



Corners



MPS World Summit

Please visit us at <https://mpsworldsummit.com/mps-world-summit-2023/> to register. Our conference hotel offers a limited number of rooms for our delegates: <https://bit.ly/3K1iZHN>

Announcement of the Winners of the 2022 CAAT Reduction Award and the CAAT Humane Education Award

Congratulations to Prof. Dr Stefan Schildknecht from Albstadt-Sigmaringen University, Germany, who won the 2022 CAAT Reduction Award for his project proposal titled “Development of an Adverse Outcome Pathway (AOP) for the relationship between ferroptosis induction and the degeneration of dopaminergic neurons”.

Congratulations to Dr Vivian Kral and Dr Christian Zoschke from Free University Berlin, Germany, who won the 2022 CAAT Humane Education Award for their project titled “3Rs Info Hub PRO – Your go-to place for training on non-animal research”. Each award includes prize money of \$6,000 USD.

For more information on each project, please see the full announcement at: <https://caat.jhsph.edu/programs/Refinement/>

CAAT’s Alexandra Maertens Recognized by ONTOX for the 2023 International Day of Women and Girls in Science!

Every year, February 11 marks the International Day of Women and Girls in Science, celebrating women’s vital role in science and promoting gender equality in STEM fields. ONTOX included Dr Alexandra Maertens, Assistant Scientist and Head of the Green Toxicology and Big Data Initiative at CAAT in honoring the ONTOX women scientists’ contributions to the advancement of toxicology.

To read more about the incredible women recognized this year, please visit ONTOX’s website at:

<https://ontox-project.eu/international-day-women-girls-science/>

Hartung Appointed Guest Editor of New Frontiers Research Topic

Thomas Hartung has been appointed guest editor of the research topic “Multi-system Inflammatory Syndrome observed Post-COVID-19: The Role of Natural Products, Medicinal Plants and Nutrients and the use of Prediction Tools Supporting Traditional Forms of Diagnosis” with the *Frontiers in Pharmacology* journal.

For more information, or to submit your research, please visit the article collection’s page: <https://www.frontiersin.org/research-topics/49690/>

Thomas Hartung listed among best scientists in Biology & Biochemistry as well as Medicine!

Research.com published the 2022 university and scientist rankings in all fields. CAAT’s own Thomas Hartung ranked worldwide #818 (US #506) for Biology and Biochemistry with 430 publications and 34,657 citations and #5,071 (US #2,855) for Medicine with 408 publications and 28,917 citations. He was one of 250 recognized medical scholars bringing Johns Hopkins to rank 2 of best medicine universities world-wide and one of 92 scholars bringing JHU to rank 8 for biology and biochemistry globally.

Evidence-Based Toxicology Collaboration Launches New Journal

The Evidence-Based Toxicology Collaboration (EBTC) has launched *Evidence-Based Toxicology*, a new open-science journal for toxicology and environmental health research, in collaboration with Taylor & Francis. To learn more, please visit the journal’s homepage: <https://www.tandfonline.com/journals/tebt20>



SOT Award Recipients from CAAT

Congratulations to Emily Golden, Dowlette Alam El Din, and Alan Kim for receiving SOT Awards!

- Emily Golden, M.F.S., Ph.D., is the recipient of two SOT Awards! She received the Bern Schwetz Travel Award from the National Capital Area Chapter (NCAC) of SOT and received the Regulatory and Safety Evaluation SS Post-doc Excellence Award.
- Dowlette Alam El Din (PhD Student) has been awarded an SOT Travel Award as well as 2nd place in the Society of Toxicology Stem Cells Specialty Section Excellence in Research Award.
- Alan Kim (PhD Student) received the SOT Endowment Award for the 2023 Korean Toxicologists Association in America (KTAA) Young Soo Choi Student Scholarship Award.

Recent Events

JHU and CAAT Attended January 30 Senate Briefing

At a Senate briefing held on January 30, Hopkins Associate Professor (and CAAT Board member and faculty) Dr Paul Locke spoke about the state of play for using non-animal alternatives in drug discovery and development. He covered the passage of the FDA Modernization Act and focused on three action items for Congress: Eliminating animal testing requirements in current statutes; providing funding to incentivize the development of non-animal alternatives; and providing oversight of federal agencies.

CAAT and JHU PhD and master's students Breanne Kincaid, Alan Kim, Dowlette Alam El Din, Yiguang Zhu, Leah Mönkemöller, and Haley Garbus attended the briefing, along with JHU and CAAT post-doctoral fellows Rebecca Critser and Emily Golden.

3Rs Webinar on Confronting Animal Methods Bias in Scientific Publishing

On February 6, CAAT hosted a 3Rs training webinar, organized by Kathrin Herrmann, Director of CAAT's Beyond Classical Refinement Program.

Catharine E. Krebs, PhD, from the Physicians Committee for Responsible Medicine (PCRM), talked about animal methods bias in scientific publishing, which is a newly defined type of publishing bias describing a preference for animal-based methods where they may not be necessary or where nonanimal-based methods may already be suitable, which impacts the likelihood or timeliness of a manuscript being accepted for publication.

Beyond the 3Rs: Why Academia Must Zero in on Human-relevant Replacement Methods

On February 23, Kathrin Herrmann, PhD, Director of CAAT's Beyond Classical Refinement Program gave a presentation in the 3R-Network BW Webinar series on why academia needs to zero in on human-relevant replacement methods.

Toxi-City: How ZIP Code Impacts Health and Life Expectancy

On March 4, 2023, Thomas Hartung, Director of CAAT, spoke at the American Association for the Advancement of Science (AAAS) Annual Meeting. Prof. Hartung's presentation was part of the panel, Toxi-City: How ZIP Code Impacts Health and Life Expectancy.

The talk spotlighted JHU's research on enviro-genomics with examples of novel health science in Baltimore and Maryland. His presentation showcased how basic, population, and community scientists promote research in cardiorespiratory health & airborne contaminants; chemical mixtures & emerging contaminants & health community, social & environmental determinants of health; and neurological health.

Organoid Intelligence (OI) Webinar

On March 9, 2023, CAAT hosted researchers as they discussed four tracks related to the OI field: The ethics of OI; Organoids, OI Clinical Potential; Electrophysiology and Learning Environment; Data Analysis. View the recording here: <https://bit.ly/40g9hqQ>
Passcode: Yon2xgE^

World Organoid Research Day

World Organoid Research Day was on Wednesday, March 22, 2023. Organoid Spheroid hosted a free online and in-person celebration featuring over 30 talks by the world's leading organoid researchers, including Thomas Hartung.

All talks were online and in person at Qkine, Cambridge, U.K. The event featured 5-minute talks live and on demand.

Evidence-Based Toxicology Collaboration (EBTC) at SOT 2023

The Evidence-Based Toxicology Collaboration hosted a booth and a reception and mixer discussing "Shaping the Future of Toxicology with EBTC 2.0" at the SOT 2023 Conference in Nashville, TN on March 22, 2023.

21st Century Toxicology: Sneak Preview of Ongoing Relevant Activities

The Center for Alternatives to Animal Testing (CAAT) and the Humane Society International (HSI)/Animal-free Safety Assessment Collaboration (AFSA) hosted a satellite meeting at the SOT 2023 Conference in Nashville, TN. The "21st Century Toxicology" series celebrated its 15th anniversary. This annual series, launched in 2008, was inspired by the 2007 National Academies' report "Toxicity Testing in the 21st Century: A Vision and a Strategy." This event was a hybrid event.



3Rs Training Webinar on Bioprinting

Our 3Rs training webinar, organized by CAAT's Beyond Classical Refinement Program Director Kathrin Herrmann, took place on March 28, 2023. Prof. Dr Jens Kurreck from Technical University Berlin talked about bioprinting of organ models for the replacement of animal experiments.

New publications

July Carolina Romero's Paper Published in *Frontiers in Cellular Neuroscience*

Frontiers has recently published an open access article from CAAT and the Johns Hopkins Department of Ophthalmology: Romero, J. C., Berlinicke, C., Chow, S. et al. (2023). Oligodendrogenesis and my-

elination tracing in a CRISPR/Cas9-engineered brain microphysiological system. *Front Cell Neurosci* 16, 1094291. doi:10.3389/fncel.2022.1094291

Organoid Intelligence publications and news points

On Tuesday, February 28, 2023, CAAT launched their research into Organoid Intelligence (O.I.). We had more than 500 news points including the *Financial Times*, CNN, *The Wall Street Journal*, BBC, and a special press release by Johns Hopkins University. Read more via the links below:

Lead Article

Smirnova, L., Caffo, B. S., Gracias, D. H. et al. (2023). Organoid intelligence (OI): The new frontier in biocomputing and intelligence-in-a-dish. *Front Sci* 1, 1017235. doi:10.3389/fsci.2023.1017235

Editorials

Hartung, T., Smirnova, L., Morales Pantoja, I. E. et al. (2023). The Baltimore declaration toward the exploration of organoid intelligence. *Front Sci* 1, 1068159. doi:10.3389/fsci.2023.1068159
Smirnova, L., Morales Pantoja, I. and Hartung, T. (2023). Brain-cell cultures: The future of computers and more? *Front Young Minds* 11, 1049593. doi:10.3389/frym.2023.1049593

Organoid intelligence hub

<https://www.frontiersin.org/journals/science/article-hubs/organoid-intelligence-a-new-biocomputing-frontier>

Lay summary

<https://www.frontiersin.org/journals/science/article-hubs/organoid-intelligence-a-new-biocomputing-frontier/lay-summary>



European Citizens' Initiative confirmed with 1.2 million valid signatures

A European Citizens' Initiative (ECI) – launched by Cruelty Free International in collaboration with cruelty-free brands including Dove and The Body Shop and over 100 animal protection organisations across Europe – was found to have received 1,217,916 authentic signatures, making it the seventh ECI out of a total of 90 registered to successfully pass the one million signature threshold.

The ECI calls on the European Commission to strengthen and protect the ban on cosmetics animal testing, transform EU chemicals regulation, and commit to phasing out all animal testing in the EU.

The European Commission will now carefully examine the ECI and Commis-

sion representatives will soon meet with the organisers to discuss the issues in more detail. A hearing in the European Parliament will follow this spring, including a possible vote on animal testing before the Commission gives its formal response and explains what actions it proposes.

Cruelty Free International launches the UK RAT List

Following on from the success of the EU Replace Animal Tests (RAT) List, Cruelty Free International has launched the UK RAT List, which highlights five animal tests that are still being conducted in the UK despite having accepted non-animal replacements.

The list includes skin irritation, skin sensitisation, Botulinum toxin batch po-

tenency and veterinary vaccine batch safety tests as well as antibody production, which use a combined total of at least 80,000 animals per year in the UK alone.

The UK RAT List is available here: <https://crueltyfreeinternational.org/replace-animal-tests-uk-rat-list>

Judicial Review of the UK Home Office's policy on cosmetics animal testing concludes

Cruelty Free International's legal challenge of the UK Home Office's decision to abandon the ban on testing cosmetics on animals recently concluded, and the final ruling is expected in the coming weeks.

Testing cosmetics and their ingredients on animals has been banned in the UK since 1998 – the first ever ban on the



practice – but in an August 2021 letter, the Home Office admitted that it has changed its policy to allow animal testing for cosmetic ingredients in the UK under industrial chemicals legislation.

The Judicial Review – which decides the lawfulness of a public body’s actions – was brought for two reasons. Firstly, to confirm that the decision to reverse the cosmetics policy ban is unlawful; and secondly, to confirm, with safety testing more generally, that the Home Office must factor the usefulness of the product or ingredient into the harm-benefit analysis when deciding whether to grant a licence for animal tests.

Latest USDA figures show an increase in animal testing

Cruelty Free International’s analysis of the latest figures from the US Department of Agriculture (USDA) show that a total of 712,683 animals were used in experiments across the US in 2021 – an increase of 6% compared to the previous year.

The figures also reveal that 70,161 animals were used in “Category E” experiments, in which no relief is provided for animals experiencing pain or distress.

The USDA figures do not include mice, rats, fish, or birds, despite these animals being the most commonly used animals in experiments. As a result, the true number of animals used in US laboratories is expected to be far higher. Cruelty Free International has estimated the annual number to be at least 14 million (Taylor and Alvarez, 2019).

The USDA has recently changed the way it publicly provides animal use data, so that it is no longer straightforward to compare pain categories and animal use across states or to compare the data with previous years. No explanation has been provided for this new and more opaque system of providing this public information.

HEARTS Act re-introduced with support from Dr Jane Goodall

On Valentine’s Day, Congressmembers Chris Pappas (D-NH) and Ken Calvert (R-CA) announced the introduction of

the *new* HEARTS Act (Humane and Existing Alternatives in Research and Testing Sciences Act), which aims to ensure that non-animal testing methods are prioritized in research funded by the National Institutes of Health (NIH). This bill builds on the one that had previously been led by Representative Roybal-Allard (D-CA) prior to her retirement.

Dr Jane Goodall, Founder of the Jane Goodall Institute and UN Messenger of Peace has joined Cruelty Free International to welcome the reintroduction of the HEARTS Act, which directs the NIH to provide incentives to researchers to use non-animal methods and establishes a dedicated center within the NIH devoted to advancing new alternative methods and developing a plan for reducing the use of animals in federal funded research.

In addition, the bill updates the definition of “animal” to include cephalopods to ensure that these animals will receive the minimum protections afforded to other animals used in NIH-funded research. This change is consistent with regulations in the EU and UK.

Cruelty Free International is working with Congressman Pappas and Calvert’s offices to continue to build bipartisan support for the bill and encourages other organizations and interested parties to register their support for the bill.

International Collaboration on Cosmetics Safety launches

On February 8, the International Collaboration on Cosmetics Safety¹ (ICCS) – a global group of over 40 cosmetics manufacturers and suppliers, industry associations, scientists, and animal protection organizations – launched with the aim of increasing the international use of non-animal methods in cosmetics safety testing.

Cruelty Free International will join other members of the ICCS to support non-animal research and testing for cosmetics and personal care products and ingredients. The group will provide funding for new evaluation of animal-free

safety assessments and regularly share these with cosmetics and chemical regulators to increase their acceptance.

It will also fund education and training to build confidence in and demonstrate the scientific validity of animal-free safety assessment approaches.

Workshops aim to accelerate non-animal methods in drug safety testing

Cruelty Free International, in collaboration with Safer Medicines and Animal Free Research UK, organized a series of five workshops with 13 international experts, including regulators and preclinical scientists, to identify opportunities for the greater use of non-animal methods in drug safety testing. The findings were published online earlier this year in *ALTEX* (Turner et al., 2023).

The workshops focused on four of the human body’s most important organ systems concerning harmful drug side effects, which are often not identified in animal tests: the liver, and respiratory, cardiovascular, and central nervous systems. The participants worked together to create a series of maps that outline how existing non-animal test methods could be used to address the basic safety requirements for each organ system and highlight key gaps where additional human-relevant approaches are needed.

The participants also discussed ways to encourage greater adoption of non-animal testing methods in the development of human medicines, and how they can be incorporated in safety testing guidelines.

References

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- Turner, J., Pound, P., Owen, C. et al. (2023). Incorporating new approach methodologies into regulatory non-clinical pharmaceutical safety assessment. *ALTEX*, online ahead of print. doi:10.14573/altex.2212081

¹ <https://iccs-cosmetics.org/>



EUSAAT

*European Society for
Alternatives to Animal Testing*

EUSAAT Congress 2024 – new date September 18-20, 2024

The next EUSAAT congress 2024 is planned for the period from Wednesday, September 18 to Friday, September 20, 2024. We hope for a grandiose support like last year and that many stakeholders and researchers from different areas of the 3Rs community will participate. We will again pay special attention to enabling as many young scientists as possible to participate – through low participation fees and a variety of Young Scientist Travel Awards. We are already very excited about the topics and key areas at the EUSAAT Congress 2024 and are of course happy to receive thematic suggestions.

EUSAAT Society – new postal address

Following a decision by the General Assembly, EUSAAT has moved to the new postal address:

EUSAAT, Apostelgasse 13/10,
1030 Vienna

This address must be used from now on; the former one is no longer valid.

COST Action IMPROVE – update

In recent months the members of the COST Action “CA21139 – 3Rs concepts to improve the quality of biomedical science (IMPROVE)” were very busy organizing themselves. Currently, over 150 members have been approved and started to work together in the four working groups (WG) Quality and Translatability of Science, Implementation, Dissemination, and Education and have been forming subgroups to tackle the different tasks. The process was successfully completed to define several leadership positions such as WG co-leaders and coordinators for STSM (Short Term Scientific Mission), ITC (Inclusiveness Target Countries), YRI (Young Researchers and Innovators), mentorship program, website/social media, general public/outreach, database, training schools, and gender and diversity.

The next meetings planned for this year are a management committee meeting on April 18, 2023 (online), a huge WG meeting in Sarajevo (Bosnia Herzegovina) on June 5-7, 2023, and a training school in Milan (Italy) in mid-September directly ahead of the Centro 3Rs meeting on September 13-15, 2023.

Recent activities also comprised the introduction of the COST Action IMPROVE at a meeting of the Member State National Contact Points for the Implementation of the Directive 2010/63/EU at the European Commission and at the Austrian Federal Ministry for Education, Science and Research.

If you are interested in this COST Action, you can apply for participation in the working groups. More details can be found at: <https://www.cost.eu/actions/CA21139/>

EUSAAT – Job announcements

- University assistant (PhD position) at the University of Veterinary Medicine Vienna on “Tendon-on-a-chip: a biomimetic tendinopathy model”: <https://euraxess.ec.europa.eu/jobs/71937>
- PhD position at the Austrian Institute of Technology GmbH in Vienna on the blood-brain barrier in rare neurodevelopmental diseases (RASopathies such as SYNGAP-1 disorder) using *in vitro* models based on human induced pluripotent stem cells: <https://jobs.ait.ac.at/Job/204558>



Search for alternatives to animal experiments in research is further promoted

Developing replacement and supplementary methods for animal experiments and thus avoiding animal testing is the goal of the “Micro-Replace Systems” research network led by Professor Dr André Bleich, Head of the Institute of Laboratory Animal Science at the Hannover Medical School (MHH), and Professor Dr Maren von Köckritz-Blickwede from the Foundation of the University of Veterinary Medicine Hannover. In 2017, the project started under the name “R2N – Replace and Reduce based in Lower Saxony” with the support of the Lower Saxony Ministry of Science and Culture (MWK).

The main focus was on researching methods that replace experiments on living animals with studies on organ models and stem cell-derived culture systems. The research network now initiated from R2N consists of twelve working groups from the MHH, the University of Veterinary Medicine Hannover, and the Technical University of Braunschweig and will receive follow-up funding of €3.6 million from the program “zukunft.niedersachsen” of the MWK for another three years.

Stem cells grow into mini organs

The network is primarily concerned with reducing animal experiments in basic and infection research. Research foci in the first funding period were, for example, the animal-free safety assessment of gene therapies or xenogeneic transplants, the digestive tract – especially the oral mucosa, intestine, and liver – as well as the upper and lower respiratory tract. Here,

research with adult and induced pluripotent stem cells (iPSC) as well as precision cut organ slices was further developed. Based on these cultures, organoid systems have been developed that were also applied in dedicated chip-systems, as well as air-liquid interface cultures or a complex oral mucosa biofilm model. These models were used to analyze intestinal barrier defects induced by genetic or microbial factors in inflammatory bowel disease research, viral infections including SARS-CoV-2, or new material for oral implants.

Chip systems: sustainable and reusable

Now the researchers aim to develop a new, standardized chip system on which they can observe the interaction between intestinal or respiratory cells and immune cells. The chip systems will not only be equipped with human stem cells; animal models also will be replicated to allow a straightforward transfer of *in vivo* to *in vitro* research. A special focus also lies on culture conditions that mimic the (patho-)physiological situation *in vivo*, e.g., by applying hypoxia. Tailored according to tissue type, the corresponding culture conditions and the respective scientific question, the chip systems will be designed to be versatile and also reusable. Furthermore, it is foreseen to develop a biomembrane permeated by blood vessels to ensure higher complexity of the different tissues for more extensive investigations. The consortium also aims to develop non-animal antibodies tailored for its research questions. In an overarching project, the new systems will be subjected to intensive quality controls so that they can be transferred for broad use if possible.

New website 3r-forschung.de

The research consortium “R2N – Replace and Reduce based in Lower Saxony” and the research group “FOR 2591 – Severity Assessment in Animal Based Research”, both led by the Hannover Medical School, have initiated the website 3r-forschung.de, which is primarily directed at people outside science.

The DFG-funded website provides easy-to-understand answers to questions such as “What are the alternatives to animal testing?”, “How can pain and fear be alleviated for laboratory animals?”, and “How can the use of fewer laboratory animals be accomplished?” Furthermore, it explains the 3R principles “Replace, Reduce and Refine” with the help of clear graphical illustrations, reviews general facts about 3R research, and addresses current topics.

The aim is to illustrate the contribution 3Rs research brings to society and how 3Rs research reconciles science and animal welfare. If you would like to support this idea as a network partner, please contact us via the website.

Lower Saxony Animal Welfare Award for R2N

Replacing, reducing or completely avoiding animal experiments in research and improving the conditions for laboratory animals – this characterizes the work of Professor Dr André Bleich, Head of the Institute of Laboratory Animal Science and the Central Animal Laboratory of the Hannover Medical School (MHH). On January 13, the scientist was awarded the “Lower Saxony Animal Welfare Prize” in the category “Alternative Methods to Animal Experiments” for the research net-



work “R2N – Replace and Reduce from Lower Saxony”, which he heads. The prize, worth €6,000, was awarded for the first time by the Lower Saxony Ministry of Food, Agriculture and Consumer Protection. The State Commissioner for Animal Welfare in Lower Saxony, Michaela Dämmrich, presented the award. The association “Doctors against Animal Experiments” was also awarded the prize.

Boost for animal-free methods to generate antibodies for research

Animal-free generation of antibodies for research offers scientific advantages beyond saving mice and rabbits. In particular, the recombinant production of animal-free antibodies always provides sequence-defined reagents and assures unlimited reproducibility (Gray et al., 2020). Moreover, even secondary antibodies, which currently typically are blood serum

products, can be replaced by defined animal-free multiclonal antibodies (Wenzel et al., 2020). The R2N¹ research consortium “Micro-Replace-Systems”, led by the Hannover Medical School (MHH) and funded by the program “zukunft.niedersachsen” of the Federal State of Lower Saxony, now embarked to analyze the replacement of animal-derived antibodies with animal-free monoclonals and multiclons in a wide variety of applications, including for the first time the replacement of animal serum-derived secondary antibodies by vegan multiclons. These antibodies will be generated by phage display and provided by the Braunschweig University (Dept. of Biotechnology led by Stefan Dübel) in collaboration with the university’s spin-off project Abcalis². The Abcalis team has developed an antibody pipeline that completely avoids the use of animal products throughout the entire selection and production process, resulting in truly vegan antibodies.

The team won several awards including the first prize for animal-free antibodies awarded by the European Coalition to End Animal Experiments (ECEAE)³ and is currently funded by the German Federal Ministry of Economic Affairs and Climate Action. It also will provide access to worldwide distribution channels to assure future availability of animal-free research antibodies generated by the research consortium.

References

- Gray, A. C., Bradbury, A., Dübel, S. et al. (2020). Reproducibility: Bypass animals for antibody production. *Nature* 581, 262. doi:10.1038/d41586-020-01474-7
- Wenzel, E. V., Russo, G. and Dübel, S. (2020). Multiklonale Antikörper als Ersatz für Zweitantikörper aus Seren [Article in German]. *Biospektrum* 26, 416-417. doi:10.1007/s12268-020-1401-7

¹ <https://r2n.eu/>

² <https://abcalis.com/>

³ <https://www.eceae.org/antibodies.html>

The RISK-HUNT3R project’s primary objective is to create a safety testing pipeline that is sustainable, human-relevant, and does not rely on animal testing. As part of its work within the ASPIS cluster, the consortium has started building an implementable next-generation risk assessment (NGRA) workflow called the “ASPIS Safety Profiling Algorithm” (ASPIS).

During the first project period, the consortium conducted various exploratory studies to refine the structure and toolbox of the ASPA algorithm. The preliminary outcomes were presented in February at

the latest General Assembly, where one hundred participants from the 37 project partners met to discuss the project’s progress and plans. The consortium will continue to refine the modules of ASPA while a first set of overarching case studies will be started to demonstrate the general applicability in real-life scenarios.

At the assembly, the scientific and advisory board provided general feedback on the project’s progress and next steps. It became clear that the RISK-HUNT3R project has achieved increased maturity levels by clearly defining its focus. The ASPA platform will support the project

by highlighting testing priorities, gaps, and opportunities. It will also encourage communication and shared understanding among different stakeholder groups while promoting collaboration within ASPIS and other toxicological initiatives such as PARC, the European partnership for the assessment of risks from chemicals.

The next appointment for RISK-HUNT3R to disseminate its results will be the 62nd SOT annual meeting. The consortium is preparing to fly to Nashville and attend this critical conference. On this occasion, the project experts will be engaged as speakers in several ses-



sions, such as the ASPIS cluster-promoted session on challenges in developing *in vitro-in vivo* extrapolation models for next-generation risk assessment. The RISK-HUNT3R scientific outcome will also be presented via several posters, and the project material will be distributed at the ASPIS booth in the ToxExpo exhibition area.

RISK-HUNT3R press review

Recent research has explored various aspects of biomedical science and technology, providing insight into potential therapeutic interventions for diseases and improving drug and chemical safety assessment.

In Suciú et al. (2023), the authors examined the effects of the proteasome inhibitor MG-132 on human LUHMES neurons. The inhibitor can cause cell death within 24 hours but does not immediately reduce cell viability. Metabolomics and transcriptomics analysis showed how the molecular initiating event (MIE), e.g., proteasome inhibition, triggered changes in energy metabolism and activation of stress response pathways and how these led to adverse outcomes (neurodegeneration). The study provides insight into the cellular changes triggered by proteasome dysfunction and exemplifies how a chain of causal events can be assembled in an adverse outcome pathway (AOP).

An *in vitro* battery composed of six human-derived cell models was evaluated in Capinha et al. (2023) as an approach for studying the toxic effects of trichloroethylene (TCE), an industrial solvent. High throughput transcriptomics screening was performed to evaluate the effects of exposure to TCE-conjugates. Results showed that exposure to 1,2-DCVC, a TCE-conjugate, can cause stress responses in all

models but to different extents. The renal model was the most sensitive. Neuronal models also showed significant responses, supporting the hypothesis that 1,2-DCVC may be involved in the neurotoxic effects of TCE. The study highlights the value of human cell models and transcriptomics for such mechanistic studies.

A transcriptomics-based approach is also described by Callegaro et al. (2023). In this article, the authors propose a method for improving early safety screening for drug and chemical safety assessment. The strategy uses a weighted correlation network analysis (WGCNA) to identify co-regulated gene clusters associated with liver pathologies. The study identifies a module enriched for ATF4-regulated genes associated with hepatocellular single-cell necrosis and preserved in human liver *in vitro* models. The study also identifies TRIB3 and MTHFD2 as novel candidate stress biomarkers and uses BAC-eGFPHepG2 reporters in a compound screening to identify compounds showing ATF4-dependent stress response and potential early safety signals.

Finally, another high-content approach was explored by Cerisier et al. (2023). The authors investigated the implementation of high-content (cell painting) methods to explore the link between chemicals' modes of action and cell morphology. Information from already available datasets was combined with pathways, gene ontology terms, and disease enrichment analysis. The study resulted in a biological network connecting chemical-gene-pathway-morphological perturbation and disease relationships, containing 9989 chemicals, 732 significant morphological features, and 12,328 genes. The study demonstrated that drugs shared similar genes, pathways, and morphological profiles, which could help interpret chemical-phenotype observations.

News and Events

The first RISK-HUNT3R stakeholders symposium will take place on June 13-14, 2023, in Egmond aan Zee (The Netherlands). More information will be shared in the upcoming weeks. To be informed of any updates, please subscribe to the newsletter via the project website (<https://www.risk-hunt3r.eu/>).

References

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

Giorgia Pallocca and Marcel Leist