



AI-Enabled Toxicology: Opportunities, Challenges, and Best Practices

38th Annual Fall Symposium: Southern California Society of Toxicology Regional Meeting

Ionis Conference Center, Carlsbad, CA
October 7, 2026



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Morning Program

Time	Speaker / Credentials	Title / Activity	Duration
8:00–8:55 am	—	Breakfast & On-site Registration	55 min
9:00–9:15 am	Christine Hoffmaster, BS, DABT — President, SCCSOT	Presidential Address	15 min
9:15–9:45 am	Kris Thayer, PhD — Cal EPA/OEHHA	AI in Chemical Hazard Assessment: Opportunities and Challenges	30 min
9:45–10:10 am	Steve Albanese, PhD — Research Leader, Computational Chemistry, Schrödinger Therapeutics	Embracing a new era of toxicity screening: Atomic-resolution modeling to mitigate off-target liabilities	25 min
10:10–10:25 am	Aneesh Acharya, PhD — Chief Operating Officer, Molecular Instruments	Better Models Begin with Better Images	15 min
10:25–10:50 am	Coffee Break • Poster Set Up		25 min

Time	Speaker / Credentials	Title / Activity	Duration
10:50–11:05 am	Jonathan Wu — Undergraduate Student, Western University of Health Sciences	β2-Adrenergic Receptor Agonists-Induced Pro-Inflammatory Genes in Macrophages and the Effects of Carvedilol and R-carvedilol	15 min
11:05–11:30 am	Yen Wei Chen, PhD — Senior Scientist, Computational Toxicology, Preclinical Sciences and Translational Safety, J&J	Two Translational Roads, One AI Compass: Navigating In Vitro to In Vivo and Cross-Species Translatability by ToxAgent	25 min
11:30–11:45 am	Maia Green, PhD — Vice President, Toxicology, Certara	Beyond the Buzz: What’s Ready and What’s Next for AI-Enabled Toxicology	15 min
11:45 am–1:00 pm	Lunch Break		75 min
12:00–1:00 pm	Mentoring		60 min
1:00–2:30 pm	Poster Presentations & Judging (Scozzari meeting room, west side of courtyard)		90 min

Mentoring: 12pm-1pm

- Review mentor bios in lobby
- Sign-up!
- Grab your lunch and find your mentor



Amy Tran-Guzman



Rajat Gupta





Afternoon & Evening Program

Time	Speaker / Credentials	Title / Activity	Duration
2:30–2:45 pm	Matt Tate, PhD — Chief Business Development Officer, Scantox	Application of DuplexSeq error corrected sequencing for robust genomic safety assessments	15 min
2:45–3:00 pm	Jenielle Domaol — Graduate Student, UCLA/University of Washington	Ldlr-/- Mice Exhibit Sex-Dependent Microglial and Proteomic Responses to Chronic Diesel Exhaust and High-Fat Diet Exposure	15 min
3:00–3:15 pm	Xiyin Wang, PhD — Post-doctoral Fellow, Neurocrine	Integrating Target-Centric Safety Assessment with Human iPSC-Derived Neuronal MEA as a New Approach Methodology for Early De-Risking of Seizure Liability	15 min
3:15–3:40 pm	Wei-Chun Chou, PhD — Assistant Professor of Environmental Health, UCR	From Mechanistic Modeling to AI-Enabled Toxicology	25 min
4:00 pm	Poster Awards & 1st Annual SCCSOT Tox Murder Mystery Happy Hour		—



Current SCCSOT Financials



- Fall meeting 2025: \$26,071.69
- Spring 2026 awards and mixer: \$29,855.32
- Total income May 2025 to April 2026: \$66,061
 - *Total Expense May 2025 to April 2026: \$56,102.57*
- June 2026 account balance: \$39,022.14
- Expected sponsorship: \$80,000



Heather Estrella
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 **FREE
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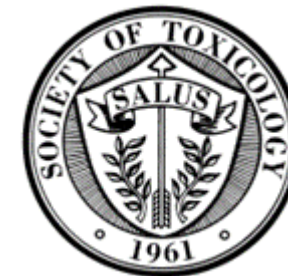
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2-year term

You can nominate a colleague or self-nominate.

Great way to get involved with chapter activities.

**Deadline to nominate:
November 15**

For questions or additional information please contact the SCCSOT at southerncaliforniaSOT@gmail.com
Or Visit the Chapter's Website: <https://www.toxicology.org/groups/rc/SouthernCal/index.asp>



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The mystery calls.
Stay for drinks as darkness falls!

TOX MURDER MYSTERY

*A deadly dose, a hidden crime.
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Unmask the culprit, win the gold,
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OCT 7 @ 4 PM

SCCSOT 38th Annual
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VENDOR BINGO PRIZE

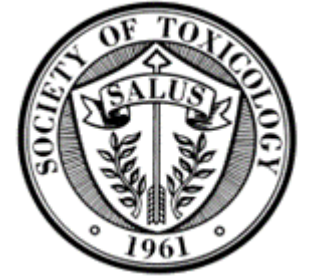
Complete your Vendor Bingo sheet
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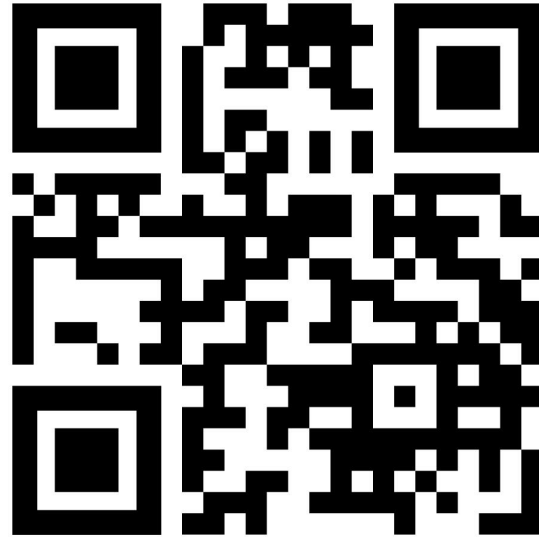


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38th Annual Fall Symposium: Southern California Society of Toxicology Regional Meeting

AI-Enabled Toxicology: Opportunities, Challenges, and Best Practices



Invited Speaker Abstracts



AI in Chemical Hazard Assessment: Opportunities and Challenges



Kris Thayer, PhD, Cal EPA/OEHHA

Artificial intelligence (AI) and automation offer opportunities to improve chemical hazard assessment by saving time, reducing repetitive work, and improving access to chemical information. This presentation examines integrated chemical assessment platforms and AI-assisted systematic review tools, including their use in problem formulation, scoping, prediction, literature screening, study data extraction, and hazard characterization. It discusses limitations in literature coverage, data accuracy, critical appraisal, and evidence synthesis, emphasizing the continuing need for expert scientific evaluation. High-quality, interoperable chemical data repositories support these applications, which are also critical to the evaluation of New Approach Methods (NAMs). Publishing structured, machine-readable study data alongside articles could further reduce extraction costs and errors. Together, these approaches could improve how scientific evidence moves from primary research into chemical assessments that inform regulatory and other decisions.



Embracing a new era of toxicity screening: Atomic-resolution modeling to mitigate off- target liabilities



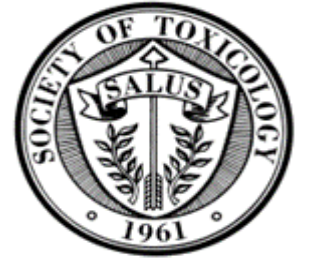
Steve Albanese, PhD Research Leader, Comp. Chemistry,
Schrödinger Therapeutics

Off-target activity remains a significant source of drug-development failure, and liabilities discovered late can be difficult to address through molecular design. In vitro safety panels have substantially improved the safety profile drugs brought to market, but there is an opportunity to identify risks earlier, before compounds are synthesized and tested.

We present a physics-based approach to prospective off-target screening that combines induced-fit docking with free-energy calculations to assess potential binding across an expanding panel of proteins and enable design for already identified liabilities. Case studies from Schrödinger's Therapeutics Group illustrate how atomic-resolution models can guide design decisions. In an NLRP3 inhibitor program, prospective modeling supported the optimization of on-target potency while reducing hERG liability. In a Wee1/Myt1 inhibitor program, we describe how this modern approach would have helped to identify kinase-selective chemical series. Additional examples examine how computational screening can inform the interpretation of competitor selectivity data and differentiation strategies.

Together, these applications show how physics-based screening can complement experimental in vitro safety panels by providing structure-based hypotheses and design tools to act on them. Future work will expand target coverage and improve screening speed and cost.





Better Models Begin with Better Images

***Aneesh Acharya, PhD Chief Operating Officer,
Molecular Instruments***

As image analysis increasingly incorporates AI, the quality of its conclusions still depends on the quality of the underlying images. This presentation will describe HCR™-based approaches for imaging challenging short RNA targets, including siRNAs and oligonucleotide payloads in AOCs, alongside flexible multiplex immunofluorescence using HCR™ HiFi Encoders. Through these examples, we will show how assay chemistry determines what can be detected, how clearly it can be measured, and how confidently the resulting images can be interpreted.



β 2-Adrenergic Receptor Agonists-Induced Pro-Inflammatory Genes in Macrophages and the Effects of Carvedilol and R-carvedilol

*Jonathan Wu, Undergraduate Student,
Western University of Health Sciences*

Background

Prolonged activation of β 2-Adrenergic receptors (β 2-ARs) by chronic stress facilitates an immunosuppressive microenvironment and cancer development. Our previous studies showed that the β -blocker carvedilol and the non- β -blocking enantiomer R-carvedilol inhibited UV-induced inflammation, immunosuppression and skin cancer development. However, the mechanisms behind these effects of carvedilol and R-carvedilol are unknown. Because β 2-ARs are expressed on many types of skin cells as well as immune cells such as macrophages, the goal of this project is to examine the effects of carvedilol and R-carvedilol on inflammation-related genes induced by β 2-AR agonists in the murine macrophage cell line RAW 264.7.

Methods

RAW 264.7 macrophages were treated with the β 2-AR agonist salmeterol (β 2-AR specific agonist) along with other β -adrenergic agonists epinephrine and isoproterenol at 1 μ M. The cells treated with salmeterol were co-treated with the β 2-AR specific antagonists ICI 118,551, the racemic mixture carvedilol (β 1/2-AR antagonists), and the enantiomers R- or S-carvedilol at 1 μ M. The MAPK/ERK pathway activity was assessed with the MEK inhibitor U0126 (1 μ M). RAW 264.7 cell lysates were generated by repeated passage through a 26-gauge needle, and total RNA was isolated using the RNeasy Mini Kit (Qiagen). cDNA was obtained with the High-Capacity cDNA Reverse Transcriptase Kit (Thermo Fisher Scientific). The cDNA was amplified with Power SYBR Green PCR Master Mix (Applied Biosystems) and primers for murine genes and β -actin. Real-time PCR was performed on a CFX96 Real-time Thermal Cycler Detection System (BioRad) and analyzed using β -actin as the normalization control with the 2- $\Delta\Delta$ Ct method. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) was used to measure the relative mRNA expression levels of IL-1 β , IL-6, and COX-2.

Results

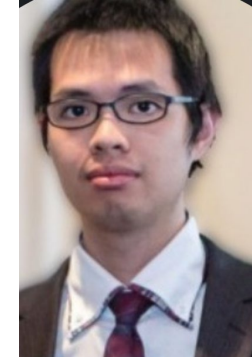
Salmeterol (1 μ M) significantly increased IL-1 β mRNA expression compared to the vehicle control ($p < 0.001$) and increased COX-2 expression ($p < 0.05$), while IL-6 levels were not statistically significant. Consistent with β 2-AR stimulation by the β 2-AR specific agonist salmeterol, treatment with the nonselective adrenergic agonists epinephrine and isoproterenol (1 μ M each) similarly increased IL-1 β levels. Pharmacological blockade using carvedilol (β 2-AR nonselective antagonist; 1 μ M) and ICI 118,551 (β 2-AR selective antagonist; 1 μ M) significantly suppressed salmeterol-induced IL-1 β expression ($p < 0.01$). While S-carvedilol (1 μ M), which has high binding affinity for the β -ARs, strongly attenuated salmeterol-induced IL-1 β , R-carvedilol (1 μ M), which lacks β -AR-blocking activity, failed to reduce IL-1 β expression. The MEK inhibitor U0126 at 1 μ M partially attenuated salmeterol induced IL-1 β expression.

Conclusions

Activating β -ARs induced increased mRNA expression of pro-inflammatory genes, particularly IL-1 β , in macrophages. S-carvedilol, but not R-carvedilol, prevented salmeterol-induced IL-1 β , implying that carvedilol's anti-inflammatory properties in the experimental conditions presented here are dependent on β 2-AR inhibition. β 2-AR activation induced by agonists may be also dependent on the MAPK pathway. Since this study did not involve UV, it remains undetermined whether UV-induced inflammation is dependent on β 2-AR and whether the anti-inflammatory activity of carvedilol and R-carvedilol is dependent on β -blockade.



Two Translational Roads, One AI Compass: Navigating In Vitro to In Vivo and Cross- Species Translatability by ToxAgent



Yen Wei Chen, PhD *Senior Scientist, Computational Toxicology, Preclinical Sciences and Translational Safety*
J&J

Translating preclinical safety findings into meaningful evidence for human risk assessment remains a central challenge in drug development. ToxAgent tackles two critical translational paths—connecting in vitro biology with in vivo outcomes and determining when animal findings may translate to humans—by unifying two complementary, openly accessible resources we developed. The Safetyome maps more than 11,000 safety-relevant proteins across 22 organ systems, prioritizes a 500-target core panel, and delivers the first systematic collection of specialized panels covering more than 3,000 phenotypes – an unprecedented scale, built to reveal mechanisms and help resolve safety issues. The full Safetyome, core panel, and specialized panels were all orthogonally validated using independent large-scale datasets. Our cross-species concordance framework links standardized preclinical and clinical safety data from 7,565 marketed and investigational drugs, creating the largest analysis of its kind. Going beyond identical adverse-event terms, it identifies biologically related findings while accounting for species, therapeutic modality, and exposure, revealing 850 significant identical-term relationships and 2,833 additional endpoints through related-term associations. ToxAgent transforms these achievements into an intuitive multi-agent AI experience, enabling toxicologists to ask practical questions across in vitro systems, animal studies, and human outcomes without navigating complex datasets. By putting these capabilities directly in the hands of the toxicology community, ToxAgent makes translational evidence easier to explore and apply, supports earlier and better-informed decisions, accelerates mechanistic issue resolution, and advances the 3Rs—all toward safer medicines.



Beyond the Buzz: What's Ready and What's Next for AI-Enabled Toxicology



Maia Green, PhD Vice President, Toxicology,
Certara

Drug safety liabilities remain a major source of attrition, delay, and increased cost throughout pharmaceutical development. Advances in artificial intelligence (AI) provide opportunities to identify and investigate potential safety liabilities during lead optimization, before a candidate enters IND-enabling studies, when structural modifications are still feasible and go/no-go decisions carry lower financial and time costs. This presentation highlights the application of AI tools for predictive safety assessment, with a focus on developmental and reproductive toxicology. In discovery, predictive models and virtual-tissue simulations help anticipate birth defects and generate mechanistic hypotheses. SEND Explorer® complements predictive approaches by leveraging Standard for Exchange of Nonclinical Data (SEND) datasets to enable rapid cross-study comparisons, identify safety signals, and contextualize findings that support more informed, data-driven decision-making. Building on the same historical data infrastructure, integration of virtual control groups apply AI and statistical modeling to curated historical control animal data to replace or supplement concurrent control animals in nonclinical toxicology studies. This approach provides an additional opportunity to reduce animal use while preserving data quality, study integrity, and interpretability. Together, these approaches provide a snapshot of how AI tools can complement traditional toxicology methods by integrating diverse data sources, improving early identification of safety liabilities, and supporting more informed decision-making. In addition, as regulatory interest continues to grow, selecting and applying fit-for-purpose predictive tools has the potential to reduce animal testing, improve the efficiency of safety assessment, and ultimately support development of safer therapeutic candidates.

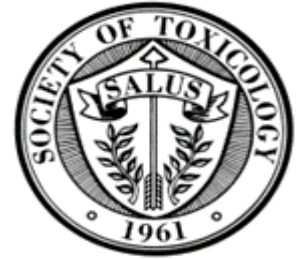


Application of DuplexSeq error corrected sequencing for robust genomic safety assessments



Matt Tate, PhD Chief Business Development Officer,
Scantox

DuplexSeq™ is a highly accurate Error-Corrected Sequencing approach, delivering an error rate of $<1 \times 10^{-7}$ nucleotides sequenced. This sensitivity enables robust genomic safety assessments for APIs and impurities, as well as in the prediction of carcinogenicity outcomes. Easily integrated into existing studies where a high-quality DNA sample can be obtained, DuplexSeq™ delivers mutagenic and broader carcinogenic assessments earlier and with greater accuracy. Recently the technology has been utilized by organizations addressing challenges within the nitrosamine and complex nitrosamine impurities assessment area, with DuplexSeq being demonstrated as a robust methodology for the detection of mutagenic hazard and the derivation of derived exposure limits.



Ldlr^{-/-} Mice Exhibit Sex-Dependent Microglial and Proteomic Responses to Chronic Diesel Exhaust and High-Fat Diet Exposure

Jenielle Domaol, Graduate Student,
UCLA/University of Washington

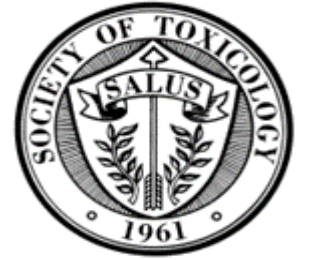
Background: Air pollution is one of the leading causes of mortality, contributing to about 6.7 million premature deaths annually. Diesel exhaust (DE) is a significant contributor to particulate matter (PM) in urban traffic-related air pollution, resulting in adverse respiratory, cardiovascular, and reproductive health effects. Research has suggested that PM, particularly ultrafine PM, can trigger neurodegeneration by affecting the brain and central nervous system. Microglia, the brain's resident immune cells, are thought to play a crucial role in this process. While important to maintain homeostasis, repair damage, and trigger the inflammatory response, continued exposure to PM can condition a pro-inflammatory environment. This glial dysfunction can lead to neuronal damage and death, which can result in neurodegenerative conditions. Susceptible populations, like those with familial hypercholesterolemia (FH), face an already elevated risk of developing adverse health outcomes, but co-exposure to environmental exposures, like a high-fat diet and PM, can further increase the likelihood of neurodegenerative effects. While there is growing research to uncover how PM affects neurodegeneration and how diet and sex play into this relationship, the specific molecular mechanisms are not well understood. The objective of this project was to characterize the effects of chronic DE exposure and a high-fat diet on neuroinflammatory and proteomic responses in the hippocampus and olfactory bulb of a mouse model of FH. Based on publications and our laboratory's preliminary data indicating that mice exposed chronically to DE develop neuroinflammation and impaired spatial memory, we hypothesize that chronic DE exposure will induce sex-specific changes in microglial states and protein pathways, particularly in the hippocampus and olfactory bulb, associated with neurodegeneration, and that HFD will exacerbate these changes in hypercholesterolemic mice.

Methods: This study examined mechanisms of PM-induced neuroinflammation and cognitive dysfunction, focusing on proteomics and microglia abundance and morphology. Six-week-old low-density lipoprotein receptor knockout (*Ldlr*^{-/-}) mice, a model of FH, were exposed to freshly generated DE (~250 µg/m³) or filtered air (FA) over 18 weeks. Mice (n = 11-14 per sex and exposure) were fed a high-fat (HFD) or standard chow diet and exposed for 6 h/day, 5 days/week. At the end of 18 weeks, mice were sacrificed and perfused with PBS. Left brain hemispheres were fixed, cryopreserved, and used for immunofluorescence. Confocal images (40x) were taken for each group (n = 1, 2 replicates), and an average cell count was taken. An additional 40x, 3x zoom image was taken, and soma and arborization area were measured for 3 random microglia (n = 1, 2 replicates). The hippocampus and olfactory bulb were isolated from right brain hemispheres, flash-frozen in liquid nitrogen, and analyzed for proteomics via data-independent acquisition liquid chromatography-tandem mass spectrometry (DIA LC-MS/MS). Resulting datasets were analyzed using the Advaita iPathwayGuide software.

Results: Sex-specific changes were found for cell count and morphology. Overall, the HFD DE female (DEF) group had the largest decrease in microglial cell count (42%, $p = 0.0463$) and the largest increase in soma (~19%) and arborization area (~66%) when compared to its control, HFD FAF, highlighting possible hyper-ramification and chronic stress. Protein expression analysis revealed significant changes ($FDR < 0.05$) based on DE exposure in the olfactory bulb between the HFD DE and HFD FA female groups and diet between the male HFD FA and control chow FA groups. In the hippocampus, protein expression changes observed were based on diet when comparing the male HFD DE and control chow DE groups and the HFD FA and control chow FA groups. Comparing all significantly changed proteins ($p < 0.05$), a similar upregulation of apolipoproteins in the hippocampus and olfactory bulb of the male groups with significant protein abundance changes was found. No similar protein expression changes were found between female and male groups with significant protein changes. Pathway analysis of the hippocampus identified dysregulation of the actin cytoskeleton pathway in the HFD FA male group, which is associated with synaptic dysfunction and possible cognitive decline. In the olfactory bulb, the pathway analysis also revealed impacts on glutamate synapses in the HFD DE female group, which is also associated with synaptic dysfunction and neurodegenerative disorders. Our results suggest that the *Ldlr*^{-/-} males may be more sensitive to errors in lipid metabolism, while the *Ldlr*^{-/-} females may be more sensitive to chronic stress.

Conclusion: Overall, the preliminary findings in this project suggest that chronic DE exposure and HFD alter microglial morphology and number in a sex-dependent manner, with significant protein expression changes in pathways relevant to neurodegenerative processes. Additional research is underway to ascertain whether DE-induced microglial states overlap with microglial signatures implicated in Alzheimer's disease and other neurodegenerative conditions, which could provide a deeper mechanistic insight into how air pollution contributes to neurodegenerative disease risk.





Integrating Target-Centric Safety Assessment with Human iPSC-Derived Neuronal MEA as a New Approach Methodology for Early De-Risking of Seizure Liability

Xiyin Wang, PhD Post-doctoral Fellow,
Neurocrine

Background and Purpose : Drug-induced seizures are an important central nervous system (CNS) safety liability that can contribute to compound attrition when identified during GLP toxicology studies and/or later stages of development. Target-centric safety assessment integrates biological, genetic, mechanistic, and internal/external evidence to identify potential safety liabilities earlier in drug discovery. While several efforts have integrated seizure-relevant targets from adverse outcome pathways (AOPs) and drug discovery resources, new approach methodologies (NAMs) have demonstrated fit-for-purpose utility for interrogating relevant on- and off-target signaling associated with compound-related seizure liability. To bridge this gap, we evaluated an integrated strategy combining target-centric safety assessment with functional testing using human induced pluripotent stem cell (hiPSC)-derived neuronal multielectrode arrays (MEAs).

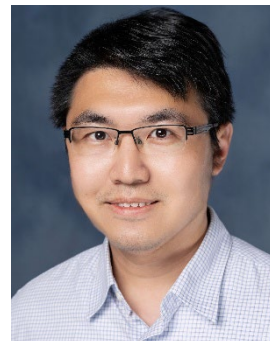
Methods: Target safety assessment evaluated the biological plausibility of seizure-related effects associated with intended pharmacology, informed by target-specific biology, seizure-relevant mechanistic evidence, and available internal and external knowledge. Functional seizure liability was evaluated using co-cultures of hiPSC-derived excitatory and inhibitory neurons with astrocytes. Five anonymized proprietary compounds representing multiple pharmacological mechanisms were evaluated by MEA at five concentrations spanning 0.3-300 μM across the compound set, with four replicate wells per concentration. Concentrations were selected based on *in vivo* toxicology findings, C_{max} values associated with convulsion and non-convulsion findings, and/or solubility. Acetaminophen served as a negative control and strychnine, picrotoxin, and pilocarpine served as positive controls. Firing, bursting, network bursting, rhythmicity, synchrony were assessed from 15-min MEA recordings before and after 1 h of compound exposure.

Results: Controls showed the expected neuronal response. All five proprietary compounds showed alterations in neuronal firing and network activity associated with a seizurogenic phenotype, with concentration-related changes across multiple MEA parameters and differences in potency among compounds. Common responses included decreased firing rate; increased burst and network-burst frequency; shorter bursts and network bursts; fewer spikes per burst and network burst; and increased regularity of network bursting. Compounds D and E showed the greatest MEA responses, Compound B an intermediate response, and Compounds A and C smaller changes. Compound D increased firing and network-burst activity at 1-3 μM , followed by decreased activity at 10 μM and loss of measurable activity at 30 and 100 μM . Prior *in vivo* findings included convulsions with Compounds A, B, D, and E in rat, dog, or monkey studies. No convulsions were observed with Compound C in nonclinical studies. Compounds A and C are in the same class. MEA detected seizure-associated changes in all four compounds with prior *in vivo* convulsions.

Conclusions: Integration of target-centric safety assessment with human hiPSC-derived neuronal MEA provides a mechanistically informed NAM strategy for early seizure-liability assessment. Target- and AOP- based information can identify seizure-relevant mechanisms warranting further evaluation, while MEA provides complementary functional evidence of compound-induced perturbations in human neuronal network activity. This proof-of-concept demonstrated seizure-associated MEA responses across compounds with diverse pharmacological mechanisms and concordance with available *in vivo* convulsion findings. Integration with systemic exposure and *in vivo* findings can further contextualize compound-specific risk. This target-to-functional-phenotype framework may strengthen weight-of-evidence assessment and support earlier identification and prioritization of seizure liabilities. Evaluation of a larger set of seizurogenic and non-seizurogenic compounds is needed to establish predictive performance and broader applicability.



From Mechanistic Modeling to AI-Enabled Toxicology



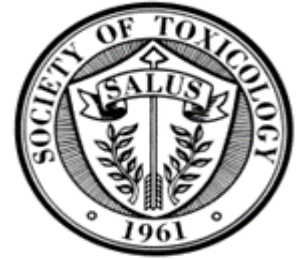
Wei-Chun Chou, PhD Assistant Professor of Environmental Health,
UCR

Artificial intelligence (AI) and machine learning (ML) are increasingly transforming toxicology by expanding our ability to analyze complex biological data, identify predictive patterns, and complement mechanistic understanding. This presentation will highlight how AI can be integrated with physiologically based pharmacokinetic (PBPK) modeling and other computational approaches to improve prediction of chemical and nanomaterial behavior in biological systems. Using nanomaterials as an example, we will illustrate how mechanistic models can describe biodistribution and tissue disposition, while AI-based approaches can capture complex relationships among physicochemical properties, biological interactions, and experimental conditions. Applications will include prediction of nanoparticle biodistribution, protein corona formation, and immunotoxicity, demonstrating a progression from exposure and disposition to biological interaction and adverse response. Rather than replacing mechanistic modeling, AI provides a complementary framework for learning from high-dimensional and heterogeneous data, reducing experimental burden, and improving predictive capacity. Together, these approaches represent an emerging direction for next-generation toxicology, with the potential to support more efficient hazard assessment, mechanistic interpretation, and data-driven decision making across diverse environmental and biomedical applications.





Poster Abstracts



#1

Heme oxygenase-1 alters IL-8 expression in endothelial cells and exacerbates early atherosclerotic plaque development

Samuel Bastidas^{1,3}, Jocelyn A Castellanos^{1,2}, Allen Louie^{1,3}, Siri Langmo^{1,3}, Julius Hernandez¹, Nina Chessa¹, Akeem Lawal¹, Min Zhang¹, and Jesus A Araujo MD, PhD^{1,2,3,4}

¹Division of Cardiology, Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles

²Department of Environmental Health Sciences, Fielding School of Public Health, University of California, Los Angeles

³Environmental and Molecular Toxicology Interdepartmental Program, University of California, Los Angeles ⁴Molecular Biology Institute, University of California, Los Angeles

Background: Heme oxygenase-1 (HO-1) has long been known to have an antioxidant and anti-inflammatory role in mammalian cells. More importantly, HO-1 has been shown to play an anti-atherogenic role in vascular cells, yet the cell type responsible for this protection remains unclear. We have previously shown that transplantation of myeloid HO-1-deficient bone marrow into low-density lipoprotein receptor knockout mice fed a high fat diet did not alter atherosclerotic plaque burden, suggesting another vascular cell could be responsible.

We **hypothesized** that endothelial cells are the critical cell type in the anti-atherogenic protection of HO-1 through antioxidant and anti-inflammatory mechanisms.

Approach: We sought to develop an *in vitro* model to better recapitulate pro-atherogenic stimuli within the vasculature by treating human microvascular endothelial cells (HMEC) with palmitic acid (PA) and 1-palmitoyl-2-glutaryl phosphatidylcholine (PGPC), thus, allowing us to study the role of HO-1 using siRNA knockdown during conditions like that *in vivo*. We also developed a novel *in vivo* endothelial cell-specific HO-1 knockout mouse in the apolipoprotein E null background (eHO-1 ^{-/-}, ApoE ^{-/-}) and aged it for 20 weeks under normal chow feeding to induce early atherogenesis.

Results: We treated HMECs with 25 μ M PGPC for 12 hours in serum-free media to simulate exposure to a pro-atherogenic oxidized phospholipid. This induced HO-1, IL-8, ATF4, and ICAM-1 mRNA expression. Next, HMECs were treated with PA for 24 hours in 1% fatty acid-free bovine serum albumin media to simulate exposure to hyperlipidemic conditions. HO-1, IL-8, ATF4, and ICAM-1 expression was induced in a dose-dependent manner. Co-treatment of HMECs with 25 μ M PGPC and 250 μ M PA synergistically upregulated IL-8 expression after 24-hour treatment. To our surprise, HO-1 deficient cells treated with PA and PGPC had significantly lower IL-8 mRNA expression as compared with control cells. Our *in vivo* model revealed that the aortic lesion area was significantly lower in female eHO-1 ^{-/-}, ApoE ^{-/-} mice as compared to control floxed HO-1, ApoE ^{-/-} mice.

Conclusion: Treatments with PA and PGPC induced inflammation and ER stress in HMECs, indicative of pro-atherogenic stimulation. HMECs treated with PA and PGPC may exhibit a synergistic mechanism of inflammation likely driven by IL-8. Interestingly, HO-1 knockdown reversed IL-8 expression upon co-treatment, suggesting HO-1 may regulate IL-8 mRNA expression in endothelial cells under pro-inflammatory conditions through an unknown mechanism.

Significance to Toxicology: Our novel endothelial cell-specific HO-1 knockout mouse can be utilized to study the pro-atherogenic and cardiotoxic effects of environmental chemicals or pharmacologic agents that exert promoting effects on HO-1 expression.

Effects of Linuron Exposure on Gene Expression in the HTR-8/SVneo Placental Cell Line

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Introduction: Racial minority women face higher rates of adverse birth outcomes than Caucasian women, including large-for-gestational-age (LGA) births, for reasons that remain poorly understood. Pesticide exposure may contribute: farmworkers and residents of agricultural communities, many of whom are Hispanic, are exposed to pesticides at higher rates than the general population, and this exposure has been linked to adverse birth outcomes. Linuron, an EPA-regulated phenylurea herbicide still used commercially to control weeds, persists in soil and water and reaches people through spray drift, inhalation, and contaminated food or water. It has been linked to LGA, reproductive and developmental harm, and, in animal studies, birth defects, and is a known androgen receptor antagonist and endocrine disruptor. Yet its effects on the placenta, an organ with endocrine functions essential for fetal development, remain unstudied. Here, we assess viability, cytotoxicity, proliferation, and gene expression in HTR-8/SVneo placental cells exposed to human-relevant linuron concentrations.

Materials and Methods: HTR-8/SVneo cells were seeded in 96-well (or 6 - well for RNA isolation) plates and incubated for 24 h prior to treatment with medium plus DMSO (vehicle control), linuron (50 μ M - 1 μ M), or camptothecin (positive control).

Viability/Cytotoxicity (Live/Dead): Live and dead cell measurements were performed using the MultiTox-Glo Multiplex Cytotoxicity Assay (Promega). Following exposure, GF-AFC reagent was added to each well and incubated for 55 min, after which live-cell fluorescence was measured (~400 nm excitation / ~505 nm emission) using a microplate reader. AAF-Glo reagent was then added and incubated for 15 min, and dead-cell luminescence was measured.

Gene Expression: RNA was isolated from cells following a 24-hour exposure using the RNeasy Plus Mini Kit (QIAGEN) per manufacturers protocol. RNA concentration and purity were assessed using a NanoDrop spectrophotometer, and samples were sent to Novogene for sequencing.

Results: Viability/Cytotoxicity (Live/Dead): Following exposure to 50 μ M of linuron, the live-to-dead ratio of placental cells increased by 84.2% compared to controls ($p=0.0001$). Following exposure to 10 μ M, the live-to-dead ratio decreased by 42.1% compared to controls ($p=0.043$).

Gene Expression: Treatment with 5 μ M linuron for 24 h yielded 1 gene that met both FDR ($FDR < 0.05$) and fold-change ($|\log_2FC| > 0.58$) criteria and 31 genes that met FDR criterion alone (total 15 upregulated and 16 downregulated). Treatment with 10 μ M linuron for 24 h yielded 12 genes that met both FDR ($FDR < 0.05$) and fold-change ($|\log_2FC| > 0.58$) criteria and 46 genes that met FDR criterion alone (total 39 upregulated and 7 downregulated).

Conclusions: Overall, placental cells showed concentration-dependent, non-monotonic responses to linuron exposure. At 10 μ M, linuron decreased the live-to-dead cell ratio relative to controls, indicating a cytotoxic effect at this lower concentration, while at 50 μ M the live-to-dead ratio increased above control levels. Gene expression changes were also concentration-dependent: treatment with 5 μ M linuron altered relatively few genes, whereas 10 μ M linuron induced more differential expression in a larger number of genes, with the majority upregulated. Future experiments will involve pathway analysis of the differentially expressed genes to better understand the mechanisms underpinning linuron toxicity to placental cells, as well as additional assaying to clarify the non-monotonic viability response observed.



#2

Two Translational Paths, One AI Future: in vitro to in vivo safetyome and cross-species concordance, unified by ToxAgent

Yen-Wei Chen, Xin Liu, Fan Fan.

Johnson & Johnson

Translating preclinical safety findings into meaningful evidence for human risk assessment remains a central challenge in drug development. ToxAgent tackles two critical translational paths—connecting in vitro biology with in vivo outcomes and determining when animal findings may translate to humans—by unifying two complementary, openly accessible resources we developed. The Safetyome maps more than 11,000 safety-relevant proteins across 22 organ systems, prioritizes a 500-target core panel, and delivers the first systematic collection of specialized panels covering more than 3,000 phenotypes – an unprecedented scale, built to reveal mechanisms and help resolve safety issues. The full Safetyome, core panel, and specialized panels were all orthogonally validated using independent large-scale datasets. Our cross-species concordance framework links standardized preclinical and clinical safety data from 7,565 marketed and investigational drugs, creating the largest analysis of its kind. Going beyond identical adverse-event terms, it identifies biologically related findings while accounting for species, therapeutic modality, and exposure, revealing 850 significant identical-term relationships and 2,833 additional endpoints through related-term associations. ToxAgent transforms these achievements into an intuitive multi-agent AI experience, enabling toxicologists to ask practical questions across in vitro systems, animal studies, and human outcomes without navigating complex datasets. By putting these capabilities directly in the hands of the toxicology community, ToxAgent makes translational evidence easier to explore and apply, supports earlier and better-informed decisions, accelerates mechanistic issue resolution, and advances the 3Rs—all toward safer medicines.



#3

Arsenic destabilizes naïve pluripotency through epigenetic disruption in embryonic stem cells

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Background and Purpose: Arsenic is among the most consequential environmental carcinogens, with groundwater contamination affecting an estimated 220 million individuals worldwide and epidemiological studies consistently linking early-life exposure to cancers of the bladder, lung, and skin in adulthood. The long latency between developmental exposure and adult malignancy points to aberrant epigenetic reprogramming during a critical window of cellular plasticity as a likely initiating mechanism, yet the molecular events connecting the two remain poorly understood. Studies have shown that arsenic impacts the ability of stem cells to maintain pluripotency, suggesting a potential avenue for long-term impacts following short-term in-utero exposure. However, studies have not identified the primary mechanisms driving this disruption or whether this effect extends to the early embryo. Mouse embryonic stem cells (ESCs) and epiblast-like cells (EpiLCs) are well suited to addressing this gap, as they represent two key, differentially methylated stages of embryonic development. In this study, we use multi-omics to investigate the epigenetic and transcriptomic effects of arsenic on mouse ESCs and EpiLCs to understand how arsenic impacts early development.

Methods: ESCs were cultured in 2i+LIF media followed by arsenic exposure (sodium arsenite) at 1 and 2 μ M for 48 hours before collection and analysis. For EpiLC differentiation, ESCs were cultured in 2i+LIF for 48 h without treatment, then switched to EpiLC induction media with simultaneous arsenic exposure at 1 and 2 μ M for 48 h. Vehicle controls at each stage received an equal volume of sterile water. All experiments were performed in triplicates. We profiled the transcriptome (RNA-seq) and methylome (RRBS) in both cell states, and chromatin accessibility (ATAC-seq) and histone modifications (CUT&Tag for H3K4me3, H3K36me2, and H3K27me3) in ESCs. Datasets were first analyzed individually using differential feature and pathway analyses before integrating the different omics to uncover shared patterns of disruption induced by arsenic using cluster and motif analyses.

Results: Arsenic destabilizes the pluripotency network of ESCs at both the transcriptomic and epigenomic levels, producing a partial loss of naïve pluripotency. Arsenic-induced DEGs and hypomethylated DMRs overlapped significantly with those defining the normal ESC-to-EpiLC transition, indicating that exposure recapitulates a substantial fraction of the priming program in the absence of a differentiation signal. Furthermore, our paired ATAC-seq and CUT&Tag data show substantial epigenetic remodeling of ESCs following exposure. ATAC-seq analysis reveals a closing of regions associated with motifs for Pou5f1 and Sox2, two of the core regulators of pluripotency and self-renewal in ESCs. H3K4me3 gains at promoters of self-renewal and formative pluripotency genes were mirrored in the RNA-seq data, where promoters gaining H3K4me3 were significantly enriched for upregulated genes (odds ratio 6.3, Fisher exact test $p = 5.3 \times 10^{-38}$). At loci that lost H3K36me2, H3K27me3 signal rose by 71% versus ~6% at unaffected control loci, and the two changes were anticorrelated genome-wide (Spearman $\rho =$

-0.42 , $n = 12,518$ regions). These regions of H3K27me3 encroachment were non-canonical for PRC2, the primary agent of H3K27me3 deposition, and instead were enriched for motifs of pluripotency transcription factors, including Nr5a2 and Esrrb.

Conclusions: Arsenic exposure pushes naïve ESCs toward a partially primed-like state without an inductive cue, producing a coordinated shift across the transcriptome, the methylome, and chromatin accessibility. Loss of H3K36me2 and the resulting redistribution of H3K27me3 provide a plausible mechanism, since H3K36me2 normally constrains PRC2 activity, and the concurrent closing of pluripotency transcription factor binding sites indicates that the network is destabilized at the chromatin level rather than through a single transcriptional target.

These findings identify premature exit from naïve pluripotency as a mechanism by which brief developmental arsenic exposure could reshape the epigenetic landscape of the early embryo, and they offer a candidate origin for the durable, long-latency phenotypes observed in exposed human populations. We plan to continue this work by targeting the H3K36me2/H3K27me3 axis to test whether restoring this balance rescues the arsenic-induced phenotype.



#4



#5

Ldlr ^{-/-} Mice Exhibit Sex-Dependent Microglial and Proteomic Responses to Chronic Diesel Exhaust and High-Fat Diet Exposure

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Background: Air pollution is one of the leading causes of mortality, contributing to about 6.7 million premature deaths annually. Diesel exhaust (DE) is a significant contributor to particulate matter (PM) in urban traffic-related air pollution, resulting in adverse respiratory, cardiovascular, and reproductive health effects. Research has suggested that PM, particularly ultrafine PM, can trigger neurodegeneration by affecting the brain and central nervous system. Microglia, the brain's resident immune cells, are thought to play a crucial role in this process. While important to maintain homeostasis, repair damage, and trigger the inflammatory response, continued exposure to PM can condition a pro-inflammatory environment. This glial dysfunction can lead to neuronal damage and death, which can result in neurodegenerative conditions. Susceptible populations, like those with familial hypercholesterolemia (FH), face an already elevated risk of developing adverse health outcomes, but co-exposure to environmental exposures, like a high-fat diet and PM, can further increase the likelihood of neurodegenerative effects. While there is growing research to uncover how PM affects neurodegeneration and how diet and sex play into this relationship, the specific molecular mechanisms are not well understood. The objective of this project was to characterize the effects of chronic DE Exposure and a high-fat diet on neuroinflammatory and proteomic responses in the hippocampus and olfactory bulb of a mouse model of FH. Based on publications and our laboratory's preliminary data indicating that mice exposed chronically to DE develop neuroinflammation and impaired spatial memory, we hypothesize that chronic DE exposure will induce sex-specific changes in microglial states and protein pathways, particularly in the hippocampus and olfactory bulb, associated with neurodegeneration, and that HFD will exacerbate these changes in hypercholesterolemic mice.

Methods: This study examined mechanisms of PM-induced neuroinflammation and cognitive dysfunction, focusing on proteomics and microglia abundance and morphology. Six-week-old low-density lipoprotein receptor knockout (Ldlr^{-/-}) mice, a model of FH, were exposed to freshly generated DE (~250 µg/m³) or filtered air (FA) over 18 weeks. Mice (n = 11-14 per sex and exposure) were fed a high-fat (HFD) or standard chow diet and exposed for 6 h/day, 5 days/week. At the end of 18 weeks, mice were sacrificed and perfused with PBS. Left brain hemispheres were fixed, cryopreserved, and used for immunofluorescence. Confocal images (40x) were taken for each group (n = 1, 2 replicates), and an average cell count was taken. An additional 40x, 3x zoom image was taken, and soma and arborization area were measured for 3 random microglia (n = 1, 2 replicates). The hippocampus and olfactory bulb were isolated from right brain hemispheres, flash-frozen in liquid nitrogen, and analyzed for proteomics via data-independent acquisition liquid chromatography-tandem mass spectrometry (DIA LC-MS/MS). Resulting datasets were analyzed using the Advaita iPathwayGuide software.

Results: Sex-specific changes were found for cell count and morphology. Overall, the HFD DE female (DEF) group had the largest decrease in microglial cell count (42%, p = 0.0463) and the largest increase in soma (~19%) and arborization area (~66%) when compared to its control, HFD FAF, highlighting possible hyper-ramification and chronic stress. Protein expression analysis revealed significant changes (FDR < 0.05) based on DE exposure in the olfactory bulb between the HFD DE and HFD FA female groups and diet between the male HFD FA and control chow FA groups. In the hippocampus, protein expression changes observed were based on diet when comparing the male HFD DE and control chow DE groups and the HFD FA and control chow FA groups. Comparing all significantly changed proteins (p < 0.05), a similar upregulation of apolipoproteins in the hippocampus and olfactory bulb of the male groups with significant protein abundance changes was found. No similar protein expression changes were found between female and male groups with significant protein changes. Pathway analysis of the hippocampus identified dysregulation of the actin cytoskeleton pathway in the HFD FA male group, which is associated with synaptic dysfunction and possible cognitive decline. In the olfactory bulb, the pathway analysis also revealed impacts on glutamate synapses in the HFD DE female group, which is also associated with synaptic dysfunction and neurodegenerative disorders. Our results suggest that the Ldlr ^{-/-} males may be more sensitive to errors in lipid metabolism, while the Ldlr ^{-/-} females may be more sensitive to chronic stress.

Conclusion: Overall, the preliminary findings in this project suggest that chronic DE exposure and HFD alter microglial morphology and number in a sex-dependent manner, with significant protein expression changes in pathways relevant to neurodegenerative processes. Additional research is underway to ascertain whether DE-induced microglial states overlap with microglial signatures implicated in Alzheimer's disease and other neurodegenerative conditions, which could provide a deeper mechanistic insight into how air pollution contributes to neurodegenerative disease risk.

Data-Driven Prediction of Nanoparticle-Mediated Tumor Volume Reduction Using Neural Ordinary Differential Equations

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Background:

Lipid nanoparticles (LNPs) are increasingly used as delivery platforms for cancer therapeutics, but their antitumor efficacy varies substantially across formulations and experimental conditions. Nanoparticle physicochemical properties, including size, surface charge, lipid composition, and surface modification, can influence systemic disposition, tumor delivery, and therapeutic response. However, experimentally evaluating large numbers of nanoparticle formulations is costly and time-intensive. Machine learning approaches may help identify relationships between nanoparticle characteristics and therapeutic outcomes, while longitudinal modeling can further account for the time-dependent nature of tumor response. This study developed a neural ordinary differential equation (Neural ODE) framework to predict longitudinal tumor volume reduction from LNP characteristics, treatment conditions, and therapeutic cargo.

Methods:

A curated LNP dataset containing 230 observations representing 38 longitudinal treatment trajectories from 12 studies was analyzed. Input variables included nanoparticle size, zeta potential, dose, therapeutic strategy, surface modification, and lipid composition, including cholesterol, ionizable lipid, and helper lipid descriptors. Missing size and zeta-potential values were supplemented using reported literature values when available; otherwise, observations with insufficient information were excluded. The outcome was tumor volume reduction relative to the corresponding vehicle control across multiple post-treatment time points. To reduce information leakage, trajectories were partitioned at the study level into training, validation, and test sets using a 50%/25%/25% group-based split. Categorical variables were encoded, continuous predictors were standardized, and time and response variables were independently scaled. A Neural ODE model was trained to learn the time-dependent rate of change in tumor response as a function of static nanoparticle and treatment characteristics. Model performance was evaluated using coefficient of determination (R^2), root mean squared error (RMSE), and mean absolute error (MAE). Feature influence was further assessed by measuring changes in test-set MAE after perturbation of individual static predictors.

Results:

The Neural ODE model captured overall temporal patterns in LNP-mediated tumor response across held-out studies. On the test set, the model achieved an R^2 of 0.64, RMSE of 0.14, and MAE of 0.11 on the scaled response. Feature-importance analysis indicated that therapeutic strategy, cholesterol composition, helper lipid composition, and surface modification contributed substantially to predictive performance. The indicator distinguishing estimated from experimentally reported zeta-potential values also affected model performance, suggesting that uncertainty in nanoparticle characterization may contain information relevant to cross-study prediction.

Conclusions:

Neural ODE modeling provides a framework for integrating nanoparticle characteristics with irregularly sampled longitudinal tumor-response data and predicting time-dependent therapeutic outcomes. The model captured meaningful cross-study temporal trends despite the limited number of available studies, but its moderate predictive performance indicates that broader experimental diversity and additional independent data will be required to establish generalizability. Future work will expand the framework to additional nanoparticle classes, including metallic and silica nanoparticles, and evaluate whether shared or nanoparticle-class-specific determinants govern antitumor response.



#6

Perinatal E-cigarette Exposure Perturbs Reproductive Development in Offspring

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Background: Recent reports indicate that about 7% of women use e-cigarettes during their pregnancy, exposing the fetus to harmful toxicants during critical developmental periods. Although prenatal e-cigarette exposure has been associated with altered developmental programming and adverse cardiovascular, neurological, and pulmonary outcomes in offspring, its effects on the development of male and female reproductive systems remain understudied. We hypothesized that perinatal e-cigarette exposure induces cell-specific transcriptomic and epigenomic alterations in the ovaries and testes of F1 offspring.

Methods: We exposed pregnant rats to daily e-cigarette aerosols from embryonic day 6 (E6) to postnatal day 21 (PND21), to HEPA filtered air (FA), 0% Nicotine Gold Leaf Blu E-cigarettes (PGVG), or 2.4% Nicotine Gold Leaf Blu E-cigarettes (NIC) (N=3/treatment). These exposures resulted in similar serum cotinine levels of a habitual smoker. Ovaries and testes were collected at PND21 and PND60, respectively, and profiled using single-nucleus RNA-seq and ATAC-seq.

Results: Although no changes to litter size between exposure groups was detected in the F1 generation, we observed fewer pups born to NIC exposed pups in the F2 generation (N=3-7; $p < 0.05$). However, our single nucleus analysis revealed that the addition of nicotine (NIC) in e-cigarette vapor in ovaries and testes closely correlates to PGVG at transcriptomic and epigenetic levels. In females, analysis of differentially expressed genes (DEGs) ($q < 0.05$) and differentially accessible peaks (DAPs) ($q < 0.05$) in early theca and preantral granulosa cells showed impairment of steroidogenesis pathways in both PGVG and NIC treated pups. Serum levels of androgenic hormones trended downwards in treated female pups, although none reached statistical significance. In males, we observed that key genes that are integral to spermatogenesis were significantly dysregulated across all detected differentiation stages in PGVG and NIC treated germ cells. Most notably, genes involved in sperm acrosome assembly and ciliary motility were particularly affected at the round spermatid stage.

Conclusions: Perinatal e-cigarette exposure in rats is associated with sex- and cell-type specific responses in the ovaries and testes of F1 pups. These molecular effects led to alterations of steroidogenesis in F1 females, dysregulation of key genes involved in spermatogenesis, and reduced fecundity evidenced by the reduction of litter size in the F2 generation. Together, these findings identify reproductive programs that are sensitive to developmental e-cigarette exposure and provide a foundation for investigating mechanisms by which e-cigarette exposures may influence reproductive phenotypes across generations.



#7

Metabolite Ratios, Not Metabolite Identity, Guide Non-NHP Species Selection: A Case Study with GATC-1021

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Background and Purpose:

GATC-1021 is a dual 5-HT_{2A} and 5-HT₆ agonist in development for opioid use disorder that engages both receptors without producing psychedelic activity and was highly active in fentanyl self-administration studies, supporting progression toward IND-enabling work. IND-enabling toxicology requires a non-clinical species that is metabolically representative of human. This study sought to identify and qualify such a species without relying on non-human primates (NHP).

Methods:

Hepatocyte stability was assessed across species using qualified half-life ($t_{1/2}$) as the endpoint, and metabolite identification was performed by a contract laboratory. An internal AI metabolism-prediction platform was used prospectively to predict cross-species metabolite differences and nominate non-NHP candidate species, informed by medicinal chemistry input. An expanded species panel (dog, mouse, minipig, rat, NHP) was profiled in hepatocytes and microsomes to test these nominations. To enable mechanistic confirmation, authentic reference standards of the N-desmethyl and O-desmethyl metabolites of GATC-1021, along with their corresponding N-¹³C- and O-¹³C-labeled isotopologues, were synthesized in-house and individually run through metabolite identification across species.

Results:

Qualified $t_{1/2}$ diverged more than sixty-fold across species (human 169 min, NHP 222 min, versus rat 2.70 min, minipig 2.07 min), disqualifying rat and minipig as metabolically representative of human. N-demethylation and O-demethylation were predicted and subsequently confirmed as the branch points driving this species divergence. Dog was the only non-primate species to qualitatively match human metabolites (N-demethylation, oxidation); mouse did not. However, metabolite proportions did not match: dog formed 75.71% of the oxidation metabolite by 20 minutes, versus 7.46% in human at 120 minutes, with no human metabolite exceeding 10%.

Conclusions:

On this quantitative basis, dog was disqualified as the primary GLP toxicology species. Qualitative metabolite overlap alone is insufficient for toxicology species selection; prospective in silico prediction, validated against cross-species metabolite identification data, effectively directed species testing rather than merely explaining results retrospectively. This workflow is generalizable to programs facing species divergence in metabolism during candidate progression.



#8

Interpretable Dual-encoder Transformer-based Deep Learning to Predict Nanoparticle-Protein Corona Inflammatory Responses

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Background & Purpose: Nanoparticle (NP)-induced inflammatory responses are determined not only by their intrinsic physicochemical properties but also by the biological identity acquired through protein corona (PC) formation. However, most computational nanotoxicology models rely primarily on NP-related features, while the contribution of PC to inflammatory responses remains poorly represented. This study aims to develop an interpretable dual-encoder deep learning framework that integrates NP physicochemical properties and PC biological identity to predict inflammatory responses, using cytokine level changes as model target.

Methods: Two curated datasets were integrated within a transfer-learning framework. A larger NP-only dataset containing 868 samples was used to pretrain an NP model using 12 physicochemical and experimental features. The model was subsequently transferred to a smaller paired NP-PC dataset containing 87 samples to get the NP encoder. In addition to NP features, 30 representative PC proteins were incorporated using protein sequence embeddings generated by ESM-3, an advanced protein language pretrained model. The dual-encoder architecture independently encoded NP and PC information and integrated their latent representations through cross-attention mechanisms. Species and cytokine type were incorporated through feature-wise linear modulation (FiLM), and the model predicted percent changes in four inflammatory cytokines: TNF- α , IL-6, IL-1 β , and IL-10. Model performance was assessed using R^2 , RMSE, MAE, Pearson correlation, and Spearman correlation. Ablation analysis, feature importance and NP-PC features interaction analyses were conducted to evaluate model components and identify major determinants of inflammatory responses.

Results: The dual-encoder model achieved a held-out test R^2 of 0.54 ± 0.02 , RMSE of 0.39 ± 0.01 , MAE of 0.27 ± 0.01 , Pearson correlation of 0.74 ± 0.01 , and Spearman correlation of 0.57 ± 0.03 . Ablation analysis demonstrated that removing NP pretraining reduced the test R^2 from 0.54 to 0.29, while removing NP information or PC information further reduced R^2 to 0.05 and 0.12, respectively. Removing ESM-3 protein representations decreased R^2 to 0.14, and eliminating cross-attention reduced R^2 to 0.42. These results support the importance of using pretrained models and the value of integrates NP-PC features. Model interpretation identified zeta potential and dose as the most influential NP-related features, whereas Apolipoprotein A-II, vitronectin, and fibronectin were among the most influential PC proteins. Interaction analysis further revealed interconnected relationships between NP physicochemical characteristics and PC components associated with predicted cytokine responses.

Conclusion: This study presents an interpretable dual-encoder framework that combines NP physicochemical properties with PC biological identity to predict inflammatory responses. Transfer learning from a larger NP-only dataset and incorporate an advanced protein pretrained model improved prediction in the limited paired NP-PC dataset. The ablation and interaction analyses demonstrated that both NP characteristics and PC information contribute substantially to model performance. By explicitly modeling NP-PC interactions, this framework provides a data-driven approach for investigating how NP properties and PC biological identity jointly shape inflammatory responses and may support mechanism-informed assessment of nanomaterial immunotoxicity.



#9

Inhalation Exposure to Ambient Ultrafine Particulate Matter and Its Impact on Red Blood Cell Homeostasis in Mice

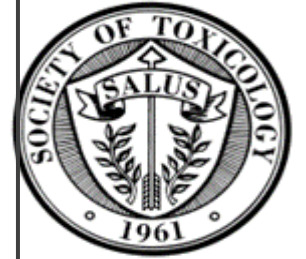
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Background and Purpose: Ambient particulate matter (PM) is a complex mixture of airborne solid particles and aerosols, and is among the criteria air pollutants regulated by the U.S. EPA. Within this mixture, the ultrafine fraction (UFP, aerodynamic diameter $< 0.1 \mu\text{m}$) is of particular concern: their small size allows more efficient deposition in the distal lung, and their large surface area per unit mass increases the area available for particle-cell interaction, giving UFP greater biological potency than the larger fine fraction on an equal mass basis. Ambient UFP exposure has been associated with cardiovascular, respiratory and metabolic outcomes. However, despite evidence that inhaled UFP enters the systemic circulation, circulating red blood cells (RBCs) remain an understudied target, even though they are the most abundant cell type in blood and are essential for oxygen transport and cardiovascular homeostasis. Their premature clearance has been linked to thrombosis, inflammation and increased iron burden on the spleen. Populations living and working near major roadways experience repeated low-level UFP exposure, making systemic targets beyond the lung relevant to real-world risk. This study investigates whether six months of concentrated ambient UFP exposure alters circulating blood cell indices, RBC turnover, splenic burden and erythropoietic signaling. We hypothesize that UFP exposure accelerates RBC turnover, producing reduced RBC counts, elevated splenic iron and a compensatory rise in plasma erythropoietin (EPO).

Methods: Wild-type C57BL/6 mice were exposed beginning at six months of age to concentrated ambient UFP in a whole-body inhalation chamber for six months, reaching approximately 12 months of age at necropsy. The Versatile Aerosol Concentration Enrichment System (VACES) drew ambient PM from a high-traffic roadway intersection and concentrated it to approximately 7–10 $\times$ ambient levels; a cascade impactor was used to size-select the PM to the ultrafine fraction ($< 0.1 \mu\text{m}$). Control animals received HEPA-filtered air. Both groups were exposed 5 hours/day, 4 days/week at a consistent time, with standard housing situations; all procedures were reviewed and approved by the University of California, Irvine, Institutional Animal Care and Use Committee (IACUC). Complete blood counts (CBC) were performed. Eryptosis was assessed by Annexin V phosphatidylserine (PS) externalization, with RBC size and membrane complexity quantified by forward and side scatter (FSC/SSC) on flow cytometry; hemolysis was quantified by free hemoglobin absorbance. At necropsy, absolute spleen weight and spleen-to-body weight ratio were recorded and splenic iron content determined by colorimetric assay. Plasma EPO was measured by ELISA. The numbers of mice were male-air ($n = 6$), male-PM ($n = 7$), female-air ($n = 8$) and female-PM ($n = 10$), with the number analyzed per endpoint varying by sample availability and volume. Data were analyzed by one-way or two-way ANOVA with post hoc pairwise comparisons in GraphPad Prism.

Results: In males, UFP exposure produced selective leukocyte changes without evidence of RBC loss. Absolute monocyte count was lower in UFP-exposed males than in air controls (0.09 ± 0.09 vs $0.20 \pm 0.00 \times 10^3/\text{mm}^3$; $p = 0.0226$). Absolute granulocyte count was also reduced (0.54 ± 0.14 vs $0.70 \pm 0.07 \times 10^3/\text{mm}^3$, $p = 0.0449$), and total white blood cell (WBC) showed a trend in the same direction but did not reach significance (3.50 ± 1.08 vs $4.74 \pm 1.39 \times 10^3/\text{mm}^3$, $p = 0.1118$). RBC and platelet parameters were unaffected: RBC count (10.83 ± 1.11 vs $10.98 \pm 1.15 \times 10^6/\text{mm}^3$), HGB (16.09 ± 1.39 vs $16.30 \pm 1.83 \text{ g/dL}$), HCT (53.27 ± 4.87 vs $53.98 \pm 6.01\%$), MCV (98.57 ± 2.94 vs $98.40 \pm 1.82 \text{ fL}$), MCH, MCHC, RDW (22.83 ± 0.61 vs $23.06 \pm 0.47\%$) and PLT (1305 ± 215 vs $1167 \pm 238 \times 10^3/\text{mm}^3$) showed no exposure effect ($p > 0.05$), indicating that circulating RBC mass and morphometry were preserved. Spleen-to-body weight ratio did not differ by exposure in either sex. Plasma EPO did not differ significantly by exposure within either sex, but concentrations were significantly higher in male than female mice among PM-exposed groups ($p = 0.0366$). Eryptosis, hemolysis and splenic iron analyses are in progress.

Conclusions: By the evidence thus far, six months of concentrated ambient UFP exposure did not significantly reduce circulating RBC counts or hemoglobin concentration, or enlarge spleen, or increase plasma EPO, indicating that circulating RBC profile was maintained under this exposure regimen. The observed reductions in circulating monocyte and granulocyte counts could indicate recruitment of these phagocytic cells from the circulation into the lung, or UFP-associated cytotoxicity. Completion of the eryptosis, hemolysis and splenic iron analyses will determine whether the preserved circulating RBC profile reflects an absence of RBC toxicity or subclinical/sublethal damage not captured by standard hematologic indices. This study examines RBC homeostasis as a potential systemic target of ambient UFP exposure, aiming to better inform health risk assessment for near-roadway populations.



#10

PM2.5 Exposure as a Modifier of Maternal Skeletal Remodeling and Postpartum Bone Recovery

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Background & Purpose: Pregnancy and lactation require extensive maternal skeletal remodeling to meet the calcium demands of fetal development and milk production. Fine particulate matter (PM2.5) exposure has been associated with adverse skeletal outcomes. However, its effects on the maternal skeleton during periods of increased calcium demand remain poorly understood. This study investigated whether gestational PM2.5 exposure alters maternal bone outcomes following lactation.

Methods: Pregnant C57BL/6 mice were exposed to concentrated ambient PM2.5 or filtered air (FA) during gestation. Exposures occurred at the Air Pollution Health Effects Laboratory (APHEL) using a Versatile Aerosol Concentration Enrichment System (VACES), which concentrates ambient particles while preserving their physicochemical properties. Maternal femurs were collected following sacrifice after weaning and analyzed using micro-computed tomography. Cortical outcomes included cortical area fraction (Ct.Ar/Tt.Ar) and polar moment of inertia (J). Trabecular outcomes included bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), connectivity, connectivity density, Euler characteristic, and relative bone mineral density (relative BMD). Linear regression was used to evaluate associations between litter size at birth and maternal bone outcomes and to determine whether these relationships differed by exposure.

Results: PM2.5 exposure did not significantly affect cortical or trabecular microarchitectural outcomes overall. However, PM2.5- exposed dams exhibited a nonsignificant directional pattern of slightly increased Tb.Th and Tb.Sp, decreased BV/TV, connectivity, and connectivity density, and a less negative Euler characteristic. Litter size was not significantly associated with these trabecular endpoints. In contrast, relative BMD decreased significantly with increasing litter size in PM2.5-exposed dams (slope = -82.01, $p = 0.010$), but not in FA dams (slope = 78.20, $p = 0.162$), with a significant exposure by litter size interaction ($p = 0.010$).

Conclusions: Gestational PM2.5 exposure did not produce significant gross changes in maternal bone microarchitecture at the examined endpoints, although coordinated directional differences in trabecular measurements may suggest alterations occurring in skeletal remodeling. Future histological analyses will determine whether PM2.5 induces changes in bone remodeling at the cellular and tissue levels, even when significant changes in bone microarchitecture are not overtly observed.



#11

Prenatal Secondhand E-Cigarette Aerosol Exposure and Offspring Responses to Allergic Airway Challenge

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Background: Electronic cigarettes (e-cigarettes) generate aerosols containing nicotine, ultrafine particles, and potentially harmful constituents, including formaldehyde and acetaldehyde, resulting in both primary and second-hand exposures. Although e-cigarettes are often perceived as less harmful than combustible cigarettes, the health effects of second-hand e-cigarette aerosol exposure during pregnancy and its potential consequences for offspring pulmonary development remain incompletely understood. This study investigated the effects of gestational second-hand e-cigarette aerosol exposure, with and without subsequent postnatal re-exposure, on respiratory function and pulmonary inflammatory markers in offspring using a mouse model. Outcomes were evaluated according to prenatal exposure (filtered air or e-cigarette aerosol), postnatal exposure (filtered air or e-cigarette aerosol), and ovalbumin (OVA) sensitization status, with analyses stratified by sex.

Methods: Pregnant C57BL/6 mice were exposed whole-body to either filtered air or e-cigarette aerosol generated from a 50/50 propylene glycerol/glycerin solution with no more than 2% nicotine concentration for 2 hours/day throughout 18 days of gestation. The aerosol was delivered from a third-generation mod with 1 puff generated for 2 seconds every 28 seconds. F1 offspring were evaluated at postnatal day 28 (PND 28) using whole-body plethysmography (vivoFlow®, SCIREQ, Canada) to assess respiratory parameters at baseline and following methacholine challenge (0–50 mg/mL). Following the initial assessment, offspring were exposed to either filtered air or e-cigarette aerosol for 2 hours/day, 4 days/week, for 7 weeks. A subset of offspring from each air/e-cig exposed group underwent OVA sensitization to induce an asthma-like phenotype. Offspring were evaluated according to prenatal exposure, postnatal exposure, OVA sensitization, and sex. Respiratory function was reassessed using a methacholine challenge, and lung tissue was collected for quantification of cytokines using a multiplex immunoassay. Plasma cotinine was also assessed as a biomarker of systemic nicotine exposure.

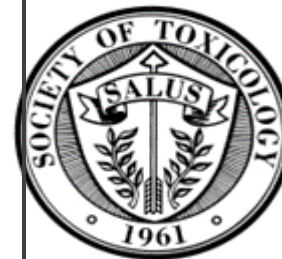
Results: Plasma cotinine was detected in offspring following secondhand e-cigarette aerosol exposure, confirming systemic nicotine exposure (air exposed groups Mean = 0 ± 0.14 , e-cig exposed groups Mean = $0.767 \mu\text{g/ml} \pm 0.19$; $p = 0.0001$; $n = 5$ per group). Respiratory function evaluated on PND 28 following methacholine challenge differed by sex within the same exposure groups, with the most notable difference observed in prenatal e-cig exposed male offspring. In prenatal e-cig exposed males, Penh, an indirect breathing-pattern-derived index, was higher compared with prenatal e-cig exposed females (Mean = 0.686 vs. 0.340 ; $p = 0.034$; methacholine conc. = 25 mg/ml ; $n = 10$ per group).

Across exposure groups, OVA-sensitized offspring had higher pulmonary IL-5, IL-2, TNF- α , IFN-

γ , and IL-1 β concentrations than the corresponding non-OVA groups (all $p < 0.05$). The largest difference was observed for cytokine IL-1 β which increased from an average mean of $22.19 \pm$

5.1 to 176.25 ± 96.7 $p = 0.0002$ ($n = 5$ per group). E-cigarette exposure without OVA sensitization produced comparatively limited changes in measured pulmonary cytokine concentrations. Among OVA-sensitized offspring, e-cigarette exposure was associated with changes in selected cytokines compared with air-exposed controls. Data was summarized as mean $\pm$ standard error. Current group comparisons were evaluated using analysis of variance (ANOVA). A probability value $p < 0.05$ was considered significant.

Conclusion: OVA sensitization was associated with the most pronounced changes in pulmonary cytokine concentrations, indicating that allergic challenge was a key contributor to the inflammatory response observed in this model. The cytokine profile reflected changes in both type 2-associated and pro-inflammatory mediators. E-cigarette exposure alone produced relatively limited alterations in the measured pulmonary cytokines, while prenatal and postnatal e-cigarette exposure appeared to modify selected respiratory and immune outcomes in OVA-sensitized offspring. Differences observed between male and female offspring warrant further investigation using formal sex $\times$ exposure interaction analyses. Collectively, these findings provide evidence that gestational second-hand e-cigarette aerosol exposure, with subsequent postnatal exposure and allergic challenge, may influence offspring pulmonary and immune responses and highlight the need for continued investigation of potential maternal and offspring health consequences of second-hand e-cigarette exposure.



#12

Shape influences toxicity of simulated environmental nanoplastics across rodent testicular cell types

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Introduction: Plastic pollution remains unchecked as greater than 400 million tons of consumer and industrial waste is produced annually. Natural weathering of plastic waste yields environmental nanoplastics (ENPs), with distinct morphologies. Studies show that in-house simulated ENPs (sENPs) have enhanced membrane permeability compared to commercially manufactured (pristine) nanoplastics used in most toxicological assessments. Amid growing concern that NPs can accumulate in tissues, our goal is to understand how sENP exposure affects the integrity and functions of testicular cell types, and whether distinct shapes influence NP toxicity.

Methods: In-house polystyrene jagged-edged and rounded, crystalline sENPs were generated by grinding sourced bulk plastic, followed by accelerated UV/ozone weathering, filtration (<800 nm), concentration, and ultrasonication. Based on environmentally relevant concentrations, ENPs were diluted to 10-400 µg/mL in culture medium. We observed cell-line and primary cell effects across three cell types, Sertoli, Leydig (with or without hCG stimulation), and spermatogonia after 24 hours of treatment and measured viability and proliferation (MTT/BrdU), steroid production (ELISA), and mRNA expression (RT-qPCR).

Results: In testicular cell line models, most concentrations decreased cell viability and altered proliferation depending on the cell type after treatment with both ENP models, with the most profound effects observed in Leydig cells starting at 50 µg/mL ENP. Testosterone level increased after exposure to ENPs in Leydig cell models. mRNA expression of cell-specific functional markers was altered, particularly in Sertoli cells and C18-4 spermatogonia. Overall, jagged-edged nanoplastics were more deleterious compared to the rounded, crystalline shape.

Conclusion: Exposure to sENPs perturbed all testicular cell types across multiple assays at environmentally relevant concentrations. This data strongly supports the hypothesis ENP morphology confers distinct levels of toxicity, with stronger effects observed after treatment with the jagged-edged formulation. Future investigation aims to understand the mechanisms underlying ENP toxicity to develop mitigation and intervention strategies.



#13

Benchmarking Uncertainty Quantification Methods for Drug-Induced Liver Injury Prediction

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Background and Purpose. Drug-induced liver injury (DILI) is a leading cause of drug attrition and post-market withdrawal. QSAR models predict DILI from chemical structure, but most reports focus on ranking accuracy and neglect calibration — the property that a model's stated confidence matches empirical accuracy. Miscalibrated probabilities mislead regulatory triage, where confidence drives whether a compound is advanced or deprioritized. Uncertainty quantification (UQ) methods such as Monte Carlo (MC) dropout and deep ensembles produce calibrated estimates and have been benchmarked on solubility and ADME, but no published work compares them head-to-head on DILI with rigorous calibration evaluation, applicability-domain analysis, and label-noise sensitivity. This study addresses that gap.

Methods. Four classifiers were trained and evaluated on DILIrank 2.0 (Olubamiwa et al., 2025): random forest (RF; bootstrap UQ), plain feedforward neural network (NN), NN with MC dropout (50 forward passes per compound), and a 5-member deep ensemble. Of 1,336 annotated drugs, 108 biologics/polymers plus 56 compounds failing molecular-weight or deduplication filters were excluded, yielding an 856-compound primary set (526 DILI-positive, 330 DILI-negative); a 535-compound verified subset (vMost + vNo only) was retained for label-noise stratification. All models used identical 2,048-bit Morgan fingerprints (ECFP4, radius 2). Five-fold Bemis–Murcko scaffold-based cross-validation ensured test compounds were structurally novel relative to training. All metrics were reported with 1,000-resample bootstrap 95% confidence intervals. Analyses covered predictive performance (AUROC, PR-AUC), calibration (expected calibration error [ECE], reliability diagrams), selective prediction (accuracy vs. coverage), and applicability domain (Spearman correlations of Tanimoto nearest-neighbor similarity with uncertainty and correctness).

Results. Predictive performance was statistically indistinguishable across methods (mean AUROC 0.727 RF, 0.717 NN, 0.720 MC dropout, 0.713 deep ensemble; bootstrap 95% confidence intervals overlapped throughout). Calibration diverged sharply: mean ECE 0.088 (RF), 0.176 (MC dropout), 0.193 (deep ensemble), 0.203 (NN). RF produced the best-calibrated probabilities; all three neural methods were systematically overconfident on low-probability predictions. Selective prediction improved accuracy for every method — RF the most (+0.110 at 50% coverage; 0.685→0.794), followed by deep ensemble (+0.085) and MC dropout (+0.079) — confirming informative uncertainty. Uncertainty also tracked applicability domain: Spearman correlations between nearest-neighbor Tanimoto similarity and uncertainty were negative for all methods (RF $\rho = -0.241$, $p < 1e-12$; MC dropout $\rho = -0.152$, $p < 1e-5$; deep ensemble $\rho = -0.182$, $p < 1e-7$), with corresponding positive correlations with correctness. Structurally novel compounds were therefore both flagged uncertain and genuinely less accurate. Restricting to the verified subset improved calibration for all three neural methods (ECE ≈ 0.14) but not RF, indicating the vLess ambiguous class injects label noise that neural calibration absorbs but RF resists.

Conclusions. On DILI prediction, the four methods differ negligibly in ranking accuracy but meaningfully in the reliability of their confidence estimates. For regulatory triage, where trust in a probability matters as much as its value, this distinction is decisive. Random forests, often treated as a baseline, produced the most reliable probabilities on DILIrank 2.0 and were most robust to label noise; neural UQ methods offered informative but overconfident uncertainty, particularly on ambiguous labels. All three methods' uncertainty estimates tracked structural novelty, supporting their use as automated applicability-domain flags. Results support routine calibration reporting alongside AUROC in DILI benchmarks and motivate cautious interpretation of neural probabilities on small, noisy toxicology datasets where miscalibrated confidence is a hidden source of downstream error.



#14

Chronic inhalation of diesel exhaust induce hypercholesterolemia and dysregulates carbohydrate metabolism

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Background and Purpose: Inhalation of ambient particulate matter (PM) induces cardiometabolic syndrome, including atherosclerosis, type 2 diabetes mellitus, and metabolic dysfunction-associated steatotic liver disease. Previously, short-term (2-week) exposure to diesel exhaust (DE) induced systemic transcriptomic changes and disrupted lipid homeostasis in Apolipoprotein E knockout (ApoE KO) mice. However, the molecular mechanisms driving the DE-induced hyperlipidemia and dysregulation in carbohydrate metabolism remain uncharacterized. This study sought to identify key genes governing alterations in lipid and carbohydrate metabolism following chronic DE exposure.

Methods: 6-week-old low-density lipoprotein receptor knockout (LDL-R KO) mice were fed either a high fat diet (HFD) or normal chow diet and exposed to filtered air (FA) or DE (at 250 µg/m³, 6 hours/day, 5 days/week) for 18 weeks. At the end of the study, plasma was collected to measure lipid and glucose levels, and liver, intestine, and aorta were harvested. To compare atherosclerotic lesions across exposure groups, whole aortas were stained with Oil Red O, and en face lesional area was quantified using BioDock AI-assisted image analysis software. Hepatic mRNA expression levels were analyzed via quantitative real-time PCR (qRT-PCR) using SYBR Green to evaluate metabolic pathways and inflammatory cytokines.

Results: Although LDL-R KO mice predictably develop dyslipidemia and atherosclerotic lesions under HFD conditions, 18 weeks of intermittent DE exposure significantly exacerbated both hypercholesterolemia and hyperglycemia compared to FA-exposed controls. Interestingly, while hepatic and plasma cholesterol levels were elevated in DE-exposed mice, en face aorta lesional areas showed no significant difference between groups. qRT-PCR analysis revealed that PI3K, a key regulatory gene within the hepatic insulin-AKT-mTORC1 signaling axis, was significantly downregulated following DE exposure, suggesting elevated hepatic gluconeogenesis. Furthermore, we observed a significant downregulation of MVD and SQLE, which both encodes rate-limiting enzymes in de novo cholesterol biosynthesis. This likely reflects a negative feedback response driven by excessive hepatic cholesterol accumulation originating from other potential mechanisms.

Conclusions: Chronic DE exposure disrupts carbohydrate metabolism via downregulation of hepatic PI3K-mediated glycemic control. While additional research is required to identify the specific mechanisms that drives dysregulation in systemic lipid homeostasis, our results show how PM exposure promotes cardiometabolic toxicity and provide therapeutic targets to address air pollution-induced metabolic disease.



#15

Beyond Clinical DILI Classifications: A Fit-for-Purpose Framework for Benchmarking the NAMkind™ Liver Model in Drug Discovery

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Background: Drug-induced liver injury (DILI) remains a major cause of compound attrition, clinical trial termination, and post-marketing regulatory action. New Approach Methodologies (NAMs) for hepatotoxicity are commonly benchmarked against clinical classification systems such as FDA DILIRank and DILIST.

Although these classifications are valuable for communicating observed human hazard, they may not fully reflect the needs of drug developers, who must identify actionable liabilities, prioritize compounds, and guide optimization before clinical exposure. Clinical classifications also may not convey differences in mechanism, biological detectability, frequency of occurrence, or confidence in the supporting evidence.

Objective: To examine whether clinical DILI classifications alone provide an adequate benchmark for evaluating the performance of NAMkind™, a 3D multicellular human liver NAM, and to propose a fit-for-purpose framework that considers both the clinical reference evidence and the intended context of use in drug discovery.

Methods: Performance of NAMkind™, a 3D multicellular human liver model that integrates albumin, ATP, glutathione, and LDH endpoints, was evaluated using a 113-compound dataset spanning multiple DILI concern categories. Clinical DILI classifications were considered alongside factors affecting their suitability as benchmarking standards, including mechanism of injury, exposure-response relationships, time to onset, biological detectability, applicability domain, translation to human outcomes, clinical frequency, and confidence in the supporting evidence. Representative compounds were used to illustrate how these factors may influence interpretation of NAMkind™ performance.

Results: Clinical severity, frequency, biological detectability, and translation to human outcomes do not necessarily align. Methotrexate illustrates a detectability challenge because its clinically relevant hepatotoxicity involves chronic fibrosis-related processes that may not be reproduced in short-duration in vitro systems. Fialuridine illustrates a human-translation challenge because severe clinical hepatotoxicity emerged despite limited warning from conventional preclinical models. Simvastatin illustrates the distinction between clinical frequency and mechanistic liability because clinically apparent DILI is rare despite evidence of hepatocellular stress and biological activity. These examples demonstrate that reference compounds can test fundamentally different properties of a NAM. Nevertheless, compounds may contribute equally to performance calculations despite substantial differences in mechanism, assay detectability, clinical incidence, and evidentiary support. Analysis of a 113-compound dataset spanning multiple DILI concern categories showed that NAMkind™ exposure-normalized multi-endpoint responses were generally associated with clinical DILI classification while also revealing biologically informative discordances. These included compounds with strong clinical concern but limited assay detectability, as well as compounds with measurable in vitro liabilities despite low clinical incidence. Such findings suggest that benchmark selection and interpretation can meaningfully influence perceived model performance. This raises a central benchmarking question: Should all clinically classified compounds contribute equally to NAM performance assessment regardless of their relevance to the model's intended context of use?

Conclusions: Clinical DILI classifications answer the question, "What risk has been observed in patients?" Drug developers must also ask, "What liabilities can be detected prospectively and acted upon during development?" We propose that clinical classifications remain an important benchmark but be supplemented by a fit-for-purpose framework incorporating mechanism of injury, biological detectability, applicability domain, exposure relevance, translation to human outcomes, clinical frequency, and confidence in the reference evidence. This approach could improve benchmark selection and interpretation of NAM performance while supporting compound prioritization and drug-development decisions.



#16

Advancing Target Identification and Lead Optimization Through Mechanistic Toxicology: An AI-Integrated Approach for Translational Safety Assessment

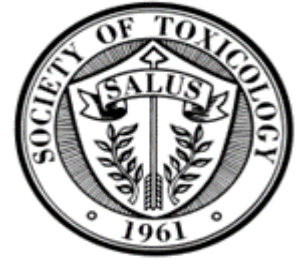
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Integrating toxicological insight into early discovery remains challenging despite persistent safety-related attrition in drug development. Many clinical safety failures originate from target biology or compound design and may be identifiable earlier through systematic mechanistic analysis. This challenge is especially relevant for antibody–drug conjugates (ADCs), in which efficacy and toxicity are shaped by antibody specificity, antigen expression, linker stability, and payload properties. Conventional models often struggle to predict ADC toxicity because chemical, biological, toxicological, and clinical evidence is fragmented across independent data sources.

We describe an agentic AI approach for translational safety assessment that coordinates specialized agents focused on chemistry, biology, toxicology, pharmacology, and clinical evidence. Each agent retrieves, evaluates, and contextualizes information from curated scientific resources, peer-reviewed literature, and clinical trial records, then contributes to iterative consensus cycles that generate mechanistic hypotheses. The approach was applied to an ADC use case involving tubulin-inhibitor payloads. Agents assessed target expression, payload cytotoxicity, linker chemistry, toxicophore patterns, and clinical adverse event profiles, with emphasis on hepatic and hematologic safety risks. The agentic workflow integrated mechanistic evidence across discovery, preclinical, and clinical domains. It identified potential contributors to toxicity, including linker instability, payload lipophilicity, and exposure of sensitive tissues such as hepatocytes and bone marrow. Toxicophore mapping and mechanistic substructure inference highlighted chemical motifs associated with cellular stress-response pathways. Cross-domain evidence synthesis showed alignment between predicted safety liabilities and adverse events reported for ADCs with similar design features. Iterative reasoning improved biological plausibility assessment and supported refinement of safety hypotheses into actionable design considerations. Combining mechanistic toxicology with agentic AI enables more systematic exploration of safety-relevant biological and chemical mechanisms during target identification and lead optimization. By emphasizing evidence synthesis, contextual reasoning, and translational interpretation, this approach supports earlier recognition of toxicity risks in complex modalities such as ADCs and may help guide the design of safer, more effective therapeutics.



#17



#18

Integrating Target-Centric Safety Assessment with Human iPSC-Derived Neuronal MEA as a New Approach Methodology for Early De-Risking of Seizure Liability

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Background and Purpose

Drug-induced seizures are an important central nervous system (CNS) safety liability that can contribute to compound attrition when identified during GLP toxicology studies and/or later stages of development. Target-centric safety assessment integrates biological, genetic, mechanistic, and internal/external evidence to identify potential safety liabilities earlier in drug discovery. While several efforts have integrated seizure-relevant targets from adverse outcome pathways (AOPs) and drug discovery resources, new approach methodologies (NAMs) have demonstrated fit-for-purpose utility for interrogating relevant on- and off-target signaling associated with compound-related seizure liability. To bridge this gap, we evaluated an integrated strategy combining target-centric safety assessment with functional testing using human induced pluripotent stem cell (hiPSC)-derived neuronal multielectrode arrays (MEAs).

Methods

Target safety assessment evaluated the biological plausibility of seizure-related effects associated with intended pharmacology, informed by target-specific biology, seizure-relevant mechanistic evidence, and available internal and external knowledge. Functional seizure liability was evaluated using co-cultures of hiPSC-derived excitatory and inhibitory neurons with astrocytes. Five anonymized proprietary compounds representing multiple pharmacological mechanisms were evaluated by MEA at five concentrations spanning 0.3-300 μ M across the compound set, with four replicate wells per concentration. Concentrations were selected based on in vivo toxicology findings, C_{max} values associated with convulsion and non-convulsion findings, and/or solubility. Acetaminophen served as a negative control and strychnine, picrotoxin, and pilocarpine served as positive controls. Firing, bursting, network bursting, rhythmicity, synchrony were assessed from 15-min MEA recordings before and after 1 h of compound exposure.

Results

Controls showed the expected neuronal response. All five proprietary compounds showed alterations in neuronal firing and network activity associated with a seizurogenic phenotype, with concentration-related changes across multiple MEA parameters and differences in potency among compounds. Common responses included decreased firing rate; increased burst and network-burst frequency; shorter bursts and network bursts; fewer spikes per burst and network burst; and increased regularity of network bursting. Compounds D and E showed the greatest MEA responses, Compound B an intermediate response, and Compounds A and C smaller changes. Compound D increased firing and network-burst activity at 1-3 μ M, followed by decreased activity at 10 μ M and loss of measurable activity at 30 and 100 μ M. Prior in vivo findings included convulsions with Compounds A, B, D, and E in rat, dog, or monkey studies. No convulsions were observed with Compound C in nonclinical studies. Compounds A and C are in the same class. MEA detected seizure-associated changes in all four compounds with prior in vivo convulsions.

Conclusions

Integration of target-centric safety assessment with human hiPSC-derived neuronal MEA provides a mechanistically informed NAM strategy for early seizure-liability assessment. Target- and AOP- based information can identify seizure-relevant mechanisms warranting further evaluation, while MEA provides complementary functional evidence of compound-induced perturbations in human neuronal network activity. This proof-of-concept demonstrated seizure-associated MEA responses across compounds with diverse pharmacological mechanisms and concordance with available in vivo convulsion findings. Integration with systemic exposure and in vivo findings can further contextualize compound-specific risk. This target-to-functional-phenotype framework may strengthen weight-of-evidence assessment and support earlier identification and prioritization of seizure liabilities. Evaluation of a larger set of seizurogenic and non-seizurogenic compounds is needed to establish predictive performance and broader applicability.

β 2-Adrenergic Receptor Agonists-Induced Pro-Inflammatory Genes in Macrophages and the Effects of Carvedilol and R-carvedilol

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Background

Prolonged activation of β 2-Adrenergic receptors (β 2-ARs) by chronic stress facilitates an immunosuppressive microenvironment and cancer development. Our previous studies showed that the β -blocker carvedilol and the non- β -blocking enantiomer R-carvedilol inhibited UV-induced inflammation, immunosuppression and skin cancer development. However, the mechanisms behind these effects of carvedilol and R-carvedilol are unknown. Because β 2-ARs are expressed on many types of skin cells as well as immune cells such as macrophages, the goal of this project is to examine the effects of carvedilol and R-carvedilol on inflammation-related genes induced by β 2-AR agonists in the murine macrophage cell line RAW 264.7.

Methods

RAW 264.7 macrophages were treated with the β 2-AR agonist salmeterol (β 2-AR specific agonist) along with other β -adrenergic agonists epinephrine and isoproterenol at 1 μ M. The cells treated with salmeterol were co-treated with the β 2-AR specific antagonists ICI 118,551, the racemic mixture carvedilol (β 1/2-AR antagonists), and the enantiomers R- or S-carvedilol at 1 μ M. The MAPK/ERK pathway activity was assessed with the MEK inhibitor U0126 (1 μ M). RAW 264.7 cell lysates were generated by repeated passage through a 26-gauge needle, and total RNA was isolated using the RNeasy Mini Kit (Qiagen). cDNA was obtained with the High-Capacity cDNA Reverse Transcriptase Kit (Thermo Fisher Scientific). The cDNA was amplified with Power SYBR Green PCR Master Mix (Applied Biosystems) and primers for murine genes and β -actin. Real-time PCR was performed on a CFX96 Real-time Thermal Cyclers Detection System (BioRad) and analyzed using β -actin as the normalization control with the $2^{-\Delta\Delta C_t}$ method. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) was used to measure the relative mRNA expression levels of IL-1 β , IL-6, and COX-2.

Results

Salmeterol (1 μ M) significantly increased IL-1 β mRNA expression compared to the vehicle control ($p < 0.001$) and increased COX-2 expression ($p < 0.05$), while IL-6 levels were not statistically significant. Consistent with β 2-AR stimulation by the β 2-AR specific agonist salmeterol, treatment with the nonselective adrenergic agonists epinephrine and isoproterenol (1 μ M each) similarly increased IL-1 β levels. Pharmacological blockade using carvedilol (β 2-AR nonselective antagonist; 1 μ M) and ICI 118,551 (β 2-AR selective antagonist; 1 μ M) significantly suppressed salmeterol-induced IL-1 β expression ($p < 0.01$). While S-carvedilol (1 μ M), which has high binding affinity for the β -ARs, strongly attenuated salmeterol-induced IL-1 β , R-carvedilol (1 μ M), which lacks β -AR-blocking activity, failed to reduce IL-1 β expression. The MEK inhibitor U0126 at 1 μ M partially attenuated salmeterol induced IL-1 β expression.

Conclusions

Activating β -ARs induced increased mRNA expression of pro-inflammatory genes, particularly IL-1 β , in macrophages. S-carvedilol, but not R-carvedilol, prevented salmeterol-induced IL-1 β , implying that carvedilol's anti-inflammatory properties in the experimental conditions presented here are dependent on β 2-AR inhibition. β 2-AR activation induced by agonists may be also dependent on the MAPK pathway. Since this study did not involve UV, it remains undetermined whether UV-induced inflammation is dependent on β 2-AR and whether the anti-inflammatory activity of carvedilol and R-carvedilol is dependent on β -blockade.



#19

Effects of SCFAs on Neoplastic Transformation of Murine Epidermal Cells JB6 P+

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Background: Short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, are naturally occurring metabolites and have been investigated for their potential effects on cancer-related cellular processes due to their ability to alter cellular membranes and shift intracellular calcium (Ca^{2+}) homeostasis. The JB6 P+ mouse epidermal cell line is commonly used to study cancer-related neoplastic transformation. Anchorage-independent colony formation in soft agar is an important characteristic associated with cellular transformation and tumorigenic potential, while the sulforhodamine B (SRB) assay can be used to evaluate cell viability and determine whether observed effects are associated with cytotoxicity.

Objective: The objective of this study was to evaluate the effects of acetate, propionate, and butyrate at various concentrations on JB6 P+ transformation using soft agar colony formation assay and the cellular viability after SCFA exposure using SRB assay. The soft agar assay was used to determine whether treatment affected anchorage-independent colony formation after 20-day incubation, while the SRB assay was used to determine whether the treatments caused loss of cell viability following 96 hours of incubation.

Methods: Soft agar assay was performed using 0.5% noble agar dilute for the bottom layer followed by seeding 40,000 cells/mL in a 0.33% noble agar diluted top layer and treatments of

0.1 and 100 μM SCFAs. SRB utilized 3,000 cells/well where SCFAs treatments were added as a serial dilution from 100 to 0.001 μM . After 96-hour incubation, cells were fixed using trichloroacetic acid and then dyed using sulforhodamine B. Finally, a 10 mM unbuffered Tris base was added for plate reading. Both assays utilized 10% FBS EMEM media for cell incubation, and all SCFA concentrations were diluted using media.

Results: Soft agar analysis demonstrated a concentration-dependent difference in the response to butyrate treatment. Treatment with 100 μM butyrate resulted in a reduction in colony formation. Because anchorage-independent colony formation is associated with neoplastic transformation and tumorigenic potential, the reduction in colonies observed with 100 μM butyrate may indicate an inhibitory effect on a cancer-related cellular phenotype. Notably, the reduction in colony formation observed with 100 μM butyrate can be accompanied by signs of significant growth inhibition resulting in decreased cell viability as shown by the SRB assay incubation with butyrate. In contrast, acetate and propionate at both 0.1 and 100 μM did not produce significant changes in colony formation. Similar results reflected in the SRB assay showing no significant loss of cell viability following treatment with acetate and propionate.

Conclusion: Overall, acetate and propionate did not demonstrate significant cytotoxicity toward JB6 P+ cells at the concentrations tested and did not produce significant changes in anchorage-independent colony formation. In contrast, butyrate produced a concentration-dependent decrease in cellular viability as measured by the SRB assay, with a corresponding reduction in anchorage-independent colony formation at 100 μM in the soft agar assay. Because soft agar colony formation is associated with cellular neoplastic transformation and tumorigenic potential, the reduction in colony formation following butyrate treatment suggests that butyrate may suppress transformation-related growth in addition to reducing overall cellular viability. However, the decrease in SRB viability indicates that the reduced colony formation cannot be attributed exclusively to an inhibition of transformation. Therefore, the results support an inhibitory effect of butyrate on JB6 P+ cell growth and anchorage-independent growth, while acetate and propionate showed comparatively limited effects under the conditions tested. Future studies should examine longer incubation periods and additional concentrations to determine whether the effects of butyrate become more pronounced over time and to establish a clearer dose-response relationship. In particular, further investigation of calcium-dependent signaling mechanisms may help explain the stronger effect of butyrate compared with acetate and propionate. Replication and validation of these findings with longer treatment periods and complementary assays will be necessary to confirm the observed effects and clarify the underlying mechanism.



#20



Thank you

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