

Corners



Upcoming Events

8th Annual 3Rs Symposium: Pandemic-Driven Advances June 3-4, 2021 (online)

The eighth annual joint 3Rs symposium, organized by the Johns Hopkins University Center for Alternatives to Animal Testing, USDA's Animal Welfare Information Center, NIH's Office of Laboratory Animal Welfare, and the Johns Hopkins Department of Molecular and Comparative Pathobiology, is scheduled for June 3 and 4, 2021. This year's virtual symposium is titled Pandemic-Driven Advances. Its goal is to bring together experts and practitioners to focus on the challenges faced during the pandemic and the successes in effectively using animal models to maximize both biomedical discovery and the goals of the 3Rs.

Registration closes on June 1, 2021.

Event website: <https://caat.jhsph.edu/programs/Refinement/3Rssymposium.html>

Microphysiological Systems World Summit

Virtual Conferences

June 24, 2021: Regulatory Acceptance

December 9, 2021: Systems Engineering of Microphysiological Systems

Hybrid Conference (In-person and virtual)

May 30-June 3, 2022

New Orleans, LA

The MPS (Microphysiological Systems) World Summit will bring together a global audience, including institutions (govern-

ment, health foundations, charities), the academic research community (universities, research institutes), environmental and human toxicity researchers, the pharmaceutical and other industries (cosmetics, chemical, and food industries), medical centers and practitioners, patient associations, and policy makers and testing centers – in a series of global annual conferences to create a roadmap for MPS technologies. This will be a first step in establishing an international MPS society.

Additionally, this series of international conferences (which include two virtual events in 2021 in the lead-up to the hybrid event in June 2022) will facilitate stakeholder communication as well as networking among young scientists and MPS thought leaders, promoting international standardization and harmonization of MPS and serving as a global training environment.

The May 30, 2022 summit is a hybrid event that will host up to 500 in-person participants and 500 additional participants online. It will be co-chaired by Suzie Fitzpatrick, FDA, Don Ingber, Harvard Wyss and Thomas Hartung, Johns Hopkins CAAT. The two preceding virtual meetings will be held on June 24, 2021 and December 9, 2021.

The 2022 meeting will start on a Monday with two pre-meeting workshop sessions focused on hands-on training and education. The scientific sessions will include varying formats: workshops, roundtables, plenary sessions, and scientific symposia. In total, we will have four keynotes, up to 24 parallel sessions, and three lunch sessions. Lunch and keynotes will be 60-minute sessions.

Sponsorship packages are available for all sessions, as well as other specialized options. Please contact Camila Sgrignoli Januario (cjanuar1@jhu.edu) for details.

Website: <https://mpsworldsummit.com>

VIDEO: Thomas Hartung and Paul Locke Join Jane Goodall for Congressional Panel on Humane Research and Testing Act

21st Century Innovations in Alternatives to Animals in Biomedical Research: Congressional Briefing – The Humane Research & Testing Act

On Wednesday March 10, 2021, Citizens for Alternatives to Animal Research (CAARE) hosted a virtual panel discussion, titled “21st Century Innovations in Alternatives to Animals in Biomedical Research,” recognizing the importance of the *Humane Research and Testing Act*. Scientists discussed the moral imperative to mitigate the use of animals in medical research and recent scientific advancements that reduce the need for animal testing. Presenters included: Dr Jane Goodall, DBE, founder of the Jane Goodall Institute and United Nations Messenger of Peace; Paul Locke, JD, DrPH, Associate Professor at the Johns Hopkins Bloomberg School of Public Health and affiliate of the Center for Alternatives to Animal Testing; Don Ingber MD, PhD, Founding Director of the Wyss Institute for Biologically Inspired Engineering at Harvard University; Azra Raza, MD, Chan Soon-Shiong Professor of Medicine and Director of the MDS Center at Columbia University; and Thomas Hartung, MD, PhD, Professor at the Johns Hop-



kins Bloomberg School of Public Health and Director of the Center for Alternatives to Animal Testing.

Watch now: <https://tinyurl.com/jnbw8rbk>

EBTC in *Nature Scientific Reports*: In Vitro Tests Outperform Animal and Human Studies in Predicting Trogliatone-Induced Toxicity

The Evidence-based Toxicology Collaboration (EBTC) has published the results of a systematic review, conducted in collaboration with the Norwegian Institute of Public Health, Safer Medicines Trust (UK), and other partners. The review found that pre-clinical animal and pre-market human trials do not predict a drug's potential to cause harm, despite being mandatory in safety and efficacy trials.

The collaboration conducted a comprehensive comparison of the *in vivo* (animal and human) and *in vitro* tests currently available to assess drug safety before market authorization and compared their findings against "real world data" on adverse drug reactions. They focused on two drugs: troglitazone, which was withdrawn from the market due to severe liver toxicity, and rosiglitazone, which remains on the market. No reported biomarker for either drug indicated a strong hazard signal in either pre-clinical animal studies or human trials. In contrast, the *in vitro* data showed that troglitazone was active in twice as many *in vitro* assays as rosiglitazone, indicating a strong signal for off-target effects. The *in vitro* data showed marked differences in the two drugs' toxicological profiles, offering a new paradigm for reducing drug attrition and preventing adverse drug reactions.

The open access publication appeared in *Nature Scientific Reports* on March 18 and may be viewed here: <https://www.nature.com/articles/s41598-021-85708-2>

Next Generation Humane Science Award Winner: Leah Wehmas

Leah Wehmas, of Oregon State University/US EPA, was awarded CAAT's Next Generation Humane Science Award for her work on recently developed genomics technologies to make use of archival tissues from toxicity studies that will minimize the

need for new animal studies in toxicology.

The Next Generation Humane Science Award is available annually to young scientists to acknowledge and encourage researchers who focus on replacing the use of animals in experiments. Please join us in congratulating Leah Wehmas!

Details about the award may be found here: <https://caat.jhsph.edu/programs/awards/HumaneScience.html>

VIDEO: Contemporary Refinement Research, its Application in Practice, and Future Directions

On March 11, CAAT's Beyond Classical Refinement Program hosted a webinar featuring four former winners of the CAAT Refinement Award, a prize awarded for outstanding research that has the potential to significantly improve the lives of laboratory animals and/or reduce the number of animals used. Becca Franks (New York University), Brianna Gaskill (Novartis), Judith de Haan (Utrecht University) and Cathy Schuppli (University of British Columbia) talked about what they have been up to since they received the CAAT Refinement Award and where their careers have taken them. They gave short presentations on their recent or ongoing research activities and engaged in a conversation on the role of contemporary refinement research and on how barriers to its implementation in practice can be overcome.

Watch now on CAAT's YouTube Channel: <https://youtu.be/udiGTIM4KMo>

VIDEO: 13th Conference on Animal Experimentation of Swiss Animal Protection STS

Video of the 13th Conference on Animal Experimentation of Swiss Animal Protection STS, which was recorded on November 13, 2020, is now available, along with a PDF of supplementary material.

Thomas Hartung: *Brauchen wir wirklich noch Tierversuche?* (video, in German): <https://vimeo.com/522847664>

Thomas Hartung and Marcel Leist Interviewed by Science Blogger Stefanie M. Hohenberger

Thomas Hartung and Marcel Leist were interviewed about their lifetime of work in developing and promoting alternatives to animal testing (in German).

Link: <http://wissenschaftsversessen.de/blog/haendel-tierschutzpreis-2020/>

Article in *Das Magazin* (German) Features Thomas Hartung and Marcel Leist

Animal testing is still the standard in research – although many are imprecise and alternatives have long been available. So why do animals have to continue to suffer? The Swiss magazine *Das Magazin* covers the research of Thomas Hartung and Marcel Leist.

Link: <https://bit.ly/3wEmfQr>

VIDEO: Thomas Hartung and Tom Luechtefeld IVAMSS Webinar January 29, 2021 (11am EST)

On January 29, 2021, the In Vitro and Alternative Methods Specialty Section (IVAM) presented a series of webinars focused on the development and application of *in vitro* methods and non-animal models for toxicology and product safety. The webinar with Thomas Hartung and Tom Luechtefeld broke the record for largest IVAMSS webinar ever, with 536 registrants.

- Thomas Hartung: A.I. and Big Data – New Kids on the Toxicology Block
 - Thomas Luechtefeld, Insilica LLC: Smart Chemistry – Algorithms for Chemical Classification and Clustering
- Watch now: <https://www.toxicology.org/groups/ss/IVSS/Events.asp>

VIDEO: EBTC Webinar on Application of US EPA IRIS Systematic Review Methods to Health Effects February 2, 2021

Scientists at the US EPA IRIS Program recently finalized a series of systematic reviews (SR) investigating the human health effects of exposure to phthalates. In this



EBTC Online Scientific Symposium, the scientists presented the main findings from their SRs, highlighting novel uses of SR methods in dose-response modeling and drawing out general lessons learned in the application of SR methods in chemical assessment.

Watch now (YouTube): <https://www.youtube.com/watch?v=IgoHiYl1BMg>

Thomas Hartung Profiled in De Standaard: “Pope of A.I. Research in Toxicology”

Thomas Hartung and CAAT’s research in toxicology and artificial intelligence was profiled in the Dutch *De Standaard*.

Full article (in Dutch): https://www.standaard.be/cnt/dmf20210204_98023736

VIDEO: EUROTOX In2Tox Followup COVID-19 Webinar

January 18, 2021

Introduction: Mathieu Vinken, Chair EU-ROTOX In2TOX Specialty Section

“New approach methods as door-openers for drugs and vaccines or the other way around?” by Thomas Hartung (CAAT)

Watch now: <https://youtube/rBxCLx4cbzM>

VIDEO: The Future of Animal Politics in the Context of Climate Change, COVID-19, and Movements Against Racial Capitalism

The webinar series is designed to bring together a diverse audience of people interested in animal studies, critical animal studies, animal ethics, animal politics, animal law, environmental studies, environmental law, migration studies, as well as climate law/studies.

This sixth and final session, held on January 21, 2021, reflected on the insights from the previous sessions and identified ways of moving forward in theory and practice. Specifically, we looked to assemble scholars to map future areas of research at the juncture of animal studies, climate change, racial injustice and global health: What areas deserve our attention going forward?

Given the manifold intersectional forms of oppression, subjugation, and indifference experienced by animals and human people affected by climate change, how can we develop a multispecies approach to climate justice and to animal and human health? What – if any – arguments are there to include animals’ voices in the regulation of climate policy? How does the global Black Lives Matter movement intersect with and shape the future of animal liberation movements? How does this relate to other questions of global justice, and the oppression of human groups (e.g., just transitions for farmers and other workers)? How is this interconnected with capitalism? How do questions of equality play into this?

Watch now: <https://animalsclimatehealth.com/session-6/>

Animal-Free Drug Safety Testing: Challenges and Opportunities

This virtual fireside chat, held January 26, 2021, invited visionaries who are leading the way toward implementing animal-free methods that better predict the patient response to new drugs. In an interactive roundtable, panelists discussed important scientific questions that all researchers involved in drug R&D should consider, especially as regulatory agencies move to stop supporting and funding research using animal models by 2035 or sooner.

Thomas Hartung presented, along with Stephan Platz (AstraZeneca) and Armin Wolf (InSphero).

Link: <https://newfrontiersin3d.com/program/#animal-free>

National Academies of Sciences, Engineering, and Medicine Webinar

Microphysiological Systems: Bridging Human and Animal Research – A Workshop

Microphysiological systems (MPS) are *in vitro* platforms (such as tissues/organs on chips) that mimic the biochemical, electrical, and mechanical properties of organ or tissue function. They hold promise for advancing understanding of the mechanisms of disease and accelerating drug development. To date, MPS has been focused on human health but could have many appli-

cations for animal health and OneHealth. It also holds the potential to reduce the use of whole animal studies in research.

This workshop, held January 19 and 20, 2021, explored the use of MPS for a range of animal species/strains and for optimizing animal model selection where whole animal models are necessary.

Thomas Hartung spoke on *Multi-organ chips and emerging applications for biological studies: Integrated multi-organ systems*.

Link: <https://tinyurl.com/yfh8jzv7>

Is Animal Testing Still Needed? (German Article)

The promise made by their developers: Tiny models of human organs will in future lead to less suffering, more knowledge, better therapies. Thomas Hartung is interviewed.

Full article from *P.M.* (German science magazine, in German):

<https://tinyurl.com/4navwt8t>

Thomas Hartung – The Promise of AI in Pandemic Times: Beyond the Hype

This ePanel, part of a virtual keystone symposium in collaboration with *Frontiers* held January 12, 2021, addressed the promise, and reality, of AI in the context of health.

Bringing together AI luminaries with diverse perspectives on AI in medicine and public health, we explored these questions in the context of the COVID-19 pandemic, in an effort to drive AI applications towards tangible impacts on global public health. The event concluded with a live audience Q&A, where the field leaders were asked burning questions and shaped the discussion around new frontiers at the intersection of AI and medicine.

Thomas Hartung shared his insights from a scientific, policy and societal perspective in a talk entitled “The Promise of A.I. in Pandemic Times: Beyond the Hype”.

Preview: <https://youtu.be/ttfhQ5gz7Mg>

Thomas Hartung on Reconstruction Podcast

The speed with which our scientific community has identified the coronavirus and developed these vaccines is unprecedented.



Never before have researchers been able to conduct thousands of tests in such a short period of time. In fact, it may be that the biggest impact over the long term will be the revolution in research science that made this unprecedented speed possible.

Thomas Hartung was interviewed.

Listen now: <https://tinyurl.com/shxmbc6c>

CAAT Paper on Paroxetine and Developmental Neurotoxicity Published in New Frontiers Ebook

Research on the antidepressant paroxetine and developmental neurotoxicity, utilizing CAAT's "mini-brain" models and originally published in February 2020, has been incorporated into a new, free ebook: *Brain Organoids: Modeling in Neuroscience*.

Get the Free Ebook (PDF and Epub):

<https://www.frontiersin.org/research-topics/9488/brain-organoids-modeling-in-neuroscience>

Zhong, X., Harris, G., Smirnova, L. et al. (2020). Antidepressant paroxetine exerts developmental neurotoxicity in an iPSC-Derived 3D human brain model. *Front Cell Neurosci* 14, 25. doi:10.3389/fncel.2020.00025

New Publications

Anderson, W. A., Bosak, A., Hogberg, H. T. et al. (2021). Advances in 3D neuronal microphysiological systems: Towards a functional nervous system on a chip. *In Vitro Cell Dev Biol Anim* 57, 191-206. doi:10.1007/s11626-020-00532-8

Aschner, M., Paoliello, M. M. B., Tsatsakis, A. et al. (2021). Social injustice in environmental health: A call for fortitude. *Environ Res* 194, 110675. doi:10.1016/j.envres.2020.110675

Calina, D., Hartung, T., Mardare, I. et al. (2021). COVID-19 pandemic and alcohol consumption: Impacts and interconnections. *Toxicol Rep* 8, 529-535. doi:10.1016/j.toxrep.2021.03.005

Farsalinos, K., Poulas, K., Kouretas, D. et al. (2021). Improved strategies to counter the COVID-19 pandemic: Lockdowns vs. primary and community health-care. *Toxicol Rep* 8, 1-9. doi:10.1016/j.toxrep.2020.12.001

Golden, E., Maertens, M., Hartung, T. et al. (2020). Mapping chemical respiratory sensitization: How useful are our current tools? *Chem Res Toxicol* 34, 473-482. doi:10.1021/acs.chemrestox.0c00320

Gupta, R., Schrooders, Y., Hauser, D. et al. (2020). Comparing in vitro human liver models to in vivo human liver using RNA-Seq. *Arch Toxicol* 95, 573-589. doi:10.1007/s00204-020-02937-6

Hogberg, H. T., de Cássia da Silveira e Sá,

R., Kleensang, A. et al. (2021). Organophosphorus flame retardants are developmental neurotoxicants in a rat primary BrainSphere in vitro model. *Arch Toxicol* 95, 207-228. doi:10.1007/s00204-020-02903

Loser, D., Schaefer, J., Danker, T. et al. (2021). Human neuronal signaling and communication assays to assess functional neurotoxicity. *Arch Toxicol* 95, 229-252. doi:10.1007/s00204-020-02956-3

Meisig, J., Dreser, N., Kapitza, M. et al. (2020). Kinetic modeling of stem cell transcriptome dynamics to identify regulatory modules of normal and disturbed neuroectodermal differentiation. *Nucleic Acids Res* 48, 12577-12592. doi:10.1093/nar/gkaa1089

Pamies, D., Zurich, M.-G. and Hartung, T. (2020). Organotypic models to study human glioblastoma – Studying the beast in its ecosystem. *iScience* 23, 101633. doi:10.1016/j.isci.2020.101633

van der Stel, W., Carta, G., Eakins, J. et al. (2020). Multiparametric assessment of mitochondrial respiratory inhibition in HepG2 and RPTEC/TERT1 cells using a panel of mitochondrial targeting agrochemicals. *Arch Toxicol* 94, 2707-2729. doi:10.1007/s00204-020-02792-5

Wang, T., Liu, H., Itoh, K. et al. (2021). C9orf72 regulates energy homeostasis by stabilizing mitochondrial complex I assembly. *Cell Metab* 33, 531-546.e9. doi:10.1016/j.cmet.2021.01.005



Global beauty brand Garnier goes cruelty free

On March 5, 2021, Cruelty Free International announced that cosmetics and hair-care giant Garnier has been approved under the Leaping Bunny programme.

Leaping Bunny certification requires brands to forensically investigate their entire supply chain, including all raw mate-

rial and individual ingredients, for any cases of animal testing. Garnier and Cruelty Free International worked together for many months to secure this evidence from more than 500 suppliers, who source over 3,000 different ingredients for the brand, from across the world.

The Leaping Bunny logo, which is the best visible and independent assurance for consumers of a company's commitment to

produce products not tested on animals, will now feature on all of Garnier's products, and it is hoped that other L'Oréal Group brands will soon follow.

Since 2017, there has been a 190% increase in brands enquiring about Leaping Bunny status, showing that consumer demand for cruelty free products across the globe can have an impact on how cosmetics brands operate.



Appeal success at ECHA to prevent conduct of unnecessary animal tests

Two cases against animal testing decisions made by the EU Chemicals Agency (ECHA) have been decided, sparing thousands of animals from unnecessary tests. Cruelty Free Europe intervened in both cases.

In a case heard by the European Court of Justice, Esso Raffinage (a division of ExxonMobil) was challenging a demand by ECHA to conduct a developmental toxicity study. After receiving a final decision, Esso decided that the test could still be avoided by demonstrating the safety of its chemical using evidence from other sources, but ECHA refused this option. The court ruled that ECHA has a duty to consider alternatives put forward by companies even after a final decision has been made. This has positive implications for the use of alternative approaches under REACH.

In another Board of Appeal case, the ZDDP group of companies challenged ECHA's decision requiring them to conduct 90-day repeat dose toxicity studies and prenatal developmental studies on 13 similar substances used in hydraulic fluids. Tests on all 13 substances would have used thousands of animals. ZDDP argued that it was possible to avoid at least some of these tests using read-across. The Board of Appeal ruled that ECHA should have given all the registrants of the chemicals an opportunity to argue for the read-across, not just the lead registrants. The ZDDP group will now have another opportunity to argue in favour of using data from similar substances to avoid some of the animal tests demanded by ECHA.

First Geoffrey Deckers Award goes to EcoVegAnimals, EVA

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

EcoVeg Animals is a young animal protection organisation working to improve animal welfare in Bosnia and Herzegovina and beyond. Last year they successfully helped to end the use of live animals in the education programme at the Faculty of Veterinary Medicine at the University of Sarajevo. The organisation plans to use the funds from the award to encourage other faculties at the university to follow suit. They also plan to outreach to companies and consumers on the importance of bringing animal testing for cosmetics to an end.

The award honours Geoffrey Deckers, the much respected and 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 will be made annually on his birthday 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.

Progress with state-level cruelty free cosmetics legislation in the US

This year Cruelty Free International welcomed the re-introduction of cruelty free cosmetics legislation in Virginia, Maryland and Hawaii and also resumed work in New

York, where a revised bill will soon be reintroduced. After the Covid-19 pandemic cut many state legislatures short last year, work on these state bills was delayed; however progress has come quickly in 2021.

In Virginia, after a whirlwind legislative session, Senate bill 1379 and House bill 2250 passed the Virginia legislature with bipartisan support and was signed into law on March 11, 2021, making Virginia the 4th state to pass legislation prohibiting the sale of new animal-tested cosmetics. The new law will prohibit the sale of any cosmetic product that has been tested on animals after January 1, 2022.

In Maryland, SB 282 passed all committee hearings and legislative floor votes including unanimous votes in the Senate. The bills are now on the desk of Maryland Governor Larry Hogan, awaiting his signature or default passage into law. Like the Virginia law, it will prohibit the sale of any cosmetic product that has been tested on animals after January 1, 2022.

The Hawaii, Cruelty Free Cosmetics Act SB345 and HB 1088 have now successfully passed all committee hearings in their opposite chambers. The bills are now headed for final floor votes and will then go to conference committee to work out small differences in the bills and an effective date before being sent to Governor David Ige.

These important bills not only create change at the state-level but create momentum for national change. State efforts on this issue have already helped inform a way forward for the national approach as the agreements reached on state legislation were adopted into the federal Humane Cosmetics Act, which will be reintroduced this year.



EUSAAT

European Society for
Alternatives to Animal Testing

EUSAAT Virtual Seminar Series started on 15.4.2021

We are very pleased to announce the EUSAAT Virtual Seminar Series 2021. As we have postponed the EUSAAT Congress from this year to 2022 in order not to compete with WC11, we have decided to offer our members and the European 3R community a replacement with our Virtual Seminar Series 2021.

The Series kicked off on April 15, 2021 at 5 p.m. CEST with two presentations and will take place every second week.

We have decided to provide a platform for younger researchers, so one young researcher and one distinguished researcher will present per session. The topics will cover current 3R topics typically dealt with in EUSAAT conferences including regulatory, ethical, educational, 3Rs center and scientific news, discussions and developments.

If you would like to attend, please register via: eusaatvirtalseminarseries2021@gmail.com by providing your name, e-mail, affiliation and career stage.

We wish many interesting presentations and discussions, and especially good health to all of you and your loved ones.

Yours sincerely,
EUSAAT, your European 3Rs society



The last year of the project has taken a highly visible start with the fourth EU-ToxRisk Open Symposium, the yearly appointment for discussion and active interaction between EU-ToxRisk and international experts. The meeting took place virtually on February 23-24, 2021, hosting more than 300 participants from all over the world.

The scientific program focused on the critical assessment of general and specific learnings of the EU-ToxRisk project to provide a foundation for its legacy. As one of the key project deliverables, the “Advisory document on Novel Approach Method (NAM)-enhanced read-across (RAX)”, was the first topic addressed. Its content and application were reported, including the feedback and endorsements from the Regulatory Advisory Board, the OECD IATA Case Studies Project, and several international regulatory toxicologists. The approach described in the advisory document had already been discussed previously at several events. Most notable of these was the EU-ToxRisk workshop held in Espoo, Finland (May 2019). The recently published

meeting report by Rovida et al. (2021) describes how more than a hundred people actively participated in the discussion on that occasion, bringing together diverse viewpoints across academia, regulators, and industry. A consensus was reached that NAMs can improve confidence in RAX, in particular in defining category boundaries as well as characterizing the similarities/dissimilarities between source and target substances.

The 4th EU-ToxRisk Open Symposium also hosted a successful session on the currently running 2nd generation case studies (CS). These studies address new challenging regulatory and scientific questions, such as testing of chemicals with little or no observed adverse effects, with multi-target organ toxicity that undergo metabolism, or needing *ab-initio* risk assessment. One of those is the case study on the developmental neurotoxicological (DNT) effects of neonicotinoid pesticides. Results from this CS were recently published by Loser et al. (2021). In this multi-disciplinary study, the question was addressed whether neon-

icotinoid pesticides can interfere with human neuronal signaling and may therefore pose a DNT risk. A broad range of *in vitro* and *in silico* approaches was used (molecular docking to the nicotinic acetylcholine receptor (nAChR), physiology-based toxicokinetic modelling, cell-based signaling studies), and the outcome was that a subgroup of neonicotinoids triggers neuronal signaling via the nAChR in the low micromolar range, a concentration that may be of human toxicological relevance.

Finally, the Open Symposium accommodated an exciting discussion on the upcoming new frameworks, projects, and partnerships, including a platform to jump-start new efforts in next-generation risk assessment. The session also included an interesting debate on validation and trust in NAM to address specific regulatory information requirements or decision-making needs.

An important conclusion was that transparency and a close understanding of NAM will lead to their increased acceptance for regulatory use.



EU-ToxRisk publications

The EU-ToxRisk research has indeed strongly focused on detailed characterization and description of the developed test systems. The better a method and its applicability domain are characterized, the more appropriate regulatory questions can be asked.

Here are some examples of this approach described in recent project publications and addressing different toxicological areas.

Ter Braak et al. (2021) describe how various adaptive cellular stress response pathways are critical in the pathophysiology of liver disease and drug-induced liver injury. The authors systematically compared the transcriptomic profiles upon chemical activation in (hiPSC)-derived hepatocyte-like cells (HLCs), hiPSC, primary human hepatocytes (PHH), and HepG2 liver cancer cells. Using targeted RNA-sequencing, benchmark concentration transcriptional response was mapped for the various stress responses in the different test systems. This study contributes to a better understanding of how HLCs can contribute to the assessment of cell physiological stress response activation to predict hepatotoxic events.

In the publication by Capinha et al. (2021), the question was addressed how to choose the most relevant test model for renal toxicity. In the publication, the authors highlight how enzymatic bioactivation processes can vary among species. The authors focused on the enzymatic conjugation of glutathione (GSH) to trichloroethylene (TCE) followed by catabolism to the corresponding cysteine-conjugate, S-(dichlorovinyl)-L-cysteine (DCVC) and subsequent bioactivation by renal cysteine conjugate beta-lyases, considered to play an important role in the nephrotoxic effects of TCE. The results suggest that humans are at much lower risk for TCE-associated nephrotoxic effects than rats.

Finally, the EU-ToxRisk researchers addressed the area of DNT. Currently available human-based test systems for neuroscience research or toxicological assays do not well represent crucial receptors, such as the ionotropic glutamate receptors (considered essential, e.g., for memory formation). Klima et al. (2021) describe the establishment and characterization of stem cell-derived neurons that show pronounced NMDA signaling, measured either by calcium imaging or by electrophysiological ap-

proaches (multi-electrode arrays). The developed model may fill an important gap in the panel of test systems available to characterize the effects of chemicals on neurotransmitter receptors.

Knowing your cell system well is crucial for its correct implementation in a toxicological test method. However, this is not sufficient. Knowing your tested chemical and its correct handling well are also important. Handling of chemicals is an often-neglected area of test descriptions. In Leist (2021), an overview is given on the need for more active involvement of all test developers and performers with this issue. The publication gives guidance and examples on how to disclose transparent information concerning the preparation and use of test and control chemical solutions.

This crucial issue also was addressed by Proença et al. (2021). Nominal effect concentrations from *in vitro* toxicity assays may lead to inaccurate estimations of *in vivo* toxic doses because the nominal concentration poorly reflects the concentration at the molecular target in cells *in vitro*, which is responsible for initiating effects and can be referred to as the biologically effective dose. Chemicals can differentially distribute between *in vitro* assay compartments, including serum constituents in exposure medium, plate plastic, headspace, and extracellular matrices. This paper reviews the mechanisms by which test chemicals distribute between *in vitro* assay compartments and also lists the physicochemical properties driving the extent of this distribution. It helps define chemical and biological applicability domains of individual models, as well as provide a perspective on how to improve model predictivity and quantitative *in vitro-in vivo* extrapolations.

Outlook

The consortium is finalizing the organization of its last year of dissemination activities. More information will be available soon, but all interested readers can already save the dates November 3-4, 2021 (The Square, Brussels) when the consortium and its stakeholders will have their last meeting at the EU-ToxRisk Final Symposium. On this occasion, the project legacy will be summarized and the next phase of toxicological research kicked-off.

References

- Capinha, L., Jennings, P., Commandeur, S. et al. (2021). Bioactivation of trichloroethylene to three regioisomeric glutathione conjugates by liver fractions and recombinant human glutathione transferases: Species differences and implications for human risk assessment. *Toxicol Lett* 341, 94-106. doi:10.1016/j.toxlet.2021.01.021
- Klima, S., Brüll, M., Spreng, A. S. et al. (2021). A human stem cell-derived test system for agents modifying neuronal N-methyl-D-aspartate-type glutamate receptor Ca²⁺-signalling. *Arch Toxicol*. Online ahead of print. doi:10.1007/s00204-021-03024-0
- Leist, M. (2021). Identifying, naming and documenting of test and tool compound stocks. *ALTEX* 38, 177-182. doi:10.14573/altex.2012311
- Loser, D., Hinojosa, M. G., Blum, J. et al. (2021). Functional alterations by a subgroup of neonicotinoid pesticides in human dopaminergic neurons. *Arch Toxicol*. Online ahead of print. doi:10.1007/s00204-021-03031-1
- Proença, S., Escher, B. I., Fischer, F. C. et al. (2021). Effective exposure of chemicals in *in vitro* cell systems: A review of chemical distribution models. *Toxicol In Vitro* 73, 105133. Online ahead of print. doi:10.1016/j.tiv.2021.105133
- Rovida, C., Escher, S. E., Herzler, M. et al. (2021). NAM-supported read-across: From case studies to regulatory guidance in safety assessment. *ALTEX* 38, 140-150. doi:10.14573/altex.2010062
- Ter Braak, B., Niemeijer, M., Boon, R. et al. (2021). Systematic transcriptome-based comparison of cellular adaptive stress response activation networks in hepatic stem cell-derived progeny and primary human hepatocytes. *Toxicol In Vitro* 73, 105107. doi:10.1016/j.tiv.2021.105107

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 681002.

Giorgia Pallocca and Marcel Leist