Corners

Upcoming Event

Microphysiological Systems World Summit
May 30-June 3, 2022: Hybrid Conference,
Hilton New Orleans Riverside,
New Orleans, LA, USA
https://mpsworldsummit.com

The MPS World Summit will bring together a global audience – including institutions (government, 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 conferences to create a roadmap for MPS technologies. NIH NCATS will support the first three MPS World Summits with $450,000. This substantial NIH contribution is still only a fraction of the anticipated costs.

Call for abstract submissions:
Abstract submission deadline: January 31, 2022, 11:59pm PST

Abstracts are invited on the topic of new developments in MPS and applications of MPS. Describe your newest developments: break-throughs, advantages, challenges, and the field of applications. Top-scored abstracts will be selected for an oral presentation at one of 14 scientific sessions.

Abstract submission guidelines:
– All abstracts will be evaluated and selected for oral or poster presentation by the MPS Scientific Advisory Committee.
– Abstracts must be in English with no more than 300 words (title, authors and references excluded).
– Pictures, figures, attachments are not permitted.
– Include no more than three references.
– Please submit your abstract following the link: https://app.oxfordabstracts.com/stages/3626/submitter
– Make sure to select the top two sessions your abstract fits the best.
– Up to two abstracts are allowed per presenting author.
– All presenters must be registered for the conference, if accepted.
– Register for the meeting here: https://mpsworldsummit.com

Themes:
– New approaches in bioengineering of MPS devices
– New cellular models (from bio-printing to organoids, etc.)
– Individual organ MPS
– Immune function and vascularization in MPS
– Quality assurance of MPS
– Reproducibility and robustness
– Standardization and harmonization, validation
– Road toward regulatory acceptance
– MPS for disease modeling and drug efficacy testing
– MPS for toxicity testing, predictive toxicology, MPS for AOP
– Case studies using MPS
– Pharmacokinetics and -dynamics in MPS
– Computational modeling and A.I. in dialog with MPS
– Precision medicine and clinical trials on chip planning
– Ethical aspects of using MPS
– Data collection, storage, management, dissemination

Address your questions to: cjanuar1@jhu.edu

Become a sponsor! Multiple sponsorship levels are available. Information on sponsorship: https://mpsworldsummit.com/s/MPS-One-Sheet-and-Sponsorship-Levels-Flyer.pdf

Please contact Camila Januario (cjanuar1@jhu.edu) if you need more information.

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VIDEO: 3Rs Training Webinar Series 2021

https://www.youtube.com/channel/UC7WgnOtO4Ez6HiDRlTLEWZg/videos?app=desktop

Out of the seven 3Rs Training webinars we organized this year, four are in English and three are in German – one being a talk by CAAT Director Thomas Hartung. They include a talk on "Human-relevant airways models for disease research" by Lindsay Marshall, PhD, HSUS/HSI/Biomed21; a talk about "Norecopa: a National Consensus Platform working to advance the 3Rs internationally" by Prof. Adrian Smith, Norecopa; a talk about by Laure-Alix Clerbaux, PhD, Joint Research Centre of the European Commission about "Depicting the pathogenesis of COVID-19 using the adverse outcome pathway"; and a talk about "The principles of the 3Rs: Lessons learned from history" by Anne van Veen, PhD, Radboud University, Nijmegen, The Netherlands. We will continue our 3Rs Training webinar series in February. Details will be announced via the CAAT Newsletter.

VIDEO: CAAT's 40th Anniversary Celebration

https://www.youtube.com/watch?v=gCAaTMbS-G8

- Opening remarks: Andrew Rowan and Alan Goldberg
- Developmental NeuroToxicity (DNT): Helena Hogberg and Tim Shafer
- Evidence-based Toxicology Collaboration (EBTC): Sebastian Hoffmann and Daniele Wikoff
- Good Cell Culture Practice (GCCP) Program: Marcel Leist and Sandra Coecke
- Green Toxicology: Nick Anastas and Alexandra Maertens
- Cosmetic and TestSmart Program: Tony Gaspari and Julia Fentem
- Education Program: Deborah Rudacille and Lena Smirnova
- Refinement Program: Joanne Zurlo and Kathrin Herrmann
- World Congress on Alternatives: Bert van Zutphen
- Policy Program: Paul Locke and Pamela Frasch
- Future Perspectives: Thomas Hartung

VIDEO: Challenges and Opportunities for Overcoming Dog Use in Agro-chemical Evaluation and Registration

https://www.youtube.com/playlist?list=PLq3BABaYKtzndvLCmE2NT5DkB_sfCNIUs

The 90-day dog study is being conducted for agro-chemical authorization when it is not always needed to adequately address hazard identification and human safety and risk. This virtual workshop hosted a series of presentations on the role the dog study has played in regulation of agro-chemicals in both the U.S. and Europe during the past 20 years and what approaches may be employed to substantially reduce its use. The public had the opportunity to submit comments and questions to be discussed during the following invitation-only workshop.

Presentations:

- "The value of the 90-day dog study in pesticide registration toxicity testing in the U.S." by Patricia L. Bishop (The Humane Society of the United States), Douglas C. Wolf (Syngenta Crop Protection) and Vicki Dellarco (Independent Consultant)
- "Incorporating toxicokinetic and toxicity data to evaluate the value added from using dogs in subchronic toxicity testing for agrochemicals" by Lynea Murphy (Corteva AgriScience), Robert Mingoia (Corteva AgriScience), Jean Domoradzki (Corteva AgriScience) and Claire Terry (Corteva AgriScience)
- "Relevance of dog studies for the derivation of health-based guidance values for Plant Protection Products Approval – EFSA" by Martina Panzarea (EFSA) and Andrea Terron (EFSA)
- "Species Liver-Chip to assess cross-species drug toxicity and human relevance" by Kyung-Jin Jang (Emulate)

VIDEO: Alternative Approaches in Developmental Neurotoxicity – A Farewell Symposium for CAAT's Deputy Director Helena Hogberg

https://www.youtube.com/watch?v=-WPowT4_UXM

There is a paucity of information concerning the developmental neurotoxicity (DNT) hazard posed by industrial and environmental chemicals, drugs, and consumer products. New testing approaches will most likely be based on batteries of alternative and complementary (non-animal) tests. This symposium had the objective to discuss and present the status of DNT and honor Helena Hogberg for her 12 years working at the Center for Alternatives to Animal Testing and moving further to NICEATM at NIEHS.

- Helena Hogberg: Opening remarks
- Alan Goldberg and Pamela Lein: DNT – How it all began
- Lena Smirnova: DNT journey of CAAT research
- Ellen Fritsche: Scientific validation of the neurosphere DNT test methods
- Iris Mangas: The DNT programme in EFSA, past, present and future
- Tim Shafer: DNT NAMs are already being used
- Magda Sachana: OECD DNT project: seeing the light at the end of the tunnel
- Anna Price: Future of DNT testing: evaluation of effects induced by mixtures using NAMs
- Mamta Behl: Beyond DNT – A great colleague and a wonderful friend
- Thomas Hartung: Director remarks
- Helena Hogberg: Closing remarks

VIDEO: EBTC Symposium. In Here to Out There: The Counterintuitive and Fascinating Challenge of Standardizing Toxicity Assays

https://youtu.be/cESbiRjAAB4

Toxicological research is often conducted according to highly uneven standards. Difficulties in comparing the methods used by individual studies often seriously limit our ability to draw firm conclusions from even quite large bodies of evidence. EBTC's December symposium was about new developments in solving this problem, in particular the marrying of novel techniques in semantic technology to traditional but challenging assay ring-testing and development.

Dr Anne Thessen (University of Colorado Anschutz) introduced some of the more surprising issues around assay consistency, including recent research that suggests scientists cannot reliably distinguish between live and dead zebrafish embryos. She described how semantic technology can help improve this situation – not only in terms of data interoperability, but in reliably performing tasks such as scoring images of larvae.

Dr Sebastian Hoffmann (EBTC) presented an overview of EBTC's recent systematic review of zebrafish embryotoxicity assays. He showed why standardization is becoming such an important issue, particularly in an age where studies are viewed collectively, and evidence synthesis is increasingly central to toxicology and chemical risk assessment.

Dr Kristen Ryan (National Toxicology Program) discussed how broader uptake of the use of zebrafish in toxicology screening is hampered by lack of harmonization in experimental protocols, data analysis, and reporting of research. Dr Ryan presented on the goals of the SEAZIT program and how it will achieve them.

Thomas Hartung reaches h-index of 100

Thomas Hartung, director of CAAT, according to Google Scholar, achieved a rare publication milestone. The h-index, aka Hirsch index, is a measure of productivity and citation impact of the publications of an individual scientist. The h-index is determined from the number of publications by a scientist and the number of times those publications have been cited. 100 means that 100 articles have been cited 100 times or more. The ranking web of universities (https://www.webometrics.info/en/hlargerthan100) listed fewer than 12,000 researchers world-wide (3,211 in the US) in all disciplines with such a score in March 2021. The total number of researchers world-wide, for comparison, is estimated at 8.8 million.

The metric is provided by Google Scholar, which lists more than 38,000 citations of Thomas' more than 600 articles.

Much of his most cited work relates to pyrogens, especially lipoteichoic acids.

- His most cited paper with nobel laureate Bruce Beutler has 998 citations:
  Hoebe, K., Georgel, P., Rutschmann, S. et al. (2005). CD36 is a sensor of diacylglycerols. *Nature 433*, 523-527. doi:10.1038/nature03253
- His most cited toxicology work with 530 citations at number 6 is:
  Hartung, T. (2009). Toxicology for the twenty-first century. *Nature 460*, 208-212. doi:10.1038/460208a
- The 2005 guidance on Good Cell Culture Practice is at number 10 with 383 citations:
  Coecke, S., Balls, M., Bowe, G. et al. (2005). Guidance on Good Cell Culture Practice. *Altern Lab Anim 33*, 261-287. doi:10.1177/026119290503300313
- So far, he has published 163 articles in CAAT's official journal *ALTEX*. The top article in *ALTEX* appears at number 25 with 250 citations:
  Marx, U., Akabane, T., Andersson, T. B. et al. (2020). Biology-inspired microphysiological systems to advance medicines for patient benefit and animal welfare. *ALTEX 37*, 364-394. doi:10.14573/altex.2001241
- Noteworthy, the article that made him cross the 100 citation bar laid out in 2014 the vision for the standardized human brain organoid, aka mini-brain:
  Pamies, D., Hartung, T. and Hogberg, H. T. (2014). Biological and medical applications of a brain-on-a-chip. *Exp Biol Med 239*, 1096-1107. doi:10.1177/1535370214537738

European Parliament Resolution of 16 September 2021 on Plans and Actions to Accelerate the Transition to Innovation Without the Use of Animals in Research, Regulatory Testing and Education

This resolution "*Calls on the Commission to improve coordination to achieve the goal set out in Directive 2010/63/EU by establishing a high-level inter-service taskforce, involving all key Directorates-General and agencies, to work with the Member States and relevant stakeholders to draw up an EU-wide action plan, with the aim of driving the active phase-out by reducing, refining and replacing procedures on live animals for scientific and regulatory purposes, as soon as scientifically possible and without lowering the level of protection for human health and the environment, while accelerating the development of the alternative animal-free methods, technologies and instruments necessary for change; stresses that a clear and ambitious timeline and list of milestones should be set out to incentivise progress*".

CAAT is proud that our European Policy Program led by Francois Busquet (AlterTox) contributed advice and suggested several accepted amendments.

https://www.europarl.europa.eu/doceo/document/TA-9-2021-0387_EN.html

Frontiers in Artificial Intelligence Thriving

The journal launched in 2018 and has published more than 300 articles and started almost 200 Research Topics. Thomas Hartung as Field Chief Editor leads the journal with 12 sections, one of them, i.e., *Medicine and Public Health*, led by him too. The journal received 192 submissions in the last six months (up 85% compared to the previous year) and posted 53 Research Topics in the last six months (up 20% compared to previous year) and is supported by 1,759 chief, associate, and review editors. With a healthy rejection rate of more than 40% and an average review time of 116 days since its start, the journal assures quality.

Major achievements in 2021 included indexing in *PubMed Central, Emerging*

Sources Citation Index and *Scopus* – it is expecting its first impact factor in 2022.

The collection *Artificial Intelligence* – Editor's Pick 2021 (https://www.frontiersin.org/research-topics/30211/artificial-intelligence---editors-pick-2021) showcases the most well-received spontaneous articles from 2021. The work highlights the broad diversity of research performed across the journal and aims to put a spotlight on the main areas of interest. All research presented displays strong advances in theory, experiment, and methodology with applications to compelling problems.

International Foundation for Ethical Research (IFER) Presents Fellowships to CAAT's Graduate Students Ishita Virmani and Alan Kim

Each year the IFER presents graduate fellowships to those master's and doctoral students whose proposals show the greatest potential to contribute towards the reduction, refinement, and replacement (3Rs) of animal use in research, testing and/or education. Ishita Virmani and Alan Kim are the proud members of the CAAT family whose proposals were selected, and we expect them to continue into a very productive year!

SOT/EUROTOX Debate: Is There a Role for Artificial Intelligence and Machine Learning in Risk Decisions?

March 28, 2022, 4:45 pm - 6:15 pm

Each year, the SOT Annual Meeting includes a debate in which leading toxicologists advocate opposing sides of an issue of significant toxicological importance. The debate continues a tradition that originated in the early 1990s.

Chair(s):
Dori R. Germolec (NIEHS/NTP);
Thomas Weiser (F. Hoffmann-La Roche AG, Switzerland)
SOT Debater:
Craig Rowlands (UL LLC, Northbrook, IL).
EUROTOX Debater:
Thomas Hartung (Johns Hopkins University Bloomberg School of Health, Baltimore, MD).

This year, the debaters will address the proposition: Is there a role for artificial intelligence (AI) and machine learning (ML) in risk decisions? The debaters will discuss the principles and limitations of these tools for decision-making. Specific questions to be addressed include: Can recent developments in AI and ML be applied in toxicology as they are being applied in precision medicine and other fields? Are current algorithms inherent to AI and ML sufficiently refined for the design of safer products? Are these applications sufficiently robust to identify toxic "signatures," providing information for safety and risk assessment? Are they sufficiently reliable to predict toxicity and can they account for genetic and other toxicodynamic variability? Are they able to predict risk associated with exposures to mixtures? Can they predict the potential toxicity of new compounds or relate chemical structure or activity to risk? How can we tell if the results of AI and ML are accurate and defensible? In addition to inclusion as a Featured Session at this meeting, this debate will again take place (with the debaters taking the reverse positions) in Maastricht, the Netherlands, during the XVIth International Congress of Toxicology, September 18-22, 2022.

New Publications

New article: "Autism in Three Dimensions: Using Brain Organoids to Study Potential Gene-Environment Interactions" in *Environmental Health Perspectives*

Lena Smirnova and colleagues compared brain organoids containing a normal version of *CHD8*, the gene encoding chromodomain helicase DNA binding protein 8, with those containing the high-risk ASD mutation *CHD8*$^{+/-}$. *CHD8* is one of more than 50 genes associated with higher risk of ASD. The number of these genes, plus more than 50 well-supported additional genes with lower penetrance, illustrates the genetic heterogeneity of ASD that contributes to its wide symptom variation.

The clinical heterogeneity of autism spectrum disorder (ASD) makes it difficult to match treatments to patients. The unique nature of ASD in humans has also hampered the use of animal models in drug development. Brain organoids, "miniature brains" derived from human cells, have emerged as a promising alternative strategy for understanding ASD. The study suggests these organoids may also be well suited for modeling the gene-environment interactions that have long been suspected of increasing ASD risk. Available at: https://ehp.niehs.nih.gov/doi/10.1289/EHP10301

New paper published about the forced swim test in rats

Kathrin Herrmann, CAAT's Beyond Classical Refinement Director, published a paper with colleagues Carvalho, Marques and Knight asking is it "Time to Abolish the Forced Swim Test in Rats for Depression Research?" The forced swim test (FST) is a controversial rodent test that has been used for decades, mainly in depression studies. The severity of the procedure makes it ethically questionable, and its validity has also been questioned. This paper contributes new data to this debate. Available at: https://doi.org/10.1163/25889567-bja10026

Beyond Cholinesterase Inhibition: Developmental Neurotoxicity of Organophosphate Ester Flame Retardants and Plasticizers

Press release:
Common Chemicals in Electronics and Baby Products Harm Brain Development

Berkeley, Calif. – Chemicals increasingly used as flame retardants and plasticizers pose a larger risk to children's brain development than previously thought, according to a commentary published today in *Environmental Health Perspectives*. The research team reviewed dozens of human, animal, and cell-based studies and concluded that exposure to even low

levels of the chemicals – called organophosphate esters – may harm IQ, attention, and memory in children in ways not yet looked at by regulators.

The neurotoxicity of organophosphate esters used as nerve agents and pesticides is widely recognized, but the neurotoxicity of those used as flame retardants and plasticizers has been assumed to be low. As a result, they are widely used as replacements for some phased-out or banned halogenated flame retardants in electronics, car seats and other baby products, furniture, and building materials. However, the authors' analysis revealed that these chemicals are also neurotoxic, but through different mechanisms of action.

"The use of organophosphate esters in everything from TVs to car seats has proliferated under the false assumption that they're safe," said Heather Patisaul, lead author and neuroendocrinologist at North Carolina State University. "Unfortunately, these chemicals appear to be just as harmful as the chemicals they're intended to replace but act by a different mechanism."

Organophosphate esters continuously migrate out of products into air and dust. Contaminated dust gets on our hands and is then inadvertently ingested when we eat. That's why these chemicals have been detected in virtually everyone tested. Children are particularly exposed from hand-to-mouth behavior. Babies and young children consequently have much higher concentrations of these chemicals in their bodies during the most vulnerable windows of brain development.

"Organophosphate esters threaten the brain development of a whole generation," said co-author and retired NIEHS Director Linda Birnbaum. "If we don't stem their use now, the consequences will be grave and irreversible."

The authors call for a stop to unnecessary uses of all organophosphate esters. This includes their use as flame retardants to meet ineffective flammability standards in consumer products, vehicles, and building materials.

For uses where organophosphate esters are deemed essential, the authors recommend governments and industry conduct alternatives assessments and make investments in innovative solutions without harmful chemicals.

"Organophosphate esters in many products serve no essential function while posing a serious risk, especially to our children," said Carol Kwiatkowski, co-author and Science and Policy Senior Associate at the Green Science Policy Institute. "It's urgent that product manufacturers critically reevaluate the uses of organophosphate ester flame retardants and plasticizers – many may be doing more harm than good."

Available at: https://ehp.niehs.nih.gov/doi/10.1289/EHP9285

Cruelty Free INTERNATIONAL
Ending animal experiments worldwide

German animal testing facility will close in 2022

Animal testing facility LPT – which earlier this year changed its name to Provivo Biosciences – will close by the end of 2022, with animal experiments ending in January 2022.

LPT was the company at the center of Cruelty Free International's investigation with SOKO Tierschutz in 2019, which exposed shocking levels of animal cruelty and alleged breaches of EU and German law. LPT was a family-owned contract-testing laboratory carrying out toxicity testing for pharmaceutical, industrial and agrochemical companies from all over the world in order to meet the requirements of governments and regulatory authorities. Graphic undercover footage of dogs left bleeding and dying, and monkeys routinely abused during handling caused shockwaves across Europe and beyond. Jane Goodall, Founder of the Goodall Institute and UN Messenger of Peace, said that the footage shows "some of the worst abuse I've ever seen on testing with animals".

Current laboratories at Neugraben in Hamburg and Gut Löhndorf in Schleswig Holstein, northern Germany, will follow their former site at Mienenbüttel in being closed, with a new site planned to conduct tests without the use of animals.

Joint calls for the European Commission to transform chemical safety management to reduce animal testing

Cruelty Free Europe, Members of the European Parliament, and CEFIC, the European Chemical Industry Council, have called for more ambition and a greater commitment from the Commission in ensuring that revised EU chemicals laws make full use of non-animal testing methods.

In a joint letter addressed to Executive Vice President Frans Timmermans and other European Commissioners, the group urge that the EU's goal of reduc-

ing and replacing animal testing be fully taken into account at all stages of the REACH revision process, including an assessment of the numbers of animal tests that may result from any changes in the legislation. Initial estimates by Cruelty Free Europe indicate that proposals to increase information requirements to address endocrine disruptors could lead to at least 3.6 million more animal tests and at least 1.6 million more for polymers.

As well as arguing for a modernized legislative framework, the letter outlines seven concrete actions to be taken to help prioritize animal-free new approach methodologies (NAMs). These include a full evaluation of the effects of animal testing in the impact assessments for all legislation linked to the Chemicals Strategy for Sustainability, as well as stricter monitoring of the animal tests required, and the number of animals used for testing.

The REACH law governing EU chemicals management is expected to be revised next year and should be a key opportunity to ensure that data generated by tests reflect the latest scientific advances, actively reduce and replace animal testing, and that both existing data and reliable NAMs are developed, promoted and accepted faster by the European Commission, EU Member States, and the European Chemicals Agency.

Another Board of Appeal case challenges ECHA's approach to animal testing

In December, the European Chemicals Agency's Board of Appeal overturned another ECHA request for new animal tests. BASF Colours had launched an appeal over a compliance check decision requiring them to conduct a long-term toxicity test on fish, as just prior to the issuing of the final decision they had reduced their production volumes to below the legal threshold for requiring the test. Cruelty Free Europe were one of the interveners in the appeal.

In its decision announced this week, the Board of Appeal agreed with BASF and Cruelty Free Europe that ECHA had "breached its duty to ensure that the Appellants carry out studies on vertebrate animals only as a last resort" and that the tests on fish should not be carried out. The Board's decision has set an important precedent in ensuring that ECHA takes reduced production volumes into account when considering orders for new tests, even if they occur during the decision-making stage.

Humane Cosmetics Act reintroduced in the U.S

In December, the Humane Cosmetics Act was reintroduced in the U.S. House and Senate by a bipartisan group of legislators including Senators as well as 70 other house co-sponsors. The bill would end the use of animal-based testing for cosmetics in the U.S. and prohibit the sale of any cosmetic product that has been tested on animals after the date of enactment.

A 2019 online poll, conducted by SurveyUSA on behalf of Cruelty Free International, found that nearly 8 out of 10 supported a federal law that would prohibit animal testing for cosmetics.

Representative Don Beyer, who reintroduced the bill, said, "Congress must bring an end to the cruel and outdated practice of animal testing. Advances in the cosmetics industry have already made the process unnecessary by offering safer and more scientifically sound methods. By passing the Humane Cosmetics Act, we are outlawing an obsolete and inhumane practice without damaging American businesses. I thank my colleagues in both parties and chambers for their support of the Humane Cosmetics Act, which I hope will receive swift consideration."

Animal protection plan to kickstart phase-out of animal experiments in Europe

In a landmark vote in September, the European Parliament overwhelmingly backed the need for an EU-wide action plan with a clear and ambitious timeline and reduction targets to drive the phase-out of animal experiments. MEPs challenged the European Commission to establish a high-level taskforce, involving all its relevant departments and agencies, to work with the Member States and other stakeholders to draw up the plan.

In an attempt to kickstart the discussion, Cruelty Free Europe has published a plan that provides suggestions for concrete actions for the Commission, EU agencies, member states, and others to take to begin this process. The plan proposes, amongst other things: an immediate review of the utility of animal "models" to assess clinical benefit, identifying and eliminating those tests that are of low benefit from the outset, either due to scientific limitations or lack of clinical need, using the harm:benefit analysis built into EU legislation on animal experiments more strictly, paying due heed to public opinion and rejecting experiments that are likely to cause high suffering and/or be of low benefit, an immediate review of the reasons behind the numbers of surplus animals bred for experiments and killed without being used and taking measures to reduce these numbers, a reviewing of the need for genetically altered animals currently bred in huge numbers for experiments in Europe, ensuring that animal tests are not conducted when a non-animal alternative is available by improving communication between the Commission, member states and companies, and, finally, a commitment to review potential redundancies in regulatory animal tests and making sure there is a joined-up approach to deleting them.

According to the most recent EU figures for 2018 released this year, there are still over ten million uses of animals in experiments. Numbers have been falling at an average of only 1% annually over the past 20 years. Were that annual pattern to continue, it would take the best part of another century to end the use of animals in science in Europe.

For a copy of the plan, please email info@crueltyfreeeurope.org

EINSTEIN CENTER 3R

Einstein Center 3R in Berlin

Academic biomedical institutions such as university faculties conduct animal experiments in both basic and translational research. In Germany, such *in vivo* studies account for more than half of all approved animal experiments.[1] Several factors hinder the consistent use and dissemination of alternative methods to replace animal experiments in academic biomedical research. Here, science is characterized by great diversity in terms of content and methodology, frequent experimental adaptations, usually relatively low numbers of animals per project, and a large fluctuation in the scientists conducting the experiments (e.g., doctoral students, post-docs). In most cases, a combination of several complementary experimental models and methods is needed to truly replace a given animal experiment. The development of several such alternative methods is a lengthy process and overtaxes the capabilities of many research groups. There are considerable deficits in the reproducibility and robustness of methods in both animal experiments and alternatives, contributing to the reproducibility crisis of biomedical academic research. Furthermore, in contrast to alternative methods that have been approved in the regulatory environment, the question of whether a potential alternative method is accepted and thus used in the academic scientific community depends on its acceptance by individual scientists and the influence of the peer review system. This means that experimental models in academic biomedicine – both animal experiments and alternatives – are rarely conducted over a longer period in the same way with the same research in high quality. These factors, among others, make it challenging to consistently reduce the use of animals in academic research. Some of the factors described above are out of the influence of individual scientists, others are inherent in the knowledge discovery process of academic biomedicine.

The new Einstein Center 3R in Berlin[2] (Fig. 1) addresses two aspects that can be directly influenced by science: It places particular emphasis on better characterization, robustness, and transferability of the models, and it involves animal experimenters as new potential users of the models right from the start.

To this end, it implements a Collaborative Research Network, which focuses on the joint development of 3D tissue culture models. Six scientific projects were selected in a competitive, city-wide tendering process followed by independent assessment. These involve investigating specific parameters that influence the formation and growth of human intestinal organoids; optimizing engineered human heart tissue for functional diagnostics, drug tests, and therapy; using human brain organoids to study neurodegenerative diseases; using functional units of human lung tissue to improve research into lung diseases with the help of non-epithelial cells; improving the investigation of neuromuscular diseases such as myasthenia gravis using human

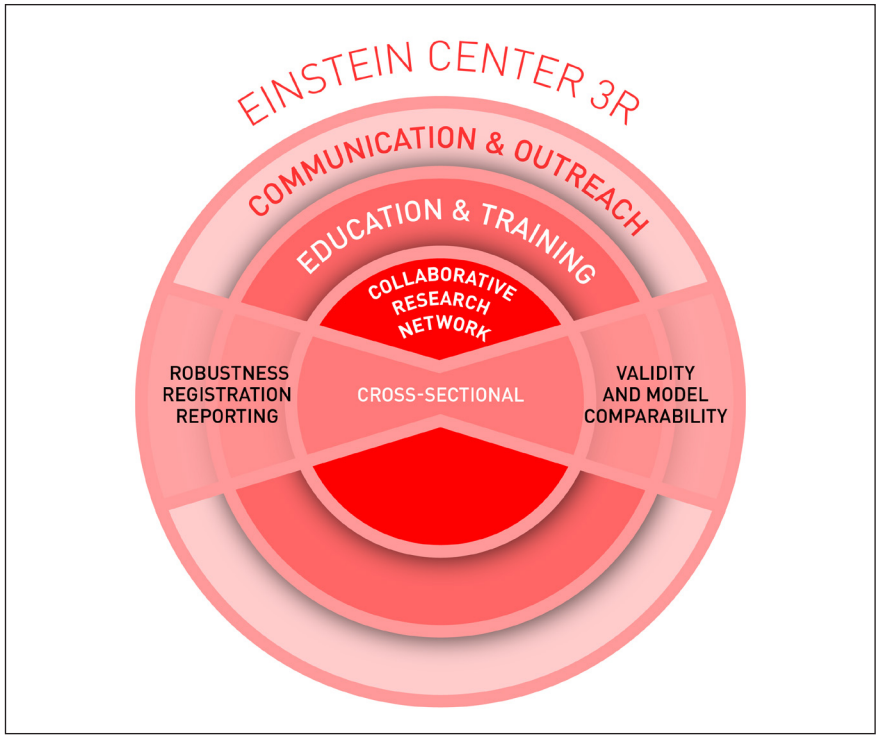

Fig. 1: Einstein Center 3R (EC3R)

[1] Use of laboratory animals in 2019, Federal Ministry of Food and Agriculture, Germany https://www.bmel.de/DE/themen/tiere/tierschutz/versuchstierzahlen2019.html
[2] https://www.ec3r.org/en

neuromuscular organoids; and bioprinting human organ models (liver) composed of several cell types with high spatial resolution. All projects have described a specific group of users of the models who are actively involved in their development. This should facilitate transfer of the complex methods, create an understanding for their strengths and weaknesses, and increase their acceptance. The Collaborative Research Network provides a platform for overarching tasks like agreement on standards and comparative validation of models. Two further cross-sectional projects aim to increase the impact of 3R research by implementing innovative imaging, artificial intelligence applications, and extended quality management (6Rs: robustness, registration, reporting).

In addition, the EC3R plans to provide a comprehensive education and training program. Consistent training in all facets of the 3Rs is the basis for high scientific quality, acceptance of the underlying science, and dissemination of the acquired scientific information. By integrating the broad spectrum of existing expertise in the Berlin 3R community and adding complementary approaches and knowledge, the EC3R aims to boost the quality, efficacy, and application of 3R methods.

Furthermore, the EC3R fosters communication and outreach. Comprehensive, honest, fact-based communication is necessary to inform scientists and the public about the great opportunities of the 3Rs and challenges to implementing them. The EC3R aims to increase visibility of the 3R activities in Berlin by communicating them in a transparent, science-based manner. Citizens receive easy-to-comprehend information and are included in a dialogue with the scientists.

The EC3R began operations in July 2021 and is funded for an initial three-year period with the option to extend the funding. Long-term financial support of interdisciplinary collaborative scientific networks with simultaneous training of young scientists is key to the consistent implementation of the 3Rs in academic biomedical research. The scientists involved in EC3R are convinced that the 3Rs contribute significantly to scientific innovation beyond improved experimental animal welfare.

EC3R receives funding from the Einstein Foundation Berlin[3].

Stefan Hippenstiel[1], Christa Thöne-Reineke[2] and Jens Kurreck[3]

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[3] www.einsteinfoundation.de

EUSAAT

European Society for Alternatives to Animal Testing

New EUSAAT website is ONLINE

On December 13, 2021, the new EUSAAT website https://eusaat.eu/ was launched after half a year of designing and with the approval of the EUSAAT members.

In addition to an attractive graphical surface, we have added important information and functions for the user. In the "About us" section, you will find useful information on our mission, the board, corporate members, and the statutes. The "3Rs Society" section provides a short introduction to the 3Rs, 3Rs societies, the 3Rs-net network, and links to relevant 3Rs resources. Moreover, there is a separate section for the EUSAAT Congresses and the Virtual Seminar Series. In the Virtual Seminar Series section, more than 20 videos covering all three Rs can be enjoyed. In addition, up-to-date news on alternatives will be presented on our website, especially on 3Rs congresses and publications. To get in touch with EUSAAT, please fill out the contact forms for the EUSAAT Newsletter as well as the EUSAAT membership application.

The EUSAAT Board hopes that the website will serve as a useful tool for the 3Rs community and to keep in touch with you.

EUSAAT Board election in 2022

According to the statutes, the EUSAAT Board must be elected every four years. The current EUSAAT Board started its term at the beginning of 2018 and, therefore, it is time to elect the next EUSAAT Board early in 2022.

The election procedure was agreed upon at the EUSAAT annual general assembly on December 16, 2021. We are very much looking forward to a high voter turnout for the 2022 EUSAAT Board election, and we would appreciate if some of you volunteer to serve as candidates for the new EUSAAT Board.

EUSAAT Congress 2022 in Linz, Austria on September 26-28

The EUSAAT Board is pleased to announce that we are again preparing an EUSAAT congress. The congress will take place on September 26-28, 2022. Selecting this date guarantees that the congress will not be over a weekend, and we think that it will be feasible as an in-person congress according to previous experience with the COVID-19 pandemic. As the next 3Rs World Congress will be organized in Canada in 2023, the EUSAAT congress will be the next international 3Rs congress worldwide. In good tradition, we have chosen the University of Linz, Austria as the venue, and rooms for our participants in the "Sommerhaus" have also already been reserved.

Topics will cover all the 3Rs – replace, reduce, refine – ranging from new technologies for risk assessment and basic science (disease models, microphysiological systems, organ-on-chips, *in silico* methods, AOPs, NAMs, QIVIVE, experimental design, Good Cell Culture Practice, etc.) to ethics, animal welfare, guidelines, 3Rs policy, 3Rs in education, etc. As in the past, the EUSAAT 2022 Congress will serve as a meeting place for all 3Rs stakeholders to exchange ideas with colleagues whom you haven't met in person for several years. Special attention will again be given to the 3Rs centers and to promoting young scientists (with, e.g., special sessions and travel awards).

We are very much looking forward to welcoming the international 3Rs community to the EUSAAT 2022 Congress in Linz again, and we appreciate any suggestions for additional sessions and topics for the program. The organizers will circulate detailed information early in 2022.

EUTOXRISK

HORIZON 2020

RISK HUNT3R

ASPIS

The EU-ToxRisk project's final symposium and the ASPIS cluster's kick-off meeting were held in Brussels, Belgium on November 3 and 4, 2021. The conference took place in a hybrid format to buffer the unstable pandemic situation. This decision allowed more than 250 persons to attend the conference either online or in person, including talks, posters, and exhibitions.

The purpose of the first day of the event was a final critical assessment of the EU-ToxRisk project. The discussion addressed the project's impact in implementing new technologies and approaches in industry and regulatory frameworks. The speakers and participants discussed and highlighted crucial key actions that allowed the EU-ToxRisk project to significantly impact the implementation of NAM.

The effort to build a proactive dialogue with the stakeholders supported by a solid scientific reputation (measurable by the number of peer-reviewed publications and sessions at international conferences) was one of the project's main learnings.

On the one hand, this was made possible by strengthening the collaboration among international experts of the consortium. This synergy has allowed the development of new, relevant approaches that will apply in specific regulatory contexts (e.g., EFSA and OECD guidelines). On the other hand, innovative approaches and technologies were applied to case studies. This strategy allowed easier interaction with the relevant stakeholders and increased confidence and knowledge exchange between regulators and research projects.

This result was facilitated by other activities aiming to increase transparency and better communication. These actions include standardized test method description templates (ToxTemp) (Krebs et al., 2019), evaluation of the test methods' readiness level (Krebs et al., 2020), and organization of workshops that enriched mutual understanding among different regulatory stakeholders about NAMs and read-across (RAx) (Rovida et al., 2021; Moné et al., 2020).

As final key learning, continuity and sustainability plans were debated. Actions are already in place to channel the outcomes of the EU-ToxRisk project into the research and dissemination plan of follow-up initiatives. Indeed, the final symposium also represented a way for the EU-ToxRisk project to formally pass the torch to the RISK-HUNT*3R* consortium and the ASPIS cluster.

The research cluster ASPIS comprises three projects, RISK-HUNT*3R*, ONTOX, and PrecisionTOX. The three consortia have identified several collaboration areas: chemical selection and risk assessment, omics and computational approach-

es, kinetics and exposure models, and dissemination and communication.[1,2,3]

Progress for NAM implementation into regulatory and industry scenarios is strongly linked to creating a scientific community trained in next-generation risk assessment (NGRA) technologies. The cluster consortia will jointly train their early-career researchers to tackle this crucial issue.

These actions and synergy will allow the ASPIS cluster to broaden its impact in the years to come.

Outlook

The material from the EU-ToxRisk final symposium, including posters and recordings, is available on the EU-ToxRisk website[4] and will soon be available on the RISK-HUNT*3R* website[5].

The first public appointment in 2022 will be the RISK-HUNT*3R*-ASPIS workshop on "Current and future regulatory needs for chemical risk assessment" (January 31, 2022). To ensure that the strategies delivered by RISK-HUNT*3R* and the cluster are fit-for-purpose, regulatory needs will guide their development. Therefore, the workshop participants will discuss and map current and future needs for regulatory risk assessment of chemical substances.

Finally, the 2nd ASPIS cluster symposium will take place in 2022. The ESTIV conference will host the meeting on November 24 in Sitges (Spain). It will represent a milestone event to present the cluster's progress to its stakeholders.

References

Krebs, A., van Vugt-Lussenburg, B. M. A., Waldmann, T. et al. (2020). The EU-ToxRisk method documentation, data processing and chemical testing pipeline for the regulatory use of new approach methods. *Arch Toxicol 94*, 2435-2461. doi:10.1007/s00204-020-02802-6

Krebs, A., Waldmann, T., Wilks, M. F. et al. (2020). Template for the description of cell-based toxicological test methods to allow evaluation and regulatory use of the data. *ALTEX 36*, 682-699. doi:10.14573/altex.1909271

Moné, M. J., Pallocca, G., Escher, S. E. et al. (2020) Setting the stage for next-generation risk assessment with non-animal approaches: the EU-ToxRisk project experience. *Arch Toxicol 94*, 3581-3592. doi:10.1007/s00204-020-02866-4

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

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

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[1] https://www.aspis-cluster.com/
[2] https://chemicalwatch.com/370080/eu-non-animal-projects-brought-together-for-aspis-cluster
[3] https://www.theparliamentmagazine.eu/news/article/delivering-a-toxicfree-environment
[4] https://www.eu-toxrisk.eu/
[5] https://www.risk-hunt3r.eu/

LUSH PRIZE

SUPPORTING ANIMAL-FREE TESTING

Lush Prize Conference Review

More than 200 people from around the world attended the Lush Prize virtual conference that was held over two days in November. This was the first time a conference had been held outside of the biennial prize cycle and provided us with the opportunity to broaden the discussion topics.

The theme, "The role of public awareness in the replacement of animals in safety testing", covered three key areas of public awareness participation: campaigners, regulators and legislators as well as scientists. We heard from experts from Europe, North and South America, and Asia with a wide perspective on how to create awareness to replace animal-based research with human-relevant science.

A fourth panel discussed the ongoing conflict between the REACH regulation (which demands animal testing despite stating it is a "last resort") and the Cosmetics Directive (which has banned animal testing of cosmetics and their ingredients since 2013). This issue was covered in a paper published in ALTEX in October, "Continuing animal tests on cosmetic ingredients for REACH in the EU" (Knight et al., 2021), and we were pleased to have one of the paper's authors, Dr Costanza Rovida of CAAT-Europe, on the panel.

There is clearly an issue of public trust at stake. As a result of legal conflict between the two pieces of legislation, animals continue to suffer for testing of ingredients that are ultimately used in cosmetics, a practice which the public believes to have stopped. Troy Seidle of Humane Society International commented during his presentation that "we are dealing with a public in Europe who believe that animal testing is a thing of the past".

Another panelist, Dr Julia Fentem of Unilever, told the conference: "The future of cruelty-free cosmetics is seriously under threat".

Several speakers referred to an important ongoing initiative – the EU Citizen's Initiative to Save Cruelty Free Cosmetics. This requires 1 million EU citizens to sign it before the deadline of 31 August 2022. If you are an EU citizen, you can add your name to this call on the EU website[1].

ALTEX editor-in-chief Dr Sonja von Aulock was one of three interviewees in our popular "fireside chat" series, bringing the valuable perspective of a science journal editor in how to promote non-animal methods of research as well as encouraging scientists to challenge reviewers and editors who request animal research be done to validate non-animal studies.

The whole conference can be watched on the Lush Prize website[2].

The conference was designed to help us better understand the varied actions required to create awareness and action across multiple audiences in this field, in particular the public, scientists, regulators and legislators. A particularly active audience resulted in lots of sharing of resources, which you can find on our website[3].

Member of the European Parliament Tilly Metz summed up the co-ordinated action that needs to be taken to not only save cruelty-free cosmetics but to also push for more support of non-animal methods of research: "We need the public, the scientists and also the companies to get loud, to get involved, to show that there is really a demand for progress".

Nominations for the next Lush Prize will open in April 2022.

Reference

Knight, J., Rovida, C., Kreiling, R. et al. (2021). Continuing animal tests on cosmetic ingredients for REACH in the EU. *ALTEX 38*, 653-668. doi:10.14573/altex.2104221

1 https://europa.eu/citizens-initiative/initiatives/details/2021/000006_en

2 https://lushprize.org/2021-conference/

3 https://lushprize.org/conference-resources-2021/