# Corners

## CAAT feed

### Virtual Seminar Honoring Marty Stephens

In recognition of the recent passing of Martin (Marty) L. Stephens, CAAT will be hosting a virtual webinar to celebrate the many contributions he made to humane science over the course of his career. The virtual event will be held Wednesday, April 17 from 3 to 5 pm ET, and will consist of presentations by past colleagues and close friends of Marty's, followed by an open forum during which attendees will have the opportunity to share memories and speak to Marty's remarkable career in alternatives and humane science. Please use the following link to register to attend this event. https://jh.zoom.us/webinar/register/WN_QeTjxxUXS4OuhYPHmkF_-g

### EBTC to Honor Marty Stephens with Annual Award

EBTC announced at the annual SOT meeting on March 13, 2024 that it is inaugurating a new award named after Marty Stephens, who passed away on February 17, 2024. The award will acknowledge individuals or organizations that made outstanding contributions to the field of evidence-based toxicology. Marty was the founding Director of EBTC and led the organization from its formation in 2011 until 2015. Under his leadership, EBTC formed a Board of Trustees and Scientific Advisory Council comprised of the leaders in the field, who continue with EBTC to this day. Marty continued contributing to the EBTC as a member of the Board until the very end. Marty's vision, insightfulness, thoroughness, and dedication were instrumental in jump-starting the EBT movement and making the EBTC the vibrant network-driven organization it is now. The EBTC leadership would like to make sure that Marty's legacy is continued through this award. The details and criteria for nominations for the award will be announced later this year, and the award will be presented at the SOT annual meeting, starting in 2025.

### MPS World Summit Late-Breaking Abstracts Open until April 20, 2024

Late-breaking abstract submissions for the 2024 MPS World Summit are open! Closing April 20, 2024 at 11:59 pm PT, this is an excellent opportunity to have your work reviewed by leaders in the field and join an engaged cohort of speakers and presenters at this year's event! The MPS World Summit will be taking place in the scenic and highly innovative city of Seattle, Washington, from June 10-14. To submit your abstract, please visit: https://mpsworldsummit.com/2024-abstract-submission/

### EBT Journal Special Issue on Preregistration templates open!

The Evidence-Based Toxicology Collaboration has launched a new format for making contributions to the EBT journal. Authors are invited to submit "Preregistration Templates", a new type of manuscript designed to make it easier for researchers to reuse each other's methods and gain the benefits of preregistering their studies. For more information (including how to win prizes!), please visit: https://think.taylorandfrancis.com/special_issues/preregistration-templates/

### NIH Announces Unprecedented Multi-Hundred-Million-Dollar New Program Targeting NAMs

From the NIH program snapshot, "*The NIH Common Fund's Complement Animal Research in Experimentation (Complement-ARIE) program will speed the development, standardization, validation, and use of human-based New Approach Methodologies (NAMs)*." Strong focus will be placed on microphysiological systems (MPS), which puts CAAT and its collaborators in an excellent position to leverage this remarkable investment. This program will certainly be a topic of discussion at the forthcoming MPS World Summit in Seattle, Washington. You will have the opportunity to hear directly from co-host and NIH program director, Danilo Tagle and team about this enormous opportunity. To learn more about this initiative, please visit: https://commonfund.nih.gov/complementarie; registration for the MPS World Summit is available at: https://mpsworldsummit.com

### 2023 Next Generation Humane Science Award Recipient Announced!

CAAT is pleased to name James Tronolone as the 2023 Next Generation Humane Science Award recipient. James is currently pursuing his PhD in Biomedical Engineer-

ing at Texas A&M University under Dr Abhishek Jain. CAAT is thrilled to support James as he continues to pursue his doctorate degree, advance MPS technology, and make meaningful contributions both within his professional community and to the broader NAMs field.

## Upcoming Events

### Save the Date: 13th World Congress on Alternatives and Animal Use

Mark your calendars, WC13 has been scheduled for August 31 - September 4, 2025 in Rio de Janeiro, Brazil! The theme of the congress will be "3Rs Integrating 3 Worlds: Human, Animal and Environmental Health." From the organizers, "*WC13 will showcase advancements in both academic and industrial sectors of life sciences, particularly focusing on sustainable alternatives to animal testing. The congress will feature focus sessions on the following topics: Reduction, Refinement and Animal Welfare, Replacement, Toxicology, Biomedical Research, Regulatory Testing, 3Rs Education, Bioethics, New Technologies, 21st Century Vision Implementatio*n." More information will be made available soon and shared both through the WC13 official newsletter and CAATfeed.

### Workshop organized by the Coalition to Illuminate and Address Animal Methods Bias (COLAAB) to Explore Animal Methods Bias in Biomedical Research Funding

"Animal methods bias" is the unfounded preference for animal-based research methods which affects the fair consideration of animal-free approaches and hampers their uptake and dissemination. Non-animal methods, also termed new approach methods (NAMs), including human cell-derived tissue chips and organoids, have advanced rapidly over the last decade, making them the first choice of model for many researchers for a variety of reasons. But because animals are still considered by many to be the "gold standard" for biomedical research, studies that do not use animals can be assessed in a biased manner. Evidence of this animal methods bias has already been established in the realm of scientific publishing – when reviewers or editors expect or request that animal experiments be included in studies as a condition for publication. Anecdotal evidence suggests that this problem may also affect the assessment of grant proposals, thus imposing a funding barrier for research using NAMs.

The Coalition to Illuminate and Address Animal Methods Bias (COLAAB) is an international collaboration of researchers and advocates aiming to provide concrete evidence on the existence and consequences of this bias and to develop and implement solutions for overcoming it.

Hosted by the COLAAB, and scheduled for May 16, 2024, 10 - 2 pm ET, a workshop will carry out the following charge:

1. Gather broad stakeholder perspectives, including from:
   a. Researchers, especially NAMs developers and users
   b. Funders, public and private across various global regions
   c. Scholars of peer review bias – Advocates, lawmakers, and other agents of change
2. Characterize animal methods bias in funding, including:
   a. Current evidence and evidence needs
   b. Its impact on research, innovation, health outcomes, and researchers' careers
   c. Parallels with animal methods bias in publishing and other contexts in which research is evaluated
3. Identify potential solutions, both individual and structural

Register for the workshop here: https://jh.zoom.us/meeting/register/tJEoc-yqqTMsHtdZ6Jrg0WBEKiOycvBN7kc0

### PCRM Summer Immersion May 30 to June 1

The Physicians Committee for Responsible Medicine is hosting a 3-day education and training program for students, postdocs, and early career researchers interested in human-based *in vitro*, *in silico*, and *in chemico* methods and technologies to advance biomedical research, improve human health and environmental safety and risk assessment, and reduce and replace animal models.

The seminar will take place in Washington, DC from May 30 to June 1, 2024, with CAAT's own Thomas Hartung and Kathrin Herrmann serving on the event's steering committee and Thomas Hartung and Lena Smirnova invited to speak. To register, please visit the following link: https://www.pcrm.org/ethical-science/summer-immersion

## Conferences and Conversations

The first quarter of the year proved to be exceptionally active for CAAT Leadership, marked by a series of significant engagements and events. Among these, CAAT members traveled to Salt Lake City for another bustling Society of Toxicology (SOT) conference. Hosting three distinct events, including the annual Satellite Meeting in collaboration with AFSA, a DIT workshop, and the EBTC mixer and award ceremony, CAAT provided ample opportunities for networking and collaboration. The enthusiastic participation underscored the value of these gatherings in fostering connections within the community. Notably, this year's SOT meeting spotlighted the burgeoning realm of artificial intelligence (AI), a focal point that complemented CAAT's scheduled talks. CAAT Director Thomas Hartung delivered multiple insightful presentations on ToxAIcology, positioning CAAT at the forefront of this intersection between AI and toxicology.

In February, Thomas Hartung and Alex Maertens left for Denver, CO, to deliver critical insights on the transformative potential of AI in toxicology. Dr Maertens' aptly titled presentation, "Chemical Safety Meets the AI Algorithm," reached many attendees and prompted later discussion on the integration of AI into safety assessments. Additionally, Dr Maertens led a very well attended workshop in collaboration with the FDA, titled "Bring Toxicology Back to the Future: Using AI to Make Toxicology Human-Focused," further emphasizing the human-centric approach facilitated by AI advancements.

In April, CAAT Leadership traveled to Germany for DNT-5, a conference series spearheaded by CAAT since 2006, see the Food for Thought … contribution in this issue of *ALTEX*. DNT-5 provided, yet again, a dynamic platform for leading experts in the field to discuss and explore new cutting-edge technologies and the

challenges facing developmental neurotoxicology (DNT). The event's agenda included sessions on the implementation of human pluripotent stem cell (hPSC)-based assays, organoids, and *in silico* methodologies for modeling developmental neurotoxicity, along with discussions on regulatory decision-making processes.

### Publications

Hartung, T., Morales Pantoja, I. E. and Smirnova, L. (2024). Brain organoids and organoid intelligence from ethical, legal, and social points of view. *Front Artif Intell 6*, 1307613. doi:10.3389/frai.2023.1307613

Kleinstreuer, N. and Hartung, T. (2024). Artificial intelligence (AI) – It's the end of the tox as we know it (and I feel fine). *Arch Toxicol 98*, 735-754. doi:10.1007/s00204-023-03666-2

Paller, C., Tukachinsky, H., Maertens, A. et al. (2024). Pan-cancer interrogation of MUTYH variants reveals biallelic inactivation and defective base excision repair across a spectrum of solid tumors. *JCO Precis Oncol 8*, e230025. doi:10.1200/PO.23.00251

Sillé, F. and Hartung, T. (2024). Metabolomics in preclinical drug safety assessment: Current status and future trends. *Metabolites 14*, 98. doi:10.3390/metabo14020098

Smirnova, L. and Hartung, T., (2024). Creating tiny human "organs" to test medicines… and more! *Front Young Minds 12*, 1320408. doi:10.3389/frym.2024.1320408

Smirnova, L. and Hartung, T. (2024). The promise and potential of brain organoids. *Adv Mater*, e2302745. doi:10.1002/adhm.202302745

Smirnova, L., Modafferi, S., Schlett, C. et al. (2024). Blood extracellular vesicles carrying brain-specific mRNAs are potential biomarkers for detecting gene expression changes in the female brain. *Mol Psychiatry*. doi:10.1038/s41380-023-02384-6

Cruelty Free INTERNATIONAL
Ending animal experiments worldwide

### Canada's Cruelty Free Cosmetics legislation comes into effect

On December 22, 2023, Canada's Cruelty Free Cosmetics legislation came into force. The law bans cosmetics testing on animals and prohibits the sale of cosmetics that rely on new animal testing data to establish the product's safety. With the passage of Bill C-47, Canada became the 44th country to pass laws to end or limit animal testing for cosmetics, which leaves the US the only country in North America without a national ban on animal testing for cosmetics. Mexico passed a ban in September of 2021.

The United States has still not passed the federal Humane Cosmetics Act despite 12 states adopting their own cruelty-free cosmetics legislation: California, Nevada, Illinois, Virginia, Maryland, New Jersey, Maine, Hawaii, Louisiana, New York, Oregon, and most recently, Washington state, which goes into effect in January 2025. In addition, two other states, Arizona and Vermont, saw similar legislation introduced for consideration this session. The state laws are broadly similar and are based on the agreements reached between Cruelty Free International and the Personal Care Products Council and others and are reflected in a refreshed version of the US Humane Cosmetics Act (HR 5399) that was re-introduced in September 2023. HR 5399 currently has 209 bipartisan cosponsors but has not yet received a hearing.

### American Bar Association calls for the advancement of non-animal research and testing methods

The American Bar Association (ABA) passed a resolution at its midyear meeting in February supporting the promotion, development, and use of methods that seek to replace, reduce, and refine animal models in biomedical research and testing. In addition, the resolution also calls for US Congress and US federal agencies to remove barriers to, and create incentives for, the application of non-animal models in regulatory testing and federally sponsored research.

Founded in 1878, the ABA provides leadership in legal ethics and professional responsibility through the adoption of standards that serve as models of regulatory law governing the legal profession. This is the first time that the ABA has spoken on animal testing.

The report accompanying the resolution highlighted how the use and further development of non-animal methods will advance animal wellbeing, foster innovation, protect the environment, and improve the value for money of taxpayer-funded research investments leading to safer products and better-quality medicines. The report also recognized the importance of the bipartisan Humane and Existing Alternatives in Research and Testing Sciences (HEARTS) Act (H.R. 1024, 118th Cong.), which addresses specific shortcomings in existing law governing research proposals funded by the National Institutes of Health (NIH).

### US NIH working group report calls for greater investment in non-animal testing methods

In early February the Director of the US NIH, Monica Bertagnolli, accepted recommendations put forth in a NIH working group report, "Catalyzing the Development and Use of Novel Alternative Methods (NAMs)", which calls for greater investment in non-animal testing methods.

The report highlights the many ways non-animal research and testing methods are already benefiting many areas of biomedical research, "*including cancer, diabetes, cardiovascular disease, Alzheimer's disease, mental illness, infectious disease, rare diseases, and more*". It also sets out specific recommendations for the NIH to prioritize and further capitalize on the benefits of NAMs in the future.

The report follows a multi-year effort by Cruelty Free International to advance the goals of the HEARTS Act and was the direct result of the language used in the Fiscal Year (FY) 2022 Labor, Health, and Human Services Appropriations bill, as requested by the HEARTS Act co-authors.

Cruelty Free International has noted that many of the recommendations included in the report also reinforce the need for actions included in the HEARTS Act. For example, the report recommended that the NIH "*establish dedicated and centralized core facilities as national or regional resources to develop and run NAM assays to reduce costs, leverage scale, and provide training*" – The HEARTS Act would establish a "National Center for Alternatives to Animals in Research and Testing" within the NIH that would achieve this goal.

Reference: Catalyzing the Development and Use of Novel Alternative Methods. Report to the Advisory Committee to the Director, December 2023: https://acd.od.nih.gov/documents/presentations/Working_Group_Report.pdf

### Cruelty Free Science Day highlights non-animal testing technology at Washington, DC's Capitol building.

On March 7, Cruelty Free International hosted a "Cruelty Free Science Day" in Washington, DC at the US Capitol – a special event held in conjunction with Representative Chris Pappas – lead sponsor of the HEARTS Act (HR 1024).

Legislators and staff were invited to learn about some of the innovative non-animal testing methods that could be used to replace animals in experiments, and how they can help advance humane science by supporting three bills that were featured at the event – the HEARTS Act, the Humane Cosmetics Act, and the Companion Animal Release from Experiments (CARE) Act (HR 2878). Attendees were also greeted by six beagles who were rescued from an Envigo breeding facility.

Co-hosted by Cruelty Free International's Head of Public Affairs for North America, Monica Engebretson, and Deputy Director of Science and Regulatory Affairs, Laura Rego Alvarez, the event featured information on cell-based methods and organs-on-a-chip from companies including XCellR8, Ananda Devices, and Emulate. Doctoral students from Johns Hopkins University also attended the event to present their work developing miniature brain models, known as organoids, to study neurotoxicity and neurodegenerative diseases.

### EVA wins 2024 Geoffrey Deckers Award

On the day of Geoffrey Decker's birthday, January 13, Cruelty Free Europe announced the recipient of the 2024 Geoffrey Deckers Award: EcoVegAnimals, EVA, from Bosnia and Herzegovina.

Established in 2019 in Sarajevo, EVA's fundamental goal is to ensure the adequate legal protection of all animal species. Their work includes the establishment and implementation of regulations and cruelty-free activities related to the use of animals for education, pharmaceuticals testing, and the cosmetics industry.

The award honors Geoffrey Deckers, the much-loved former Chair of the European Coalition to End Animal Experiments and Cruelty Free Europe, who passed away in June 2020.

The €6,000 award is given to groups demonstrating a commitment to ending animal tests and projects likely to make the most efficient and effective use of funds towards this goal.

EVA will use the funds to continue the introduction of non-animal approaches to replace the use of animal experiments across biomedical faculties at the University of Sarajevo. The funds will also support EVA's 3rd Conference on Humane Alternatives in Education.

### Cruelty Free International launches policy asks ahead of imminent UK general election

With less than 12 months to go before the UK holds nationwide parliamentary elections, Cruelty Free International has delivered a series of initiatives aimed at raising the profile of animal testing as a political issue and persuading key political parties to make policy commitments in their election manifestos.

Building on the launch of the Pledge Cruelty Free campaign in 2024, Cruelty Free International kicked off the year with an event held in Parliament. Members of Parliament (MPs) were urged to pledge their support for policies which would: strengthen the UK cosmetics testing ban; end the use of animals in chemicals testing; and initiate a transition away from the use of animals in all science and education. Four MPs signed the pledge following their attendance at the event. MPs were also briefed on Cruelty Free International's "Target Zero" report, which sets out concrete steps towards the achievement of the organisation's headline transition goal.

In February, Cruelty Free International joined with ten animal protection and welfare NGOs asking for all parties "*to commit to developing a nationwide roadmap for phasing out all experiments on animals*" before the next General Election.

### Cruelty Free Europe urges EU MEPs to uphold plans to end tests on animals in Europe

With little over two months before European Parliament elections, Cruelty Free Europe has urged Members of European Parliament (MEPs) to pledge their commitment to policies which will: protect cruelty-free cosmetics; end the use of animals in the regulatory system; and promote EU-wide action for animal-free science. In coordination with its member NGOs from across Europe, Cruelty Free Europe will be seeking commitments from sitting MEPs and candidates for election to ensure a strong base of support in the next Parliament. With clear resolutions from the current Parliament, commitments from the Commission, and the support of 1.2 million EU citizens, there is a strong mandate to pursue changes across all elements of animal testing in the EU. However, with predictions of shifting political dynamics in the next Parliament and the prospect of a new Commission in the coming months, Cruelty Free Europe aims to secure as much support as possible at this early stage in the process.

## EUSAAT

*European Society for Alternatives to Animal Testing*

### COST Action IMPROVE – update

Currently, over 200 members of the COST Action "*CA21139 – 3Rs concepts to improve the quality of biomedical science (IMPROVE)*" are busy working on different projects and publications, organizing events, etc. in the four working groups (WG) Quality and Translatability of Science, Implementation, Dissemination, and Education.

For example, currently the survey entitled "*Framing the Role of Animal Care Staff and Lab Technicians in Experimental Planning and Conduct of Animal Studies*" is running and inviting animal care staff and lab technicians to take part. This anonymous survey explores the involvement of animal care staff and lab technicians in discussions around planning and conducting research projects involving animals. The survey is available in eight languages predefined for this study, takes about 10-15 minutes to complete, and is open under the following link until the end of April 2024: https://umfragen.unimedizin-mainz.de/index.php/355157?lang=en

### Next meetings of the COST Action IMPROVE

A training school about 3D cell culture methods in Kaunas, Lithuania, will take place on May 9-10, 2024 including talks from different stakeholders (e.g., company MatTek), a WG 1 & 2 meeting will take place in Utrecht, the Netherlands, on June 17-18, 2024 right before a 3Rs Centre meeting, and a cross-WG Workshop on "Ethics and 3Rs" will take place in Istanbul, Turkey, on September 2-3, 2024.

In addition to these in-person meetings, an online-webinar series dedicated to young researchers and innovators is being organized, and some other activities such as online webinar series and basic science conferences will be supported by the COST Action IMPROVE in 2024. For example, IMPROVE is looking forward to participating in the ESTIV (European Society for Toxicology in Vitro) conference in Prague on June 3-6, 2024, and in the networking event "How to organize and respond to the pressure to phase out animal experimentation in neuroscience" at the FENS FORUM in Vienna on June 25-29, 2024.

### More information on the COST Action IMPROVE

*Website:* https://www.cost-improve.eu
*LinkedIn:* https://www.linkedin.com/company/improve-3rs-concepts-to-improve-the-quality-of-biomedical-science-ca21139/
*X (Twitter):* https://twitter.com/caimprove
*Facebook:* https://www.facebook.com/profile.php?id=100094711647507

Anyone can apply to participate in the working groups. More details and the Memorandum of Understanding can be found at https://www.cost.eu/actions/CA21139/

Further, we would like to highlight that COST Action Members can apply for Short Term Scientific Missions (STSMs), for which financial support will be granted for stays of, e.g., up to 3 months in a host lab. In addition, there is the possibility to receive ITC grants to visit conferences for young scientists from ITC countries. More information about STSMs: https://cost-improve.eu/calls-grants/#section-3andITC grants: https://cost-improve.eu/calls-grants/#section-1

### EUSAAT/EU3Rnet at the 1st International Conference of the Würzburg Initiative 3R (WI3R)

EUSAAT and EU3Rnet were invited to present their activities and participate in the 1st International Conference of the Würzburg Initiative 3R (WI3R) in Würzburg, Germany, on June 5-7, 2024 (https://wi3r.de/). Winfried Neuhaus will give the keynote "3Rs – past and progress" to open the scientific program on the second day. Within the 1st WI3R symposium the German Research Foundation (DFG) will grant the 10th Ursula M. Händel Animal Welfare Prize, which recognizes scientists who have made exemplary and sustained efforts to improve the welfare of animals in research. The €80,000 prize is currently the largest prize of its kind in Germany and is awarded biannually (https://www.dfg.de/en/research-funding/funding-opportunities/prizes/haendel-prize)

### EUSAAT Congress 2024

The next EUSAAT congress 2024 will take place on September 18-20, 2024. We hope for extensive support and that many stakeholders and researchers from different areas of the 3Rs community will participate. We will again pay special attention to enabling the participation of as many young scientists as possible through low participation fees and a variety of Young Scientist Travel Awards. We are very excited about the topics and key areas in 2024 and are happy to receive thematic suggestions.

We are delighted to have found a thematically diverse and scientifically excellent team for our scientific committee: A high-ranking field of about 50 distinguished colleagues have agreed to support the EUSAAT Congress 2024 in Linz. You will find the list of members of the Scientific Committee at: https://eusaat.eu/eusaat-congress/24th-edition/scientific-committee-2024/

We are very pleased to announce that the EUSAAT Congress 2024 will once again be supported by an overwhelming number of co-organizers and sponsors. You will find the list of co-organizers and sponsors at: https://eusaat.eu/eusaat-congress/24th-edition/organizers-sponsors-2024/

The tentative topics can be found at: https://eusaat.eu/eusaat-congress-2022/highlights/announcements/eusaat-congress-2024-practical-info-tentative-topics/

If you would like to propose a session, please contact us via: congress@eusaat.eu

Information about registration can be found at: https://eusaat.eu/eusaat-congress/24th-edition/registration-2024/

General information about the EUSAAT congress 2024 can be found at: https://eusaat.eu/eusaat-congress/24th-edition/congress-2024/

EUSAAT and the organizing committee are very much looking forward to hosting the 3Rs community at the European 3Rs Congress 2024 in Linz and to meeting you there!

LUSH PRIZE

SUPPORTING ANIMAL-FREE TESTING

The shortlist for the Lush Prize 2024 was announced in January, with 55 projects from around the world being shortlisted across eight categories, including the new "Major Science Collaboration Award".

This new award category celebrates international collaborations looking to develop non-animal techniques or approaches more widely. It is part of our non-financial "Recognition Awards" that also include one recognizing the important work politicians do to help end animal testing and support human-relevant science.

The Lush Prize rewards initiatives across science and campaigning that work to end or replace animal testing, particularly in the area of toxicology research.

Since 2012 we have provided £2.69 million to 126 projects in 35 countries.

Winners of six of the Lush Prize categories will share the £250,000 prize fund. All winners will be announced at an awards ceremony in London on 21 May.

The full shortlist can be seen on our website www.lushprize.org.

The mission of the RISK-HUNT*3R* project is to innovate safety testing strategies. The aim is to increase human relevance while overcoming reliance on animal testing. The project's ambition is to introduce a cutting-edge approach to risk assessment through the Alternative Safety Profiling Algorithm (ASPA). During the general assembly in February 2024, the consortium reaffirmed its commitment to refining ASPA modules. Real-life case studies will be used to demonstrate its versatility and applicability. A highlight was the showcasing of NAMASTOX, a user-friendly tool to guide the user through the ASPA workflow. The software, designed by the University Pompeu Fabra group, keeps track of each step of the risk assessment process and helps to manage uncertainties.

At the next general assembly, scheduled for June 2024 in Egmond aan Zee (NL), the consortium will convene to give updates on the ongoing case studies and ASPA workflow progress. A stakeholder symposium will allow experts from industry and regulation to actively give their input on ASPA. Crucial to a broad acceptance and use of ASPA is a participation of diverse stakeholder groups in its further development and refinement.

The June general assembly will also host a joint Summer School for ASPIS Academy and Young TPI (Transition Animal-free Innovations) researchers. The aim is to train early-stage researchers in the field of NAMs and to provide them with networking opportunities. Training courses, research exchanges, and discussions on data management and interpretation will form the core of these activities, all aimed at promoting the transition towards animal-free testing.

In March, a delegation from RISK-HUNT*3R* convened in Salt Lake City, USA, to participate in the 63rd annual SOT meeting. Our partners played pivotal roles as speakers and poster presenters in various sessions dedicated to new approach methodologies (NAMs) and new kinetic modeling approaches. Project material was also distributed at the ASPIS booth, including the latest newsletter issue #5, also accessible on our website (https://www.risk-hunt3r.eu/media-centre/).

## RISK-HUNT3R press review

RISK-HUNT*3R* partners have again published important work in mechanistic and applied toxicology:

Vlasveld and colleagues (2024) introduce a method using transcriptomics to identify potential drug-induced liver injury. In this article, the authors show that CHOP IDDIT3 (a transcription factor that promotes cell death and indicates a mitochondrial stress response) is a marker for drug effects linked to liver injury. Their discovery suggests that transcriptome analysis with the TXG-MAPr tool is useful to categorize compounds as potential hepatotoxicants.

Lambrecht et al. (2024) reveal a new non-apoptosis related role for BIM (a pro-apoptotic protein of the BH3-only family). BIM is shown to regulate mitochondrial dynamics and cellular energy metabolism, leading to oncotic necrosis. BIM deficiency shifts energy production away from mitochondria, reducing the dependency on malfunctional mitochondria and encouraging recovery from drug-induced liver injury. This was exemplified for paracetamol-induced liver injury.

Using metabolic profiling of neuronal cells, Suciu et al. (2024) identify a common metabolic signature of the mitochondrial complex-I inhibitors berberine, rotenone, and MPP+. Several of the perturbed biomarkers are associated with neurodegeneration: saccharopine, aminoadipate, and branched-chain ketoacids. Their upregulation occurred at concentrations that inhibited neurite outgrowth but did not affect cellular ATP levels. The study suggests that neurotoxicity of mitochondrial inhibitors may result from a combination of metabolic changes, rather than simple ATP depletion.

Finally, in their review, Rodriguez-Belenguer et al. (2023) explore the challenges of implementing NAMs to replace animal testing in toxicology. They discuss the use of combining multiple quantitative structure-activity relationship (QSAR) models (referred to as metamodels) to improve the predictivity of computational methods. Overall, metamodeling is presented as a promising approach for toxicity prediction that can leverage the strengths of different models.

## References

Lambrecht, R., Rudolf, F., Ückert, A. K. et al. (2024). Non-canonical BIM-regulated energy metabolism determines drug-induced liver necrosis. *Cell Death Differ 31*, 119-131. doi:10.1038/s41418-023-01245-7

Rodríguez-Belenguer, P., March-Vila, E., Pastor, M. et al. (2023). Usage of model combination in computational toxicology. *Toxicol Lett 389*, 34-44. doi:10.1016/j.toxlet.2023.10.013

Suciu, I., Delp, J., Gutbier, S. et al. (2024). Definition of the neurotoxicity-associated metabolic signature triggered by berberine and other respiratory chain inhibitors. *Antioxidants 13*, 49. doi:10.3390/antiox13010049

Vlasveld, M., Callegaro, G., Fisher, C. et al. (2024). The integrated stress response-related expression of CHOP due to mitochondrial toxicity is a warning sign for DILI liability. *Liver Int 44*, 760-775. doi:10.1111/liv.15822

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement n° 964537.

*Silvia Tangianu and Marcel Leist*

# The 3Rs Collaborative

## Introduction to the 3RC Artificial Intelligence Initiative

In a world where artificial intelligence (AI) increasingly intersects with critical aspects of safety evaluation and risk assessment, the 3Rs Collaborative (3RsC) is proud to announce the launch of a pioneering AI Initiative (https://3rc.org/ai/) co-led by Dr Szczepan Baran from VeriSim Life and Dr Weida Tong from the FDA. This initiative is designed to harness the power of AI to enhance the safety and risk assessment of consumer products, including drugs, environmental, and industrial chemicals. Our goal is to facilitate productive interactions between developers, end-users (e.g., researchers in academia, industry, government, and other scientific organizations), and other key stakeholders in the AI space, while encouraging appropriate use of AI technologies as an independent new approach methodology (NAM) for safety and risk assessment. By doing so, we aim to:

1. *Encourage collaboration:* Strengthen the network between technology developers, regulatory bodies, and end-users.
2. *Overcome barriers:* Identify and address challenges in the implementation of AI technologies.
3. *Promote responsible usage:* Advance the development and ethical application of AI in safety evaluation and risk assessment.

AI technologies, including Assistant AI, Predictive AI, and Generative AI, offer unique opportunities to revolutionize drug development and the 3Rs by:

- Extracting and integrating existing data to complement traditional studies.
- Predicting toxicity or efficacy, thus reducing reliance on animal testing.
- Generating synthetic data to simulate experimental studies, including virtual models.

As of March 2024, our initiative boasts 18 members from 13 diverse institutions, including pharmaceutical companies, government agencies, and non-profits. We had our first small in-person meeting at SOT in Salt Lake City in March. The collective vision is to achieve a broader understanding, acceptance, and application of AI in safety and risk assessment. We aim to foster confidence, collaboration, and regulatory acceptance, thereby advancing science and animal welfare. Despite AI's growing importance, challenges such as transparency, explainability, and bias need addressing, especially in regulatory contexts. Our initiative seeks to tackle these challenges through collaboration, aiming for an improved understanding and implementation of AI methods.

Individuals passionate about leveraging AI to improve safety evaluation and risk assessment are invited to join this initiative. If you are interested, please reach out to info@na3rsc.org for more information.

## Upcoming Events

The 3Rs Collaborative members are looking forward to hosting the 3Rs Sharing Conference in collaboration with the New Jersey Society for Biomedical Research on April 17 in San Francisco. We also look forward to presenting at the MPS World Summit on June 10-14 in Seattle, and having representatives from our initiative present at the European Organ-on-Chip Society Annual Meeting on July 3-5 in Milan.

## 3RsC & IQ MPS Webinar Series: Immune Competent & Lymph Node Models

In a continuation of past workshops on MPS models and various organs (https://3rc.org/mps/presentations/), the 3Rs Collaborative and IQ-MPS initiative hosted the first workshop in our 2024 workshop series on March 26, 2024 (https://www.youtube.com/watch?v=1H1bRAQNilQ&t=6381s). This year we are expanding topics to include disease states and other important MPS themes such as cell suppliers. This first workshop was comprised of six 3RsC member companies that each presented 15-minute data-driven presentations on their MPS. Summaries from each presentation can be found below.

The workshop began with event organizers Lauren Young, from the 3Rs Collaborative MPS initiative, and David Kukla, from the IQ-MPS affiliate, introducing the workshop series for 2024. Upcoming workshops include neuromuscular & fibrosis MPS on May 21 and 22, suppliers of MPS cells on September 17, and oncology MPS on November 12.

### *Hesperos*

Chief scientist James Hickman began the webinar by describing Hesperos' human-on-a-chip system. Functional immune cell recirculation is facilitated through various tissues such as liver, cardiac tissue, skeletal muscle, and kidney using gravity-induced flow technology on a rocking platform.

A presented case study showed addition of amiodarone caused selective infiltration of THP monocytes into cardiac tissue modulated by cytokine release from the tissues, without affecting skeletal muscle. A specific cytokine footprint and the interaction between immune and non-immune could be detected and measured.

A second case study showed compromised neuromuscular junction (NMJ) fidelity and increased NMJ fatigue in a model of ALS using a platform consisting of two molded chambers separated by microtunnels, allowing motoneurons to send axons through and form NMJs.

Finally, Dr Hickman discussed an *in vitro* central nervous system (CNS) model that assesses drug efficacy and toxicity measured by long-term potentiation. After biological signals including neuronal and astrocyte activity are confirmed, long-term potentiation is shown via AMPA and NMDA receptor manipulation. This model was used to identify the protective effects of Alzheimer's disease drugs such as donepezil.

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### *Mimetas*

Lenie van den Broek, director of biological discovery at Mimetas, provided a general introduction to their technologies, involving

several key building blocks within one system: epithelia, connective tissue, vasculature, and the immune system.

First, their immune-competent gut model, comprised of Caco-2, HT29, THP1, and MUTZ-3 cell lines, was described as a model for inflammatory bowel disease (IBD). Induction of inflammation by TNFα and IL-1β resulted in downstream IL-8 release and TEER reduction, which were rescuable by tool compounds. Additional work was presented on the development of IBD models featuring primary colon organoid tube in the Mimetas chip; plasticity of macrophages in proximity to the colon tube as well as recruitment of circulating monocytes to the colon tube were demonstrated.

A second vascular barrier model was discussed that allows for real-time measurement of barrier disruption by cytokines and quantification of stimulated T cell extravasation. Finally, immune compatibility of their vessel-tubules kidney model and vascularized liver was demonstrated.

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### *AlveoliX*

Giulia Raggi, AlveoliX project lead and operations manager, discussed their immune-competent lung-on-chip used for immunotherapy studies. This $^{AX}$Barrier-on-chip technology involves a 96-well plate with a porous flexible membrane; 3D stretching creates a bio-inspired breathing concept that mimics the lungs and connects to a variety of other tissue systems.

Cell sources for this model include peripheral blood mononuclear cells (PBMCs; mixed population of lymphoid and myeloid cells) and THP-1 cell line-derived macrophages. Cells were implanted into the epithelial or endothelial compartment of the organ chips, allowing for LPS-stimulated reduction in epithelial barrier integrity and transmigration of immune cells.

A case study was discussed for IL-2 cancer immunotherapy, currently approved for metastatic melanoma and associated with vascular leak syndrome. Using patient-derived primary alveolar epithelial cells, endothelial cells, and PBMCs, placebo comparisons to IL-2 therapy showed clinically relevant donor-donor variability.

A second study was conducted to investigate T-cell bispecific antibody toxicity – a therapy with high efficacy in mouse models but severe lung toxicity in monkey models. Lung-on-chip models predicted toxicity in humans measured by loss of barrier function, pro-inflammatory cytokines, cytotoxicity, and activation of CD4 and CD8 T cells.

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### *ImmuONE*

Louis Scott, commercial lead in immunology at ImmuONE, discussed their profiling of alveolar macrophages in response to inhaled compounds. Their technologies involve immune-focused cell culture models and tailored novel assessment strategies and approaches. A layer-by-layer development platform is used to develop various models from a monoculture of alveolar macrophages termed ImmuPHAGE™ to their innovative ImmuFIBROSIS™, which contains macrophages, epithelial cells, and fibroblasts in an inflamed system.

ImmuPHAGE™ includes terminal differentiation, phagocytic function, and a standardized response to challenges. ImmuLUNG™ retains an alveolar epithelial barrier and appropriate immune responses to various stimuli.

A major concern for safety in preclinical studies is the development of "foamy" macrophages which hinder progression to humans. Amiodarone addition to human macrophages allows for the determination of multiple profiling characteristics and endpoints, which is important as a potential non-clinical early screening tool. Known foamy-inducing and non-foamy-inducing inhalant compounds could then be compared in both human and rat immuPHAGE™ models for outcomes.

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### *Emulate*

Gautum Mahajan, principal scientific lead for Emulate, presented data on their lymphoid follicle chip. Important requirements for immunocompetent organ-chips include the development of resident immune cells, circulating immune cells, and vaccine response. A primary use of this model is in vaccine development.

Model characteristics include PBMC-isolated B and T cells at a 1:1 ratio along with monocyte-derived dendritic cells. Under flow conditions 3D nascent lymphoid follicles spontaneously form, unlike under static conditions. When the chip is exposed to bacterial antigens, class switching and plasma cell differentiation occur appropriately. Finally, a commercially available influenza vaccine applied to the chip produces anti-influenza antibodies, modulated by dendritic cells and CXCL13, with both high- and low-response groups representing donor-donor variability. When the cytokine response between the chip and serum was compared, similar clinically relevant responses were seen, however, GM-CSF was increased in the chip but not serum.

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### *React4Life*

Silvia Scaglione, chief scientist from React4Life, concluded our webinar with a discussion of their multi-organ *in vitro* platform that incorporates physiological flow and high-throughput systems for predictive and faster assays. Their immune-on-chip can create 3D cancer models, including neuroblastoma and pancreatic cancer, where cells are shown to reproduce *in vivo* behavior.

More complex environments are created with a separated tumor compartment to visualize tumor-specific immune cell infiltration: for example, a culture of natural killer cells is visible with apoptosis induction capabilities.

Alternatively, these systems can be used for testing drug efficacy, which they tested in an ovarian cancer organ-on-chip model. This emphasized the importance of a dynamic, capillary-like flow field to mimic the bloodstream, especially with 3D models. Other applications of this multi-organ cancer-on-chip were discussed, including cancer metastasis assays for cancer morphology, spread, and invasion capabilities. Finally, there is promise for personalized therapies by combining patient tissue explants and PBMCs to model accurate flow rate and cell recruitment.

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## More about the 3RsC

If you are interested in finding out more, visit our website: https://3rc.org/

Information about how to join can be found here: https://3rc.org/mps/faq-how-to-join/