENS-IS assay. Advanced artificial intelligence (AI) and machine learning techniques were then applied to analyze gene expression patterns and concentration data, allowing us to derive continuous PoD values. This AI-enhanced model was then compared to traditional in vivo data from LLNA EC3 values and human No-Observed Effect Level (NOEL) data to assess consistency and predictive accuracy.
Results: The application of AI-driven analysis successfully allowed the derivation of continuous PoD values for each tested chemical. Our findings revealed a strong correlation between the SENS-IS-derived PoD and traditional LLNA EC3 and NOEL values, confirming the assay's predictive accuracy. This correlation suggests that SENS-IS,

enhanced with AI, could reliably serve as an in vitro alternative for assessing chemical sensitization risk and provide refined NESIL values across a broader range of concentrations, supporting more nuanced risk classifications.
Conclusions: Integrating AI analysis with SENS-IS assay data represents a significant advancement in skin sensitization risk assessment, achieving high accuracy in hazard identification and potency classification. This approach allows for the derivation of reliable PoD values across various concentrations, offering an improved tool for regulatory toxicology. It thus contributes to the movement toward animal-free testing and presents a robust, ethical alternative for sensitization testing, with the potential to replace traditional animal-based methods in regulatory settings.

LP-68

Cytotoxicity of Psilocybin and Psilocin in SH-SY5Y Neuronal Cells

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The psychedelic compounds psilocybin and psilocin have gained attention not only for their recreational use but also for their therapeutic applications in treating neurological and psychiatric disorders [1]. Despite this interest, their toxicological profiles remain poorly understood. This study utilized the SH-SY5Y human neuroblastoma cell line to evaluate the cytotoxicity of these compounds.
SH-SY5Y cells were seeded at 50 000 cells/well in 96-well plates and allowed to adhere overnight. Cells were then exposed for 48 h to the following concentration ranges: 6×10^{-6}–0.5 mM for psilocybin (n = 22) and 1.3×10^{-5}–1.5 mM for psilocin (n = 16). MTT and NR uptake assays were employed to evaluate cell viability (seven independent experiments). A neuroprotection study was also performed by pre-treating SH-SY5Y cells with each psychedelic, followed by glutamate exposure (five independent experiments). To assess membrane integrity, LDH leakage was also measured (six independent experiments). From the MTT curves, EC_{20}, EC_{40} and EC_{60} values were selected for mechanistic assays of psilocin. Psilocybin was excluded from further tests due to low cytotoxicity. ROS/RNS generation, TMRE uptake (five independent experiments each) and ATP levels (three independent experiments) were assessed.
As referred, psilocybin showed low toxicity, and it did not allow for a complete concentration-response analysis. At the highest tested concentration (i.e., 0.5 mM, due to solvent toxicity at higher concentrations), only 26% (MTT) and 16% (NR) mortality was achieved. Psilocin exhibited EC_{50} values of 0.42 mM (MTT) and 0.69 mM (NR). Pre-treatment with psilocybin and psilocin failed to mitigate glutamate toxicity, indicating limited protective potential. Psilocybin did not induce any significant LDH leakage, while psilocin induced significant cell release of LDH at 1.5 and 0.75 mM, consistent with its toxicity seen in MTT and NR assays. Psilocin significantly increased ROS/RNS at all concentrations tested (0.18–054 mM), in a concentration-dependent fashion; at the EC_{60}, psilocin triggered nearly a nine-fold rise versus control, demonstrating strong oxidative potential. However, no TMRE changes were observed. ATP data showed a significant reduction ($p<0.05$) for psilocin at EC_{60}.

These findings indicate that psilocybin presents low toxicity in SH-SY5Y cells. The dissociation between psilocin's massive ROS burst (with ATP loss but no TMRE rise) suggests that this compound may provoke early mitochondrial depolarisation and energetic collapse.

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LP-69

3D human intestinal organoids as a platform to assess compound-induced gastrointestinal toxicity

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A major challenge in the clinical translation of cancer therapies is the overlooked toxicity in healthy tissues, particularly the gastrointestinal (GI) tract. Adverse events such as diarrhea, mucosal ulceration, and intestinal inflammation are frequently associated with tyrosine kinase inhibitors (TKIs) and immunotherapies, including bispecific antibodies. These effects are often linked to compromised epithelial barrier function and immune-mediated tissue damage. Given the intestine's central role in drug absorption and metabolism, it is essential to assess compound-induced GI toxicity (GIT) early in the drug discovery and development pipeline. Here, we utilize 3D human induced pluripotent stem cell-derived intestinal organoids (HIOs) as a physiologically relevant preclinical model to evaluate gastrointestinal toxicity. To quantify toxicity of clinically relevant small molecules (Afatinib, CPT11 and 5FU), we applied complementary cell viability assays and image-based analyses, including Ki-67 immunostaining to assess epithelial proliferation and cleaved caspase-3 staining to evaluate apoptosis. HIOs viability (IC50) were normalized to clinical Cmax exposure and the resulting IC50/Cmax ratio plotted to score diarrheagenic and non-diarrheagenic drugs. Furthermore, we established a co-culture system of HIOs with peripheral blood mononuclear cells (PBMCs) to model immune-mediated epithelial injury and assess the GIT potential of bispecific antibodies (EpCam). Our results demonstrate that human intestinal organoids provide a scalable and sensitive platform for detecting epithelial damage, capturing both direct cytotoxic and immune-related adverse effects. This organoid-based assay framework offers a powerful tool for early-stage screening of therapeutic candidates, enabling prediction and mitigation of GI liabilities prior to clinical translation.

LP-70

Data-driven Safe-by-Design approach for Solid Oxide Electrolyzer Cell anodes using a combination of quantum mechanical simulations and machine learning algorithms

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Solid Oxide Electrolyzer Cells (SOEC) are emerging as a compelling alternative to conventional energy technologies, offering the ability to both generate and utilize hydrogen in a reversible cycle—making them highly relevant for sustainable energy systems. However, the practical implementation of more efficient SOEC systems remains limited due to knowledge gaps. This study takes a computational approach to propose lanthanum-free anode materials for SOECs, in line with the Safe and Sustainable by Design (SSbD) framework.

For the first time, we integrate quantum mechanical calculations with machine learning techniques to support the design of SOEC anodes, offering a resource-efficient alternative to traditional experimental workflows. This strategy significantly reduces the need for costly lab-based synthesis and testing, narrowing down candidates for experimental validation.

The research involved generating molecular models for 240 potential doped Brownmillerite-based SOEC anode structures, each consisting of 29 atoms (including 20 oxygen atoms—5 in octahedral and 4 in tetrahedral coordination). Quantum mechanical properties were calculated using density functional theory (DFT) at the MN15/LANL2DZ/6-31G level via the Gaussian16 software. The resulting quantum descriptors were used in a machine learning workflow to cluster the structures. Five distinct groups were identified, and representative candidates were selected through k-means clustering for future experimental validation.

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LP-71

Evaluation of the Biological Activity of Plant Extracts Used in Traditional Ophthalmic Medicine in the Department of Bolívar, Colombia

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Abstract.
Introduction. There is currently a growing interest in identifying organisms that produce bioactive molecules with potential for inclusion in the design and development of new promising drugs applicable to modern medicine,

including the field of ophthalmology (1). Ocular pathologies not only lead to visual impairment but also significantly affect individuals' quality of life. In general, these conditions involve important components such as oxidative stress, inflammatory processes, and the activation of matrix metalloproteinases (2). The aim of this study was to identify and prioritize plant extracts used in the traditional medicine of the Montes de María region (Bolívar, Colombia) for the care and treatment of ocular diseases. **Methodology.** Ethnobotanical surveys were designed and conducted to identify plant species with ophthalmic applications. The selected species were subsequently collected, washed, and subjected to maceration to obtain hydroethanolic extracts using a 7:3 ethanol-to-water solution. These extracts were then lyophilized for subsequent bioassays. Toxicity evaluation was carried out using the *Caenorhabditis elegans* (wild-type N2 strain) model, with extract concentrations ranging from 50 to 5000 µg/mL and an exposure time of 24 hours at 20 °C. Antioxidant activity was assessed using the DPPH assay (50–300 µg/mL).
Results. Twelve plant species with ophthalmic applications were identified, with *Spondias mombin* (Hobo), *Plantago* sp. (Yanten), and *Cordia dentata* (Uvito) being the most frequently used, primarily for the treatment of eye redness, blurred vision, and ocular discharge. Toxicity assays in *C. elegans* revealed low toxicity (less than 10% lethality). Antioxidant activity assays indicated that the extracts with the highest free radical scavenging capacity were *Cordia dentata* (89.73%), *Anacardium excelsum* (82.47%), and *Aristolochia anguicida* (81.68**%).**
Conclusion. These results demonstrate a favorable preliminary safety profile, supporting the potential use of these extracts in the development of new ophthalmic phytopharmaceuticals targeting oxidative and inflammatory processes. Furthermore, these findings highlight the need for chemical characterization to identify the principal active compounds.

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LP-72

Integrating proteomics, metabolomics, and network pharmacology to investigate the mechanism of *Cordyceps sinensis*in the treatment of COPD rats

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Chronic obstructive pulmonary disease (COPD) causes significant morbidity and mortality, ranking as the third leading cause of death globally. *Cordyceps sinensis* (*C. sinensis*), has long been used in Asia as a tonic of traditional Chinese medicine, to alleviate lung ailments, and exhibits a potential therapeutic effect on COPD. This study aimed to explore the protective effects of *C. sinensis* on COPD and elucidate the underlying molecular mechanism. In this study, COPD was induced in rats via cigarette smoke exposure combined with intratracheal lipopolysaccharide (LPS) administration, followed by *C. sinensis* treatment. Pathological alterations in lung tissue and inflammatory cytokines in serum and lung tissue were assessed, and an integrated analysis combining proteomics, metabolomics, serum pharmacochemistry, network pharmacology, and molecular biology was conducted. The result showed that *C. sinensis* treatment significantly alleviated lung tissue injury in COPD rats, with the medium dose exhibiting particularly notable efficacy. Furthermore, *C. sinensis* administration reduced the levels of inflammatory factors (TNF-α, IL-8, MMP-9) in serum and lung tissue, suggesting a potential anti-inflammatory role in COPD. Integrated metabolomics and proteomics analysis revealed that the protective effect of *C. sinensis* against COPD critically depended on regulating glycerophospholipid and sphingolipid metabolism by targeting PLA2G4E and B4GALT4 proteins, which confirmed by WB and qRT-PCR. The analysis of serum samples identified 20 blood-entering compounds from *C.*

sinensis. Network pharmacology analysis revealed that sphingolipid components in *C. sinensis* exert therapeutic effects against COPD through multi-target interactions, primarily involving AKT1, ESR1, TLR4, and MMP9. The findings further revealed that *C. sinensis* mainly modulated COPD through the PI3K-AKT signaling pathway. Additionally, WB experiments verified that *C. sinensis* reversed p-AKT in the lung tissue of COPD rats. In conclusion, *C. sinensis* may influence the PI3K-AKT signaling pathway in COPD rats, potentially affecting glycerophospholipid metabolism and sphingolipid metabolism by targeting PLA2G4E and B4GALT4 proteins, thereby alleviating the inflammatory response and mitigating lung tissue damage caused by COPD.

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LP-73

Mechanistic Anchoring of Cell Painting to Transcriptomics: A Proof-of-Concept for Robust Mutagenicity Prediction

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Regulatory agencies are increasingly embracing New Approach Methodologies (NAMs) for chemical safety assessment. Among these, transcriptomics-based tools such as TGx-DDI and GENOMARK biomarkers for DNA damage detection are gaining regulatory interest. However, these approaches rely on specific gene sets and may benefit from complementary, orthogonal modalities, in line with the principles of Integrated Approaches to Testing and Assessment (IATA). The Cell Painting (CP) assay captures a broad spectrum of cellular morphological changes and offers a phenotypic layer to support mechanistic interpretation. This study aims to integrate transcriptomic and morphological data to establish a mechanistically anchored Line of Evidence for mutagenicity prediction. To achieve this, a set of 77 Ames-positive and 35 Ames-negative chemicals was curated from the Class A mutagen list defined by NIHS Japan and EURL ECVAM Genotoxicity and Carcinogenicity Consolidated Database with various mutagenic mechanisms, including alkylation, intercalation, and oxidative stress. U-2 OS cells were exposed in 10-point doses, and both CP and transcriptomics (Screen-seq, Evotec) were run in parallel on separate 384-well plates with at least three biological replicates. Both experiments and transcriptome analysis were performed by Evotec. Quality control of CP data confirmed that 111 chemicals yielded GRIT scores >1.0 in any of the doses, indicating robust morphological responses. To establish a mechanistic anchor, we first defined a ground truth dataset based on the transcriptional activation of the DNA damage response, leveraging the TGx-DDI biomarker concept. In this step, 29 out of 77 Ames positive chemicals were selected. With this subset, doses above the phenotypic point of departures (PoDs) in each chemical were selected to train an XGBoost classifier on CP features. After preprocessing, up to 32 morphological features were selected with the minimum Redundancy Maximum Relevance algorithm. The best resulting model achieved a balanced accuracy >0.85, sensitivity >0.90, and F1 score >0.85. Notably, the top three most influential features originated from DNA or RNA channels, aligning with well-established cellular responses to genotoxic stress and thereby reinforcing the mechanistic relevance of the model. This proof-of-concept shows that

integrating transcriptomic and morphological data provides a mechanistically interpretable approach to mutagenicity assessment. The strategy supports development of quantitative phenotypic PoDs for mutagenicity and lays groundwork for future integration into in vitro to in vivo extrapolation (IVIVE) models. Ongoing work will focus on expanding the training chemicals to establish the applicability domain and refining the model to enhance its regulatory applicability, ultimately contributing to next-generation risk assessment frameworks aligned with the 3Rs.

LP-74

Investigation on the Inhibitory Effect of Bergeniapurpurascens Water Extract on COVID-19 XBB1.5Variant and Its Blocking ACE2 Mechanism

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Objective: Bergenia purpurascens, a medicinal herb indigenous to southwestern China, has garnered interest for its potential therapeutic properties. This study investigates the herb's water extract's capacity to interfere with the interaction between the COVID-19 S1 Receptor Binding Domain (RBD) and the ACE2 receptor, a critical step in the virus's infection process. Methods: Quality standards for the Bergenia purpurascens extract were established to ensure consistency. The extract's efficacy was assessed using a Vero-E6 cell infection model to inhibit live virus infection of the COVID-19 XBB1.5 variant. Surface Plasmon Resonance (SPR) was employed to explore the mechanism of action. The extract's composition was further analyzed using Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS), with active compounds identified through a combination of in silico screening and actual activity testing. Results: The water extract of Bergenia purpurascens demonstrated significant inhibitory activity against the COVID-19 XBB1.5 variant, with stability and high potency, reflected by an IC50 value of 0.037 mg/mL. The selectivity index for the antiviral effect is approximately 9. The extract's composition was predominantly Phenylpropanoids and Carbohydrates, accounting for over 60% of the total. Interestingly, a majority of the high-content compounds were inactive, while the low content compounds, exemplified by Ellagic acid, showed synergistic activity. The extract's inhibitory mechanism was characterized as a non-specific inhibition of ACE2 with a steady-state binding affinity (KD) of 7.9 × 10−7 M. Conclusions: The study concludes that the water extract of Bergenia purpurascens is a promising, cost-effective non-specific ACE2 inhibitor with potential applications in COVID-19 therapy. The identification of active compounds and the elucidation of its inhibitory mechanism provide a foundation for further research and development of this traditional medicinal herb in combating viral infections.

LP-75

An Integrated Approach Involving Metabolomics and Transcriptomics Reveals Arsenic-Induced Toxicity in Human Renal Cells

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Accumulating epidemiological evidence has indicated that arsenic exposure can lead to kidney injury. However, the underlying mechanisms of arsenic-induced nephrotoxicity have not been fully elucidated. In this study, the effect of sodium arsenite on the cell viability of HEK-293 cells was studied using a CCK-8 assay. Metabolomic and

transcriptomic analyses were applied to identify differential metabolites (DMs) and differentially expressed genes (DEGs) in human renal cells exposed to arsenite, respectively. The results showed that the IC_{50} of arsenite on HEK-293 cells was 25 μM. A total of 621 DMs were identified in arsenic-treated cells (VIP > 1, $p < 0.05$). The results of the metabolome analysis revealed that purine metabolism was the major affected pathway, with 21 DMs enriched within this pathway. Additionally, 9831 DEGs were obtained after arsenic exposure ($|\log_2 FC| > 1$, Padj < 0.05). The results of the transcriptome analysis showed that ECM–receptor interaction and cell adhesion molecules were the major altered KEGG pathways, with 54 and 70 enriched DEGs, respectively. Integrated metabolomics and transcriptomics analyses revealed that the predominant mechanisms underlying arsenic-induced nephrotoxicity were associated with the perturbations of lipid metabolism and purine metabolism. Overall, the present study provided comprehensive insights into the metabolic and transcriptional alterations in human renal cells in response to arsenic exposure, providing a referable scientific basis for subsequent arsenic-induced nephrotoxicity studies.

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LP-76

Stage-Specific Toxicity of Surfactants in Chironomus riparius Larvae

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Synthetic surfactants are extensively used in various consumer products and are frequently released into aquatic environments, raising significant ecological concerns. Due to their molecular structures, these compounds often exhibit high toxicity to aquatic organisms. In this study, *Chironomus riparius*, a widely accepted freshwater indicator species, was selected to evaluate the acute toxicity of three common surfactants—sodium lauryl ether sulfate (SLES), cocamidopropyl betaine (CAPB), and ammonium lauryl sulfate (ALS)—across different larval stages (1st to 4th instars).

Egg ropes were collected from culture tanks, and larvae were reared under controlled laboratory conditions. Acute 48-hour toxicity tests were performed using fish culture water as the medium. Stock solutions (100 mg/L) of each surfactant were serially diluted to 6.25, 12.5, 25, 50, and 100 mg/L, with 0 mg/L as the control. Five larvae were exposed to 20 mL of each concentration in 100 mL Duran beakers, with eight replicates. Swimming inhibition and mortality were recorded at 24 and 48 hours, and LC_{50} values with 95% confidence intervals were calculated using the EPA Probit Analysis Program (version 1.5).

To ensure the reliability of the test system, cadmium chloride was used as a positive control according to OECD TG 235/218. For 1st instar larvae, LC_{50} values were 36.27 mg/L at 24 hours and 6.19 mg/L at 48 hours, indicating a 5.9-fold increase in toxicity over time.

Results showed stage-dependent sensitivity: the 1st instar larvae exhibited the highest vulnerability, particularly to ALS, while the 4th instar showed the lowest sensitivity, especially to SLES. Abnormal behaviors and elevated mortality rates were observed at higher concentrations, and occasional cannibalistic behavior occurred in later stages, likely due to toxic stress and/or food limitation. These findings emphasize the necessity of considering developmental stages in ecotoxicological evaluations and environmental risk assessments.

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LP-77

Comprehensive In Silico Toxicological Assessment of Porcine Collagen-Derived Peptides: A Non-Animal Strategy to Predict Mycotoxin-Like Risks and Dermal Safety

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Background:
Bioactive peptides derived from porcine collagen are increasingly utilized in dermocosmetic applications due to their anti-aging and regenerative potentials. However, systematic toxicological evaluation remains scarce, particularly concerning metabolic activation pathways associated with mycotoxin-like toxicity. We present an integrated in silico workflow to assess ADMET properties, systemic toxicity, and mycotoxin metabolism potential of short-chain porcine peptides.

Methods:
Eighteen di- to hexapeptides were selected from porcine collagen enzymatic hydrolysates and subjected to computational screening using four complementary platforms:

- MetaTox for Phase I/II biotransformation and reactive metabolite prediction.
- SwissADME for physicochemical profiling, skin permeability (LogKp), and oral bioavailability.
- pkCSM for ADMET endpoints including mutagenicity (Ames), hERG inhibition, hepatotoxicity, and LD_{50}.
- PASS Online to evaluate broader toxicological endpoints and identify structural alerts mimicking aflatoxin or ochratoxin metabolism.

Results:
Fourteen peptides demonstrated favorable toxicokinetic profiles and low toxicological concern. MetaTox indicated minimal risk for bioactivation and no toxic intermediates. SwissADME predicted high aqueous solubility and

acceptable dermal penetration. PkCSM indicated no Ames mutagenicity, no hERG I/II inhibition, low hepatotoxicity potential, and LD_{50} > 2000 mg/kg (oral, rat). Lastly, PASS results showed probabilities of activity (Pa) < 0.3 for carcinogenicity, cytotoxicity, and mycotoxin-associated effects. No peptides showed metabolic fingerprints analogous to known fungal toxins, indicating negligible risk of biotransformation into aflatoxin- or ochratoxin-like intermediates.

Conclusion:

This study underscores the utility of an integrative in silico framework for peptide safety assessment, aligning with the 3Rs (Replacement, Reduction, and Refinement) principle and modern regulatory toxicology. The absence of genotoxic or mycotoxin-like metabolic liabilities supports the safe topical application of porcine collagen peptides. Further in vitro validation is warranted for translational and regulatory confirmation.

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LP-78

Evaluation of US EPA's TSCA Rule for the Occupational Use of Carbon Tetrachloride and Proposal for a Revised Occupational Exposure Value

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Carbon tetrachloride (CTC) is classified by the United States Environmental Protection Agency (US EPA) as "likely to be carcinogenic to humans," based on evidence of increased liver and adrenal tumors in mice. Under the Toxic Substances Control Act (TSCA), US EPA derived a chronic cancer occupational exposure value for CTC (referred to as an existing chemical exposure limit [ECEL]) of 0.03 ppm. However, US EPA's ECEL of 0.03 ppm is considerably lower than global occupational exposure limits (OELs) for CTC, and arguably not consistent with the best available science as mandated by TSCA, as it was not derived using methodology recommended in US EPA's own technical guidance or used in the agency's prior derivations of CTC toxicity reference values (*e.g.*, physiologically based pharmacokinetic [PBPK] and benchmark dose [BMD] modeling). To derive the liver cancer ECEL, US EPA applied the lowest observed adverse effect concentration (LOAEC)/no observed adverse effect concentration (NOAEC) approach, rather than PBPK and BMD modeling, and did not consider total liver tumors (*i.e.*, adenomas and carcinomas combined). In this analysis, using the total liver tumor data from the same study (Nagano *et al*., 2007) as US EPA, a revised cancer ECEL for CTC was derived by applying BMD and CTC-specific PBPK modeling and following US EPA's own technical guidance. CTC-specific mouse PBPK models were used to estimate internal liver CTC doses based on the mean rate of metabolism. BMD modeling of the internal doses was then used to derive $BMDL_{10}$ values, which were converted to human equivalent concentrations (HECs) using interspecies conversion factors derived from CTC-specific human PBPK models. A total uncertainty factor (UF) of 30 was applied to the resulting liver tumor point of departure (POD) to account for differences between animals and humans (UF_A of 3) and for sensitivity within human populations (UF_H of 10), with the subsequent value converted to account for human occupational exposure, resulting in a revised liver cancer ECEL for CTC of 1.5 ppm. Modified UFs were also considered, based on a healthy worker population (UF_H of 5) and an adjusted pharmacodynamic UF_A of 1.5. The revised ECEL for CTC is 50-fold higher than that derived by US EPA under TSCA and is more consistent with global OELs, including those in Canada (2 ppm) and the European Union (1 ppm), but still below the Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) of 10 ppm and the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) of 5 ppm. Overall, based on the best

available science for CTC, including CTC-specific PBPK and BMD modeling, the revised cancer ECEL is more feasible and achievable for industry and worker compliance. This analysis should be considered by US EPA as it seeks to implement the final CTC rule under TSCA.

LP-79

Neurobehavioral and Developmental Effects of Silica Nanoparticles in *C. elegans*: A High-Content Screening Approach

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The potential effects on development, reproduction, or neurobehaviour triggered by silica nanoparticles (SiO_2) on *Caenorhabditis elegans* (*C. elegans*) were investigated in this study. We used wild-type (N2) and neurodegenerative disease models—GMC101 (Alzheimer's disease) and NL5901 (Parkinson's disease). To systematically assess physiological and behavioural endpoints, we employed the high-content screening platform SydLab™ One (Nagi Bioscience SA, Switzerland), an automated microfluidic system that enables real-time imaging, behavioural tracking, and multi-parametric data acquisition under precisely controlled environmental conditions. Developmental delays and impaired reproductive capacity were observed in wild-type *C. elegans* continuously exposed to SiO_2 nanoparticles at concentrations ranging from 0.005 to 50 µg/mL, starting from the L1 larval stage and monitored over 128 hours. Significant toxicity was evident from 5 µg/mL, with growth rates declining from 3.01 ± 0.71 µm²/hour (control) to 2.22 ± 0.82 µm²/hour, and progeny accumulation decreasing from 4.41 ± 1.44 to 3.25 ± 1.45 progeny/hour. Neurobehavioral assays revealed significant impairments in locomotor function—measured by body bending frequency (Hz) and locomotion velocity (mm/sec)—in all three strains (N2, GMC101, and NL5901) following continuous exposure to 5 µg/mL SiO_2 for 11 days. Notably, survival rates remained unchanged across both wild-type and disease-model strains, indicating that the observed neurotoxicity was not accompanied by increased lethality. Collectively, our findings demonstrate that SiO_2 nanoparticles induce both developmental and neurobehavioral toxicity in *C. elegans*, supporting a generalized mechanism of neurotoxicity that does not selectively exacerbate phenotype in neurodegenerative disease models.

LP-81

Effect of ATF6 expression changes on MEG-01 cells differentiation through regulation of SNURF and EGR1 expression

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Environmental endoplasmic reticulum(ER) stress in SD rat bone marrow induced by intubation-mediated intratracheal instillation of polyhexamethylene guanidine phosphate suggested that ATF6 is a potential regulator of megakaryocyte(MK) maturation. To verify this, human MEG-01 cells were transiently engineered to over-express or silence ATF6, and their changes in molecular mechanisms were profiled by RNA-seq with Ingenuity Pathway Analysis. Over-expression of ATF6 drove a classical unfolded-protein-related response: ER chaperones, ER-associated degradation factors, BRCA1/ATM DNA-repair genes, NRF2 detoxification targets, AMPK- and sirtuin-linked autophagy, and nonsense-mediated mRNA decay were activated, whereas IL-15 production, MHC-I antigen presentation, Golgi remodelling pathways, and MITF-M-dependent α/δ-granule programs remained inactive. This pattern defines an early quality-control state that safeguards ER expansion and genomic integrity. In contrast, ATF6 knock-down provoked p21-mediated cell-cycle arrest and polyploidisation, strong activation of vesicle and Golgi

trafficking, cytoskeletal reorganisation, accelerated mRNA processing, induction of MITF-M granule genes, and enhanced IL-15 and MHC-I expression while suppressing canonical quality-control modules, marking a late execution phase that commits to proplatelet formation. Furthermore, transcriptomic analysis revealed that SNURF expression rose and fell in parallel with ATF6, while EGR1 displayed the opposite pattern, decreasing when ATF6 increased and increasing when ATF6 was down-regulated. Because the expression patterns of SNURF and EGR1 in MKs closely parallel the mechanistic shifts triggered by ATF6 modulation, our data indicate that ATF6 partly governs the maturation switch in this lineage through coordinated regulation of these two genes. In conclusion, High ATF6 activity preserves progenitors under proteostatic stress, whereas reduced activity unlocks the platelet-assembly machinery. Therapeutic control of this axis could therefore restore balanced MK maturation in states of both inadequate and excessive differentiation.

LP-82

Overexpression of Metallothionein 1B Promotes Cell Proliferation, Migration, and Invasion in A549 Lung Cancer Cells

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Metallothionein (MT) is a metal-binding protein that regulates metal homeostasis by directly binding intracellular metal ions such as zinc (Zn) and copper (Cu). It also binds to toxic heavy metals such as cadmium (Cd) and lead (Pb), thereby exerting a protective effect against cellular toxicity. In addition, MT plays an antioxidant role by neutralizing reactive oxygen species (ROS).

MT is classified into four major isoforms: MT1, MT2, MT3, and MT4. Among them, MT1 comprises nine subtypes. In this study, we observed that exposure of pulmonary epithelial cells to polyhexamethylene guanidine-phosphate (PHMG-p), a major component of humidifier disinfectants, led to a selective increase in the expression of four MT1 subtypes: MT1B, MT1F, MT1G, and MT1H[1]. However, the underlying mechanism by which only these four subtypes are upregulated in response to PHMG-p exposure remains unclear. It is also unknown whether this upregulation serves as a defensive response against cytotoxicity or if the abnormal increase in expression leads to cellular dysfunction.

Interestingly, previous studies have reported that the expression of MT1B, MT1F, MT1G, and MT1H is also elevated in tissue samples from patients with non-small cell lung cancer (NSCLC)[2]. Based on this, we hypothesized that PHMG-p-induced upregulation of these MT1 subtypes may contribute to cellular dysfunction and promote cell proliferation and tumorigenesis.

To test this hypothesis, we selectively overexpressed MT1B in A549 lung cancer cells, which resulted in a significant increase in cell viability and migration. Moreover, MT1B-overexpressing cells exhibited enhanced invasiveness and markedly increased colony-forming ability. Additionally, the expression levels of epithelial-mesenchymal transition (EMT)-related proteins, including Snail1, Vimentin, and N-cadherin, were elevated in MT1B-overexpressing cells. We also observed upregulation of metastasis-related proteins MMP-2 and MMP-9.

Collectively, these results suggest that the increased expression of MT1B enhances the proliferative and invasive capacities of lung cancer cells, potentially contributing to the malignant progression of lung cancer.

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LP-83

In Silico Off-Target Predictions in Drug Safety Assessment: Closing the loop for predicted CYP17 & CYP11B1/2 inhibition of a drug candidate

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Early drug development in the pharmaceutical industry is constantly evolving to increase the likelihood of success and reduce the time to bring a drug candidate to the market. Artificial intelligence (AI) and *in silico* prediction tools leveraging internal and external “big data” play an emerging role in early safety assessment.
Here, we report the *in silico* prediction and *in vitro* validation of a drug off-target that could explain the adrenal cortical hypertrophy observed for the drug candidate NVP001 in a 2-week dog toxicity study.
Using a Naïve Bayes multi-category model trained with chemical fingerprints as descriptors and biological targets as categories, CYP17A1 & CYP11B1/2 were identified as potential off-targets for NVP001. This compound notably displayed high chemical fingerprint similarities with an internal dual CYP17/CYP11B1/2 inhibitor (NVP002). Both compounds induced hypertrophy in the adrenal glands in non-rodent toxicity studies. Inhibition of those cytochrome P450 could impair adrenal corticosteroid synthesis and may explain the observed histopathology findings in that organ.
To investigate this hypothesis, we have established an enzymatic LC-MS *in vitro* inhibition assay using human recombinant CYP17A1 enzyme and the positive controls abiraterone (potent CYP 17 inhibitor, lower potency on CYP11B1) and NVP002 to benchmark potencies. In addition, we developed a similar inhibition assay with dog adrenal homogenates to assess potential effects on corticosteroid synthesis including the CYP11B1 and CYP11B2 (aldosterone) pathway. Our results confirmed off-target inhibition of human recombinant CYP17A1 *in vitro* by NVP001 with an IC50 in the mid to high micromolar range, whereas the two positive controls showed potencies at low nanomolar concentrations. In addition, the *in vitro* synthesis of the glucocorticoid cortisol was inhibited by NVP001 in dog adrenal homogenates with concomitant increases in its precursor 11-deoxycortisol suggesting additional inhibitory effects on CYP11B1. No clear inhibition of aldosterone synthesis was observed with NVP001 in rat adrenal homogenates.
In conclusion, *in silico* off-target predictions based on chemical structural similarity flagged an internal drug candidate for potential effects on adrenal steroidogenesis, which was confirmed by a tailored *in vitro* inhibition assay, providing a mechanistic explanation for the observed cortical hypertrophy in the *zona fasciculata* of the adrenals in a 2-week dog toxicity study.

LP-84

Methylglyoxal (MGO) Disrupts Mitochondrial Metabolism and Activates the P62-Nrf2-Keap1 Axis to Protect against Ferroptosis in HK-2 Cells via pentose phosphate pathway Suppression.

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In this study, we investigated the mode of action underlying the cellular responses towards methylglyoxal (MGO) in HK-2 renal tubular epithelial cells. U-^{13}C glucose metabolic flux analysis showed that MGO exposure significantly

redirected synthetic pathways of citrate and glycerol-3-phosphate, indicating mitochondrial pyruvate dehydrogenase (PDH) pathway inhibition in the TCA cycle and pentose phosphate pathway (PPP) promotion[1]. At the same time, MGO drives Nrf2 nuclear translocation and the sustained expression of downstream antioxidant genes (HO-1, ACSL4 and SLC7A11) by activating the P62-dependent Keap1-Nrf2 dissociation. In addition, MGO induced glutathione (GSH) depletion and aggravated lipid peroxidation, and also induced ferroptosis in HK-2 cells, which was manifested by ROS outbreak and destruction of mitochondrial crest structure[2]. These results reveal that MGO disrupts mitochondrial energy metabolism by inhibiting PDH activity, thereby blocking pyruvate entry into the TCA cycle and triggering metabolic reprogramming[3]. This disruption leads to a compensatory reliance on glutamine anaplerosis, which replenishes TCA intermediates (m+2 citrate, m+4 isocitrate) and transiently sustains cellular energy homeostasis. These findings position mitochondrial bioenergetics as the central target of MGO toxicity, with PPP-Nrf2 axis acting as a cellular-surviving strategy[4]. In conclusion, our research illustrated the MGO-induced mitochondrial dysfunction via metabolomic analysis, providing potential novel targets to intervene in MGO induced nephrotoxicity.

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LP-85

Safety Evaluation of Sodium L-Methylfolate (L-5-MTHF-Na) for Oral Dietary Use

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Sodium L-methylfolate (L-5-MTHF-Na; CAS No. 151533-22-1) was assessed for safety in support of its use as a dietary source of folate. Genotoxicity and repeated oral dose toxicity studies were conducted in accordance with Organisation for Economic and Cooperative Development (OECD) Test Guidelines (TG) and under Good Laboratory Practice (GLP). L-5-MTHF-Na demonstrated no mutagenic activity in the bacterial reverse mutation assay (OECD TG 471) and elicited no cytogenetic effects in both *in vitro* chromosome aberration (OECD TG 473) and micronucleus assays (OECD TG 487). In 14-day and 90-day oral gavage (OECD TG 408) studies in rats, doses up to 1000 mg/kg body weight/day produced no treatment-related adverse effects across clinical, physiological, ophthalmological, neurological, and histopathological endpoints. The no-observed-adverse-effect-level (NOAEL) was determined to be 1000 mg/kg bw/day—the highest dose tested—supporting the compound's safety for oral intake.

LP-86

The presence of pyrrolizidine alkaloids and their *N*-oxide in (herbal) teas, and plant food supplements made from non-PA producing plants, and related risks

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Pyrrolizidine alkaloids (PAs) and their *N*-oxide forms (PANOs) are natural toxins produced by plants as a defence mechanism against herbivores [1-3]. Exposure to such compounds raises a concern given their genotoxic and carcinogenic potential [4]. Humans are exposed to PAs/PANOs either directly by consuming food derived from PA-producing plants or indirectly through contamination due to co-harvesting of these PA-plants with food crops [4]. The latter is concerning from a risk assessment point of view since many studies reported high levels of PAs/PANOs in food products containing non-PA producing plants [4-9]. Therefore, the present study aimed to quantify the levels of 42 PAs/PANOs in 254 (herbal) teas and 26 plant food supplements (PFS) made from non-PA producing plants, and to perform a risk assessment using the margin of exposure (MOE) approach. The results show that about 36 % of the (herbal) teas and 46 % of PFS contained PAs/PANOs exceeding the maximum levels established by Regulation (EU) 2023/915 [10]. The MOE values based on lifetime consumption revealed that use of about 16% of the (herbal) teas and 31% of the PFS would raise a health concern. In the risk assessment, less than lifetime (LTL) exposure scenarios were also considered by using Haber's rule and the threshold of toxicological concern (TTC) for LTL exposure assessment. Overall, the results indicate a priority for risk management.

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LP-87

Comparative Mutagenic Analysis of Chemically and Green Synthesised Graphene Quantum Dots using Mini-Ames Test

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Graphene quantum dots (GQDs) exhibit unique electronic, optical, and chemical properties that make them promise for various applications, including bioimaging, drug delivery, and environmental sensing. GQDs are expected to be nontoxic because of their inert and biocompatible carbon-based makeup resulting unfavorable biological interactions. This is the first report on the green synthesized GQDs using Aloe barbadensis (Aloe Vera) which exhibit enhanced quantum yield, stability, functional capabilities as compared to chemically synthesized. Scanning electron microscopic analysis confirmed the GQDs in 50 – 80 nm range.

The mini-AMES test was employed to assess mutagenic potential using Salmonella typhimurium TA98, TA100, TS1535, TA1537 and E. coli WP2 pKM101 strains. The strains were exposed at 0.5 to 16 µg/mL concertation range in presence and absence of metabolic activation for 90 min followed by 48 h incubation. Indication of a colour changes from purple to yellow were considered as positive mutagenic response.

Mutagenic potential was detected in TA1535 in absence of metabolic activation treated with chemically synthesized GQDs at the concentrations of 2 and 4 µg/mL while, green synthesized GQDs, mutagenicity was detected at the concentrations of 1 and 2 µg/mL in TA1537 in presence of metabolic activation system.

The results indicate mutagenic potential in both type of GQDs with possible mechanism of oxidative damage and DNA adduct. Mutagenic molecules have a potential towards carcinogenicity, hence thorough risk assessment by conducting follow-on in vitro and in vivo mutagenicity assays such as definitive Ames and Micronucleus tests.

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LP-88

Neuronal *in vitro* model for ferroptosis research

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Ferroptosis is a type of cell death triggered by iron-dependent formation of reactive oxygen species (ROS), culminating in lipid peroxidation and membrane damage. In the present study, LUHMES cells, as a model for human nigrostriatal dopaminergic neurons, were employed to investigate factors influencing ferroptosis activation and prevention. Differentiated LUHMES cells exhibited high sensitivity towards ferroptosis induction by Erastin and RSL-3, with no evidence of quantitative apoptotic cell death. The cells could be rescued from ferroptotic cell death e.g. by lipoxygenase inhibition, the presence of coenzyme Q_{10}, or ebselen.In the context of ferroptosis, special attention is paid on the role of the so-called "labile iron pool," which is largely responsible for the formation of hydroxyl radicals. Experimental modulation of iron in the LUHMES model revealed an inverse correlation between intracellular iron levels on the one hand and lipid peroxidation and ferroptotic susceptibility on the other. Reduction of intracellular iron, by iron chelation or limited iron supply in the culture medium, conferred complete protection against ferroptosis. We optimized methods for the quantification of both labile and bound iron. Experimental increase in iron supply leads to an increase in the intracellular labile iron pool without affecting basal cell viability or mitochondrial activity. However, elevation of labile iron resulted in a significantly increased susceptibility towards ferroptosis activation by Erastin or RSL-3. It was further observed that factors such as medium changes and the use of different media supplements (e.g., N2 or serum) can have a significant impact on intracellular iron levels and, consequently, on the sensitivity to ferroptosis induction. These findings underscore the necessity for systematically characterized and standardized protocols in the field of *in vitro* ferroptosis research to improve reproducibility and comparability between studies.

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LP-89

Messenger RNA Vaccine Drive Granulocytic Skewing of Hematopoiesis and Inhibit Erythropoiesis

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Purpose

Hematopoiesis is tightly regulated by intrinsic and extrinsic pathways to maintain blood cell homeostasis. In recent years, several clinical reports have described hematological abnormalities, including thrombocytopenia and anemia, in individuals receiving SARS-CoV-2 mRNA vaccines. However, the underlying mechanisms remain poorly understood.

Methods

Four to five-year-old female cynomolgus macaques and seven-week-old ICR mice were used. A SARS-CoV-2 Omicron variant S protein coding nucleoside-modified mRNA vaccine candidate was injected intramuscularly 100

(Mice) or 800 µg (Monkeys)/animal and hematological changes were evaluated by complete blood count, H&E stain, bulk RNA sequencing, and single cell RNA sequencing.

Results

Here, we demonstrated that intramuscular administration of mRNA vaccines, encoding either SARS-CoV-2 spike protein or luciferase, significantly reduces circulating reticulocyte levels in mice and monkeys. Bone marrow histological analysis in both mice and monkeys revealed myeloid expansion and erythroid suppression, resulting in an elevated myeloid-to-erythroid (M/E) ratio. Furthermore, mRNA vaccination markedly upregulated pro-inflammatory genes, including type I interferon (IFN) in the bone marrow. Notably, injection of lipid nanoparticle (LNP) alone was insufficient to induce this phenotype, implicating the LNP-mediated delivery of synthetic mRNA as the key driver of hematopoietic disorder.

Single-cell RNA sequencing coupled with pseudotime trajectory analysis showed that mRNA vaccination biases hematopoietic differentiation toward granulopoiesis while repressing erythropoiesis. This effect was accompanied by robust type I IFN signaling across hematopoietic stem and progenitor cell populations, including self-renewing HSCs. We demonstrated that administering a type I interferon receptor (IFNAR)-blocking antibody mitigates the mRNA vaccine–induced hematopoietic disorder in mice, thereby providing direct evidence that type I IFN signaling triggered by mRNA vaccination drives the skewing of bone marrow hematopoiesis.

Conclusion

These findings highlight a previously unrecognized impact of mRNA vaccines on hematopoietic lineage dynamics and underscore the need for further studies to assess long-term effects on stem cell function, particularly in individuals with pre-existing hematologic conditions. Future mRNA-based therapeutics should be considered to evaluate for bone marrow tropism and innate immune activation to ensure safety.

LP-90

The use of biopolymers as encapsulation material for bioactive compounds: safety considerations

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Biopolymers, due to their ability to allow the incorporation of antibacterial compounds, but also the possibility of encapsulating bioactive substances, such as probiotics, have promoted their use in recent times [1]. Biopolymer formulations can be optimized to promote targeted and sustained release of the compound they contain. Thus, formulations can be developed to pass the gastric passage and to be solubilized in the intestine part [2]. Studies have shown that coating composition can be tailored in order to allow the release of the drug in the areas of interest [3]. It has been demonstrated that edible biopolymers, such as sodium alginate and starch, can be consumed in *quantum statis* doses [4]. For the present study, sodium alginate, starch and water were used as encapsulation agents for *Lactobacillus clausii*. The capsules were stored under refrigerated conditions for further testing. To identify their solubilization capacity, simulated gastrointestinal fluids were used. According to the results obtained, the capsules were protected in gastric fluids when they were kept for 1 hour. The microscopic images observed showed that the capsule wall did not undergo any changes. Also, the mass and diameter were reduced by max 5%. Once immersed in intestinal fluids, they completely solubilized within 4 h. To test the microbiological safety of use, the capsules were solubilized in sterile solution and vigorously homogenized for 10 min. 1 ml of the solution thus obtained was poured onto plates with dehydrated culture medium, specific for *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, coliforms, yeasts and molds. None of the pathogen strains grew on the specific media. To highlight the presence of other types of microorganisms that could have been developed both inside the capsules and on the surface, their contents were inoculated onto appropriate culture media (MRS and nutrient agar), subsequently observing the types of microbial colonies that developed. For this purpose, the capsules were subjected to microbiological testing at time 0, as well as at intervals of 7 and 14 days after obtaining, after storing them under controlled refrigeration conditions. Analysis of culture media did not reveal the presence of other contaminating microorganisms at baseline (day 0) or after 7 days of refrigerated storage. However, after 14 days, colonies

corresponding to different strains of lactobacilli were observed, suggesting possible variations in the microbiological composition of the capsules over time. The experimental results support the safety of using the investigated materials for encapsulating compounds with biological activity. Future research directions aim at in vitro testing on cell lines, as well as a detailed characterization of the composition of the composite materials.

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LP-91

Evaluation of Fingerprints and Similarity Metrics Suitable for Read-Across of NOAEL in Repeated-Dose Toxicity.

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The category approach is a promising in silico method for evaluating repeated-dose toxicity of chemical substances lacking toxicological data. This approach infers the toxicity of a target compound by referencing toxicological information from structural and physicochemical properties similar source compounds. While alternative strategies may be applicable when toxicological mechanisms are well understood, the initial step in the category approach typically involves identifying structural analogs from toxicity databases. Structural similarity is often assessed using chemical fingerprints and similarity metrics. However, the selection of fingerprints and metrics is seems generally empirical and case-by-case, and their suitability for read-across of NOAEL in repeated-dose toxicity has not been systematically evaluated.

In this study, we conducted a comprehensive analysis using two commonly employed fingerprints (MACCS and Morgan) and two similarity metrics (Dice and Tanimoto) to determine the optimal approach for identifying category members relevant to select analog compounds for read-across of repeated-dose toxicity. Toxicity data of 90-day rat studies from 354 pesticides, as published by the Food Safety Commission of Japan, were used for analysis. Chemical structures were retrieved in SMILES format from PubChem and processed using RDKit to compute two fingerprints and calculated pairwise Dice and Tanimoto coefficients. The absolute differences in log-transformed NOAEL values were calculated to quantify toxicological divergence, and their distributions were analyzed in relation to similarity scores.

Furthermore, we examined the effectiveness of read-across predictions based on the similar compounds identified using each fingerprint and metric combination. Our results revealed that MACCS fingerprints tend to yield higher similarity scores overall, but also exhibit larger discrepancies in NOAEL values between target and source

compounds. In contrast, Morgan fingerprints produced lower similarity scores yet identified source compounds with NOAEL values more closely aligned to those of the target compounds.
In conclusion, our findings suggest that structural analog identification using Morgan fingerprints and Dice coefficients offers a more reliable first step for NOAEL-based read-across in repeated-dose toxicity assessment.

LP-92

Automated high throughput inflammatory disease modelling on synovial spheroids as a new approach methodology

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Synovial inflammation drives joint destruction in arthritis, yet current in vitro models lack the physiological complexity needed for reliable drug screening. Traditional 2D cultures fail to recapitulate the cellular interactions and inflammatory cascades in diseased synovial tissue. Meanwhile, animal models present ethical concerns and poor translation to human pathophysiology. Synovial spheroids from patient-derived cells offer a promising middle path, capturing tissue-like architecture while remaining amenable to high-throughput screening. However, manual spheroid generation suffers from variability and low throughput, limiting their application in pharmaceutical research.
We leveraged our MO:BOT platform to automate spheroid culture with precision liquid handling and complementary automated brightfield image acquisition. Here we demonstrate its application for synovial spheroid generation and inflammatory disease modelling, using both patient-derived synovial cells from a healthy adult donor and established fibroblast-like synoviocyte lines.
Cells were seeded at 500-1000 cells per well in 96-well U-bottom low-attachment plates to generate uniform spheroids. Automated seeding showed superior consistency in size and morphology compared to manual preparation. Critically, we identified a 2-6 day optimal window for drug screening, beyond which spheroids decreased in viability.
For disease modeling, we stimulated spheroids with TNF-α (10 ng/mL) to trigger inflammatory responses, measuring IL-6 secretion via ELISA and morphological analysis via brightfield imaging. Anti-inflammatory drug screening included concentration gradients, testing corticosteroids, methotrexate, and ibuprofen. Automated liquid handling ensured precise dosing while automated imaging tracked spheroid integrity throughout treatment and handling steps (e.g. media exchange).
Results show a complete and automated workflow from spheroid formation through inflammatory stimulation and drug response assessment, yielding a high-density data set to track spheroid integrity and morphology with high temporal resolution. Patient-derived spheroids recapitulated key inflammatory signatures while maintaining reproducible drug responses across replicates. The optimal screening window proved effective for capturing acute inflammatory responses before spheroid degradation.

This work demonstrates automated synovial spheroid culture as a promising New Approach Methodology (NAM) for inflammatory disease research, aligning with 3R principles while providing clinically relevant human tissue models. The platform enables standardized, high-throughput screening of anti-inflammatory compounds using patient-specific cells, offering a bridge between simplified 2D cultures and complex animal models. Future applications include personalized medicine approaches and mechanistic studies of joint inflammation using an automated, reproducible system.

LP-93

The Critical Role of Expert Review in the *In Silico* Qualification of Impurities in Medicinal Products

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In silico toxicological models are increasingly used to support the qualification of impurities in medicinal products, particularly when levels exceed ICH Q3B thresholds and experimental data are lacking. The recent *Draft Reflection paper on the qualification of non-mutagenic impurities* recognises the role of such models as part of alternative approaches to identify and evaluate toxicophores and to support impurity qualification when no safety concerns are identified [1]. While computational tools can identify structural alerts and predict potential toxicities, their outputs often require expert interpretation to ensure regulatory relevance and scientific robustness. The need for expert judgment is a recurring theme in guidance across regulatory frameworks [1-5], particularly in relation to model evaluation, domain applicability, and mechanistic interpretation. This poster presents three real-case examples illustrating the significance of expert review in *in silico* impurity assessment. The examples highlight distinct scenarios: (1) the dismissal of a model-generated alert based on toxicological irrelevance, leading to a negative prediction; (2) the reinterpretation of a negative model output as a potential positive concern, based on the known mechanism of action of the parent compound; and (3) the evaluation and justified dismissal of a prediction for an out-of-domain structure. These case studies demonstrate that expert input remains essential to interpret *in silico* findings, mitigate the risk of false conclusions, and ensure compliance with current regulatory expectations.

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LP-94

Creating a validation dataset for mechanisms of drug-induced toxicity

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Toxicity remains one of the primary causes of drug failure in the drug discovery pipeline [1-2]. While individual relationships between drugs, targets, and adverse effects are captured in various databases, there is no comprehensive resource that traces the full mechanistic path linking a drug to a toxic outcome [3]. This fragmentation limits our ability to model and understand the underlying mechanisms of drug-induced toxicity. To address this

problem, we present ToxMech, a tool designed to extract, integrate, and structure mechanistic toxicity information into a graph-based format of nodes and edges following the BioLink Model standard [4].
ToxMech aggregates data from multiple sources, including scientific literature, abstracts, public repositories, FDA black box warning labels, and curated Adverse Outcome Pathways (AOPs) [5]. To supplement these structured datasets, we also deploy deep research agents capable of capturing relevant information from unstructured sources such as clinical news and online articles. This combined dataset can be used to train and validate knowledge graph-based path-learning methods such as reinforcement learning, and to improve the performance of machine learning models in toxicology. By integrating information from resources like PubMed, AOP-Wiki, and the FDA Black Box dataset, ToxMech enables researchers to understand the mechanistic paths linking drug exposure to toxic outcomes.

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LP-95

Cytotoxic profile of indoor dust particles: acute, long-term repeated exposure and breathing-induced effects.

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Humans are daily exposed to a variety of chemicals that accumulate in indoor dust (ID). Lung *in vitro* models offer the opportunity to study the effects of inhaled ID. However, most of them fail to address the influence of breathing motion on the observed effects. In this work, we study the effects of ID on the AX12C iAlv lung-on-chip (LOC) model with breathing motion ability, following acute and long-term repeated exposure.
The AX12C iAlv model is composed of primary cell-derived immortalized alveolar epithelial cells (^{AX}iAECs) pre-seeded on the AX12, by AlveoliX. The LoCs were shipped to INL and acclimatized for 10 days at 37ºC, exchanging the media every 2-3 days. The exposure to 2 different ID samples (NIST SRM 2583 and SRM 2585) was tested under acute and 7-day long-term exposure in submerged conditions. For acute exposure tests, cells were exposed to 5 and 33 µg/cm^2 and incubated for 24 h in static conditions or under three-dimensional cyclic stretch (^{AX}Actuator), which generates negative cyclic pressure, mimicking the human breathing motion. A repeated 5-time cumulative exposure scenario, reaching 5 µg/cm^2 by day 4 and inquired after 72 h, was compared with an acute 72 h exposure to the same concentration of ID. We studied effects on transepithelial electrical resistance (TEER), cellular metabolic

activity (PrestoBlue), paracellular transport of Lucifer Yellow (LY), and lactate dehydrogenase (LDH), as well as immunostaining and RT-qPCR of relevant cytotoxicity markers.
The results show that cyclic stretching may influence the cellular response to ID. Compared to the static model, cyclic stretching induced a higher cytotoxic profile, particularly for SRM 2583 at the highest concentration. This was evidenced by a decrease in TEER and increased permeability (LY), suggesting a compromised barrier integrity. Additionally, distinct cytotoxic profiles between acute and long-term repeated exposure were also observed. Interestingly, repeated exposure resulted in a decreased cytotoxic profile compared to the acute scenario, which exhibited a decreased TEER and increased permeability at 72 h. Immunostaining, RT-qPCR and LDH quantification are still ongoing. Further studies will investigate the effect of cyclic stretching on long-term repeated exposure, as well as exposures at the air-liquid interface.
These findings highlight the importance of incorporating mechanical cues, such as breathing motion, to *in vitro* models. Overall, the AX12C iAlv model can be a powerful tool to assess health-related effects of ID under breathing-like conditions.

LP-96

Optimised iPSC-derived hepatocytes demonstrate functional Phase I and Phase II drug metabolism offering a novel platform for DILI screening studies.

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Background And Purpose: Drug-induced liver injury (DILI) is one of the leading causes of acute liver failure and a major reason for drug development failures. Current *in vitro* liver models, including primary human hepatocytes (PHH) and hepatocellular carcinoma cells, come with limitations, including compromised liver function, uncontrolled donor variability, and short cell life-span. Previous efforts to differentiate induced pluripotent stem cells (iPSCs) to hepatocytes have also led to the generation of cells with incomplete hepatocyte features and absence of drug metabolizing pathways. We have hypothesized that modification of iPSC differentiation protocols can lead to the generation of mature hepatocytes with improved metabolic function offering a sustainable *in vitro* platform for large-scale DILI screening studies.
Methods: Wild-type iPSCs were differentiated to hepatocytes using either conventional and Pixl Bio's optimised (pixHEP) differentiation protocols and differences in cell function were investigated by RNA-sequencing. Functionality characterisation was subsequently assessed, including liver maturity marker expression by immunocytochemistry and ELISA, urea synthesis by western blotting and secretion assays, P450 expression and activity by qPCR and luciferase assays, and Phase I and II metabolism by liquid chromatography-mass spectrometry (LC/MS). PHH and HepG2 cells were used as controls. Suitability of pixHEP to predict DILI was evaluated by cell viability following 48h treatment with 7 drugs of known DILI liability.

Results: pixHEP demonstrated a significantly different transcriptome profile compared to conventional iPSC-derived hepatocytes as shown by PCA plotting, differential gene expression, and pathway enrichment analysis. pixHEP, PHH, and HepG2 cells expressed similar levels of ALB, A1AT, and HNF4A, with pixHEP secreting higher levels of albumin compared to PHH. Urea-synthesizing and Phase I metabolic gene expression were higher in pixHEP compared to conventional iPSC-derived hepatocytes and HepG2 cells, consistent with high urea secretion levels and basal CYP3A4 activity, respectively. pixHEP treatment with the CYP3A4 inducer 1α,25-hydroxy-vitamin D3 resulted in comparable CYP3A4 activity increases to those seen in PHH, confirming successful CYP3A4 induction. Importantly, pixHEP treatment with midazolam, diclofenac, amodiaquine, phenacetin, and dextromethorphan resulted in the formation of their respective metabolites in a dose-dependent manner, demonstrating functional Phase

I and II metabolism. Finally, cell viability assessment across increasing concentrations of 7 DILI-related showed that pixHEP accurately predict drugs of high-, medium-, and low-DILI concern.
Conclusion: pixHEP is a superior hepatocyte model with liver functionality comparable to PHH. This data alongside the expansion capacity of pixHEP showcase the opportunities this model can offer in large-scale DILI screening.

LP-97

Integrating TK modeling and IVIVE to address systemic exposure challenges in genotoxicity testing of complex food mixtures

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Background: Risk Assessments of complex chemical mixtures in food present significant challenges, primarily due to the frequent lack of whole mixture data. Within the EU, genotoxicity data play a pivotal role in EFSA's safety assessments, especially with recent changes in guidance documents. A key concern in in vivo genotoxicity studies of mixtures is whether the administered dose is sufficient to elicit a genotoxic response from individual constituents commonly referred to as the "dilution effect." In a recent evaluation of three smoke flavourings, EFSA deemed the in vivo micronucleus (MN) assay in bone marrow "reliable with restrictions," due to the absence of unequivocal evidence of bone marrow exposure to all potentially genotoxic components. It is theoretically possible to confirm systemic exposure of each constituent in the mixture to the bone marrow, yet impractical for a complex mixture with a portion of unidentified constituents, such as smoke flavorings. **Purpose:**To support the future integration of new approach methodologies (NAMs) into regulatory decision making, an in vitro to in vivo extrapolation (IVIVE) was conducted to enhance confidence in the results of an in vivo genotoxicity assay. This work provides justification to EFSA that doses used in the in vivo MN assay for one of the three smoke flavorings (i.e., SF-002) evaluated was sufficiently high to detect a potential genotoxic response from the mixture's constituents despite no genotoxic response occurring in the in vivo MN assay. **Methods:** EFSA's open access toxicokinetic and toxicodynamic modeling platform, TKPlate, was utilized to perform reverse dosimetry. External exposure estimates were derived from concentrations used in a positive in vitro MN assay for SF-002. Plasma data for a biomarker of exposure (2,6-dimethoxyphenol) from the in vivo study were used to validate TKPlate application. Reverse dosimetry was then performed for two additional constituents (catechol and 2,5(H)-furanone) in the mixture outlined by EFSA to be known in vivo genotoxicants. **Results:**Using positive in vitro assay exposure concentrations, TKPlate estimated external exposures (i.e., equivalent doses that would be delivered in an in vivo assay) for 2,6-DMP, catechol and 2,5(H)-furanone. These estimated external exposures were lower than the actual exposures of these constituents in the in vivo MN study that was negative. **Conclusion:** Thus, the doses used in the in vivo MN study were sufficient and valid to elicit a genotoxic response if one were present, but none was observed. The application of NAMs such as TKPlate modeling, can enhance confidence in interpreting genotoxicity study results by supporting systemic exposure assessments especially when direct confirmation of exposure to all mixture constituents is not feasible. This approach strengthens the reliability of in vivo data in the risk assessment performed by EFSA for smoke flavorings and can be applied to other complex chemical mixtures.

LP-98

Microfluidic tools for droplet generation and perfusion cell culture

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Purpose: There is an urgent need for improved in vitro models to test the effect of toxins, pollutants and molecules on humans and the environment, including drugs, nanoparticles, viruses and pesticides. The combination of microfluidics and cellular models, known as microphysiological systems (MPS), is a developing technology that brings the ability to better control environmental parameters on the microscale. Microfluidics adds flow and mechanical stimuli to models, as well as high precision and options for automation to reduce manual handling steps and improve reproducibility. This technology can be used for applications ranging from droplet-based encapsulation to cell culture and screening. Currently, two outstanding needs for microfluidic models are tailored instrument modules to control the flow rate of liquids of different viscosities with high accuracy, and the need for chip fabrication methods to replace the use of polydimethylsiloxane (PDMS) for rapid prototyping of designs.

Methods: Here we present the design and testing of a microfluidic setup for droplet- and cell-based measurements. It features the development of a novel flow rate sensor (Galileo), and microfluidic chips made in-house from the thermoplastic, polymethyl methacrylate (PMMA). Here, microfluidic circuits were assembled with a pressure-driven flow controller and inline Galileo flow sensor. Commercially sourced or PMMA chips produced in-house were connected to the flow system and tested for compatibility for precise control of water-in-oil droplet production, and for perfusion culture of U-251 MG cells.

Results: Here, we demonstrate the technical performance of our new Galileo flow sensor to within 5% accuracy with a variety of viscous liquids at flow rates from 0.5-50 µL/min. The setup was then used to benchmark the suitability of PMMA chips of cross-junction and straight channel designs, made with a straightforward hot embossing protocol, in the domains of droplet generation and perfusion cell culture. Galileo flow rate sensors provided real-time flow measurement and/or control of hexadecane (oil) and water phases for stable droplet generation. Separately, the fluidic setup successfully controlled the stable flow rate of medium over cells in culture at 1 µl/min over 24 h.

Outlook: The performance of the Galileo sensor and PMMA chips with a range of viscous liquids opens avenues for controlling particles in suspension, emulsion formation and automating cell perfusion, for more physiologically relevant microenvironments.

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LP-99

AI-Powered Cell Painting in iPSC-Derived Hepatocytes for Liver Toxicity Profiling

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Background And Purpose: Drug-induced liver injury (DILI) remains a major challenge in drug safety. Conventional liver cell lines like HepG2 lack key hepatocyte traits. In contrast, induced pluripotent stem cell (iPSC)-derived hepatocytes (pixHEP) more closely resemble primary human hepatocyte function in metabolic, transcriptomic, and morphological features. At the same time, standard DILI-related endpoint assays (e.g., cell viability) often miss sub-lethal changes. Cell Painting (CP), a multiplexed assay staining several organelles, can detect morphological alterations linked to DILI risk and subtype. Advances in AI-based image analysis enable scalable phenotypic profiling. This study integrates pixHEP, CP, and AI-driven analysis for liver toxicity assessment.
Methods: Wild-type iPSCs were differentiated towards pixHEP using a three-step direct differentiation protocol. Upon differentiation, cells were matured for 14 days in 384-well plates and treated with 20 compounds, classified across all four DILIRank categories (Most, Less, No, and Ambiguous-DILI-concern), at 2.5 µM and 10 µM for 48 hours. A fully automated CP protocol captured 9 fields per well across five channels. Due to segmentation challenges from cell overlap, DINOv2, an AI foundation model for cross-domain image feature extraction, was used for per-channel feature extraction.
Results: DINOv2 extracted distinct morphological features per channel. Effective rank analysis (Gavish–Donoho) showed 30–33% of features per channel were informative. Canonical correlation analysis found that joint channel variance exceeded permutations by 6–16%, though most variance per channel remained unique. Cell counts were unchanged across treatments, indicative of sub-lethal doses. Similarly, most compounds failed to elicit pronounced morphological alterations. However, treatment with the alkaloid drug Colchicine triggered significant ($p<0.05$) cellular clustering.
Conclusion: Colchicine, labeled "Ambiguous-DILI-concern", caused pronounced morphological shifts without affecting cell count, illustrating the sensitivity of this approach. The phenotype likely reflects cytoskeletal stress, potentially impairing bile acid transport or vesicular repair. The low inter-channel shared variance and consistent channel contributions imply that DINOv2 captures non-redundant, complementary information. This supports analyzing all CP channels without early dimensionality reduction. Most tested compounds elicited no significant changes in either cell viability or morphology under current conditions. Increasing concentration and treatment duration may improve the assay's sensitivity. Ongoing optimization efforts will aim to expand the range of compounds that elicit measurable responses. These findings highlight the utility of combining pixHEP, CP, and AI-driven image analysis for DILI risk detection.

LP-100

Innovative Approaches in Future Colorectal Cancer Management

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The pursuit of novel anticancer agents with high selectivity and minimal toxicity to normal cells remains a cornerstone of toxicological research. Quinazolinone and hydrazone scaffolds are recognized for their pharmacological versatility, particularly in oncology. In this context, original N-acyl-hydrazones derivatives were synthesized and evaluated for their cytotoxic potential against human cancer cell lines and non-tumoral cells.
The novel compounds were synthesized via microwave-assisted condensation using substituted salicylaldehydes and benzaldehydes. Cytotoxicity was assessed using the MTT assay on HT-29 (human colorectal adenocarcinoma), A431 (human epidermoid carcinoma), and VERO (normal kidney epithelial) cell lines. Cells were exposed to increasing concentrations (3.125–50 μM) of each compound for 24 hours. Cell viability was quantified spectrophotometrically and expressed relative to untreated controls.

Several tested compounds demonstrated significant, concentration-dependent cytotoxicity against both HT-29 and A431 cancer cell lines. Among them, one compound reduced HT-29 viability to approximately 50% at 50 μM, establishing its IC_{50}. Another exhibited potent cytotoxicity across both cancer lines, particularly at higher concentrations, while also increasing VERO cell viability at lower doses, suggesting a degree of selectivity. A third compound showed consistent inhibitory effects across all concentrations tested, though the IC_{50} threshold was not reached. A fourth compound demonstrated robust cytotoxic activity, with notable reductions in cancer cell viability across all concentrations. In contrast, one compound induced only weak cytotoxicity in cancer cells but was markedly toxic to VERO cells at concentrations ≥12.5 μM, raising safety and selectivity concerns. Two additional compounds did not display any cytotoxic effects; instead, they slightly enhanced viability in all tested cell lines, indicating a non-toxic and potentially proliferative profile. Overall, two compounds emerged as particularly promising candidates due to their selective anticancer activity and minimal toxicity toward normal cells. To further explore their translational potential, we plan to expand our investigations to ex vivo models using primary cell cultures derived from human tissue biopsies, with a focus on colon epithelium.
This work was supported by a grant of the Romanian Ministry of Education and Research, CCCDI-UEFISCDI, project number: PN-IV-P7-7.1-PTE-2024-0715, within PNCDI III (project no. 50PTE/2024).

LP-101

Stepwise Evaluation of 4,4′-Bis(diethylamino)benzophenone-Induced Liver Fibrosis Using *In Silico*, *In Vitro*, and *In Vivo* Models

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Benzophenones (BPs) are widely used as photoinitiators for printing inks in food contact materials, although the toxicological data are limited for some BPs. Some studies showed that a variety of unevaluated BPs were readily detectable in human specimens. Utilizing *in silico* models, including DILI Predictor and GenRA, we predicted that four BPs might be hepatotoxic. Then we further evaluated cytotoxicity of four BPs in mouse macrophage Raw 264.7 and hepatocyte AML12. Among these BPs, only treatment with 1 μg/ml 4,4′-bis(diethylamino)benzophenone (DEAB) was cytotoxic and induced the expression of proinflammatory cytokines, including interleukin-6 (IL-6), chemokine (CC motif) ligand 5, tumor necrosis factor-alpha, and transforming growth factor beta 1. In a 28-day oral exposure mouse model, administration of 50, 100 and 200 mg/kg/day DEAB caused hepatocyte inflammation, Kupffer cells/macrophage activation, an increase in IL-6 protein, hepatic stellate cell activation and early-stage liver fibrosis, along with collagen deposition. Utilizing transcriptomic and mechanistic pathway analyses for the livers of DEAB-treated mice, we found that DEAB downregulated the expression of fibrosis-related genes such as *CDKN1A* and *CBS*, while upregulating *ANGPTL8* mRNA levels in mouse liver tissues. Based on these findings, we concluded that oral administration of DEAB initiated liver injury and inflammation, activated hepatic stellate cells, and subsequently induced liver fibrosis. Our results highlight the successful application of alternative approaches in prioritizing chemicals of concern for safety assessment.

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LP-102

Practical implementation and impact of virtual control groups in nonclinical studies

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Benzophenones (BPs) are widely used as photoinitiators for printing inks in food contact materials, although the toxicological data are limited for some BPs. Some studies showed that a variety of unevaluated BPs were readily detectable in human specimens. Utilizing *in silico* models, including DILI Predictor and GenRA, we predicted that four BPs might be hepatotoxic. Then we further evaluated cytotoxicity of four BPs in mouse macrophage Raw 264.7 and hepatocyte AML12. Among these BPs, only treatment with 1 μg/ml 4,4′-bis(diethylamino)benzophenone (DEAB) was cytotoxic and induced the expression of proinflammatory cytokines, including interleukin-6 (IL-6), chemokine (CC motif) ligand 5, tumor necrosis factor-alpha, and transforming growth factor beta 1. In a 28-day oral exposure mouse model, administration of 50, 100 and 200 mg/kg/day DEAB caused hepatocyte inflammation, Kupffer cells/macrophage activation, an increase in IL-6 protein, hepatic stellate cell activation and early-stage liver fibrosis,

along with collagen deposition. Utilizing transcriptomic and mechanistic pathway analyses for the livers of DEAB-treated mice, we found that DEAB downregulated the expression of fibrosis-related genes such as *CDKN1A* and *CBS*, while upregulating *ANGPTL8* mRNA levels in mouse liver tissues. Based on these findings, we concluded that oral administration of DEAB initiated liver injury and inflammation, activated hepatic stellate cells, and subsequently induced liver fibrosis. Our results highlight the successful application of alternative approaches in prioritizing chemicals of concern for safety assessment.

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