# Corners


### Call for Proposals: Reduction and Refinement Award

*Formerly Science-based Refinement Award*
**Deadline: January 31, 2021**

Attention veterinarians, animal care technicians, researchers, and those who care for the well-being of animals used in science: The Johns Hopkins Center for Alternatives to Animal Testing (CAAT) is now accepting proposals for the 2021 Reduction and Refinement Award. This award focuses on research projects that help reduce animal use by, for example, identifying areas of research and testing where animal models lack reproducibility and translational value or that enhance the housing, handling, and/or experimental procedures for laboratory animals who are still deemed necessary. Hence, the grant is intended for researchers who conduct systematic reviews, meta-analyses, or citation analyses of animal studies or similar work with the goal to reduce animal use in science.

This award is also for those who work hands-on with animals, such as animal welfare scientists, veterinarians, and animal care technicians, whose projects can improve the animals' living situation in the laboratory. The award includes prize money of $6,000. There are no facilities and administrative costs allowed on this award.

Details and application instructions: https://caat.jhsph.edu/programs/awards/AWE/index.html

### Thomas Hartung and Marcel Leist Receive Doerenkamp-Zbinden Prize 2020

Thomas Hartung and Marcel Leist, along with the other three endowed chairs of the Doerenkamp-Zbinden foundation, have received the Doerenkamp-Zbinden Prize 2020 in acknowledgement of their outstanding work in promotion of alternatives to animal testing. The prize, presented by the Doerenkamp-Zbinden Foundation for Animalfree Research, was presented on November 5, 2020.

More information about the prize can be found here: http://www.doerenkamp.ch/en/default.html?id=15

### VIDEO: Thomas Hartung and Marcel Leist Receive Ursula M. Händel Animal Welfare Prize

Thomas Hartung and Marcel Leist received the Ursula M. Händel Animal Welfare Prize of the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation). The €80,000 prize is awarded to researchers who improve research animal welfare in line with the 3Rs principles: Replacement, Reduction, and Refinement.

This award honors the life's work of both scientists in making major contributions to animal welfare. Hartung was recognized for using artificial intelligence ("read-across") to predict the toxicity of chemicals without using animals. Instead of using animals, data from a particular chemical is compared to similar chemical structures in toxicological databases to determine possible toxicity.

The awardees were also recognized for their international networking with multiple international stakeholders (researchers, regulatory authorities, non-governmental organizations, and industry) to advance the acceptance of alternative methods.

You can watch the video of the award ceremony here (in German): https://www.dfg.de/gefoerderte_projekte/wissenschaftliche_preise/haendel-tierschutzpreis/2020/

### Thomas Hartung in *Toronto Star*: COVID Could Spell the End of Animal Testing as Drug Makers Turn to Human Organs on Microchips

*Excerpt:*
Dr Thomas Hartung is the director of the Center for Alternatives to Animal Testing (CAAT), a laboratory for developmental neurotoxicity research based on genomics and metabolomics at Johns Hopkins University. Hartung points to the slow trajectory of drug and vaccine development using animal modelling. Conventional drug development relies heavily on animal testing to understand the molecular mechanisms of disease and potential treatments,

helping to explain why it takes more than 10 years to get a medication to market, while vaccines typically take 12 years, says Hartung. Such lengthy timelines translate into a hefty medical bill: roughly $2 billion per drug.

With COVID-19, "we cannot wait that long for treatments," says Hartung, who spoke to online delegates at the 11th World Congress on Alternatives and Animal Use in the Life Sciences in August. "We have to be faster than we were in the past."

Hartung, highly regarded in the field of animal-testing alternatives, pioneered a patent on brain organoids, which are tissue cultures made from human stem cells that simulate the human organ. Developed four years ago, mini brains, which can be mass-produced, have been used to study infections caused by viruses such as HIV, dengue and Zika.

This past spring, Hartung and his team proved that SARS-CoV-2 can infect and damage human brain cells by testing about 800 mini brains – each the size of a housefly eye – that were "identical in composition" to the human organ. Observing evidence-based effects of COVID-19 in the human brain will help researchers jumpstart important therapeutics and medical care.

"It will be difficult not to use them in a similar, fast way for drug and vaccine development and regulation in the future," Hartung says.

Full Article (Toronto Star): https://www.thestar.com/business/2020/12/05/could-covid-spell-the-end-of-animal-testing.html

## Thomas Hartung Joins InSphero Scientific Advisory Board

InSphero AG, a 3D cell-based assay technology company, announced the strengthening of its Scientific Advisory Board (SAB) with the appointment of Thomas Hartung, MD PhD, Matthias von Herrath, MD, and Gerd Kullak-Ublick, MD.

"We are pleased to welcome these distinguished researchers to our SAB," says InSphero CEO and co-founder Jan Lichtenberg, PhD. "They are all leading experts in their respective fields and will provide invaluable insights as we continue to advance our tailored drug development efforts at the forefront of preclinical research, with an emphasis on metabolic diseases, diabetes, oncology, and safety programs."

InSphero Chief Scientific Officer, Prof. Armin Wolf, PhD, who looks forward to engaging and productive discussions with these new SAB members, says, "Visionary science has always been a driving force for InSphero innovation in drug discovery and safety. Our SAB will not only guide us, but also serve as a sparring partner to challenge us and bring added value to our collaborative research partnerships with leading pharma and biotech organizations."

Full press release: https://tinyurl.com/y84sxxyf

## VIDEO: Centennial Celebration of Hildegard Doerenkamp

Hildegard Doerenkamp, a lifelong animal lover, was active in supporting alternatives to animal testing since she first saw a televised interview with toxicology Professor Gerhard Zbinden in 1982. They combined their individual assets to form the Doerenkamp-Zbinden Foundation to support the 3Rs of alternatives (Reduction, Refinement, and Replacement), and have endowed five university chairs in the US, Germany, The Netherlands, Switzerland, and India.

On November 9, 2020, the foundation honored what would have been Ms Doerenkamp's 100th birthday and her legacy with a webinar showcasing the five active professors' ongoing work and announced the application process of the 2021 Doerenkamp-Zbinden Award.

Speakers:

- Franz Gruber, President of the Doerenkamp-Zbinden Foundation: *Hildegard Doerenkamp, Born 100 Years Ago*
- Marcel Leist, The Doerenkamp-Zbinden Chair of in-vitro Toxicology and Biomedicine at the University of Konstanz (Germany): *Teratogenicity in a Dish*
- Thomas Hartung, The Doerenkamp-Zbinden Chair of Evidence-Based Toxicology at the Johns Hopkins University in Baltimore (USA): *The Hopkins Doerenkamp-Zbinden Chair – Evidence-based Toxicology to Replace Animal Testing*
- Bas Blaauboer, The Doerenkamp-Zbinden Chair of Alternatives to Animal Testing in Toxicological Risk Assessment at the University of Utrecht (The Netherlands): *In Vitro Toxicology in Risk Assessment: It is All About Biokinetics*
- Pierre Cosson, The Doerenkamp-Naef-Zbinden Chair for Alternative Methods at the University of Geneva (Switzerland): *In Vitro Antibodies for Academia*
- Mohammad A. Akbarsha, The Mahatma Gandhi-Doerenkamp Center MGDC) for Alternatives to the Use of Animals in Life Science Education & Gandhi-Gruber-Doerenkamp Chair for Life Science Education and In Vitro Toxicology at the Bharathidasan University in Tiruchirappalli (India): *Ms. Doerenkamp: Animal Savior Par Excellence*

Watch the video here (YouTube): https://www.youtube.com/watch?v=Tr9_6qgOpeE

## Thomas Hartung: "I am not a funny guy"

A close-up and personal interview with Thomas Hartung. Did you know he was a stand-up comedian in the 1980s? That his first car was a VW Golf?

All this and many more fascinating facts about CAAT's director can be found in this revealing Chemistry World interview. Link: https://tinyurl.com/ycwjvs9l

## CAAT's Coursera Courses Pass 10,000 Learner Mark

CAAT's Coursera courses, Toxicology 21: Scientific Applications and Evidence-based Toxicology, have now surpassed the 10,000 learner mark.

Both classes are free and have received highly favorable ratings from students.

You can find all our academic courses here: https://caat.jhsph.edu/programs/academics.html

### Webinar Series: Animals, Climate Change, and Global Health

This webinar series is presented by CAAT, Universität Bern, and Wageningen University and Research. CAAT's Kathrin Herrmann, Director of the Beyond Classical Refinement program, was an organizer.

Animals, climate change and global health are a nexus of high, contemporary relevance in the context of the coronavirus crisis since most infectious diseases are zoonotic, meaning they move from animals to humans and threaten to cause epi- and pandemics. Habitat loss, industrial animal agriculture, and a collapsing climate all threaten to increase zoonotic disease outbreaks in incidence, number, and severity. This crisis is not a one-time outlier and cannot be studied in isolation. Instead, it forces us to consider the bigger picture, including our relations to nature, our treatment of non-human animals, and the fact that the world, as we have come to know it, is not infinite.

More information, along with recordings of the webinars, can be found here: https://animalsclimatehealth.com/webinars/

### European Commission Publishes Chemicals Strategy for Sustainability

The European Commission has published its Chemicals Strategy for Sustainability as part of the European Green Deal.

ECHA has promised full support in particular to stimulate innovation towards safer alternatives and speeding up chemicals risk management. This is achievable only through the application of NAMs, as specified on page 21 of the Commission document.

CAAT-Europe will coordinate actions to monitor the future developments, with François Busquet working in close contact with the EU institutions in Brussels and Costanza Rovida as stakeholder at ECHA.

### Chemists Amid Coronavirus: Thomas Hartung (Chemistry World)

*Excerpt:*
During this difficult time, *Chemistry World* is checking in with notable chemists around the globe to see how they are weathering the coronavirus pandemic.

Johns Hopkins University (JHU) in Maryland, US is still under partial lockdown after stopping non-essential research and ending in-person classes in mid-March due to the pandemic. Prominent toxicologist Thomas Hartung hasn't been to his office or lab for more than seven months. He is chair of evidence-based toxicology at JHU's school of public health.

The university reopened its labs in a limited capacity in June, but some key research in Hartung's group was never really paused. That is because members of his team, which comprises about 20 researchers including his wife, pivoted to address Covid-19.

"In February or early March, it became clear that our work on mini-brains would be very relevant to Covid-19," he recalls. Hartung and colleagues created these so-called "mini-brains" – made up of neurons and other human brain cells that can be used for neurological research – several years ago. The research in question, published in June, suggested the virus that causes Covid-19, SARS-CoV-2, can infect human brain cells.

Full article at Chemical Watch: https://tinyurl.com/yax6zns3

### Mini-Brains Help Uncover COVID-19's Neurological Impact (Hopkins Bloomberg Public Health)

*Excerpt:*
In 2016, Hartung's team devised a solution based on what are known as induced pluripotent stem cells. Derived from genetically reprogrammed skin cells from adult donors, these cells can, like embryonic stem cells, produce virtually any other cell type. Hartung's team created a strategy for coaxing these cells to form brain "organoids" – tiny balls of neurons and other cells that replicate key features of the human brain.

Having used "mini-brains" to study viruses like Zika, his team recognized a clear opportunity to apply their technology to COVID-19. In a June study in *ALTEX: Alternatives to Animal Experimentation*, Hartung and colleagues offered the first substantive evidence that SARS-CoV-2 can infect the brain. "Some brain cells were clearly infected [and] the virus had multiplied a hundred- or thousand-fold in these cells," he says, adding they also saw evidence of the virus spreading within the organoid.

Full article: https://magazine.jhsph.edu/2020/mini-brains-help-uncover-covid-19s-neurological-impact

### New Publications

Hogberg, H. T., de Cássia da Silveira E Sá, R., Kleensang, A. et al. (2020). Organophosphorus flame retardants are developmental neurotoxicants in a rat primary brainsphere in vitro model. *Arch Toxicol*, online ahead of print. doi:10.1007/s00204-020-02903-2

Kappenberg, F., Brecklinghaus, T., Albrecht, W. et al. (2020). Handling deviating control values in concentration-response curves. *Arch Toxicol 94*, 3787-3798. doi:10.1007/s00204-020-02913-0

Cruelty Free INTERNATIONAL
Ending animal experiments worldwide

## Review published to analyse the clinical impact of animal-based "breakthroughs"

Dr Jarrod Bailey, former Senior Research Scientist at Cruelty Free International, and Professor Michael Balls, former head of ECVAM and Emeritus Professor of medical cell biology at the University of Nottingham, have published a critical review in *BMJ Open Science* of 27 animal-based biomedical "breakthroughs" reported in the UK national press in 1995. The reports had all claimed that the findings in animals were key and that improvements in treatments for human diseases such as cancer and Alzheimer's disease would soon follow.

The study reveals that of these 27 "breakthroughs", after 25 years, only one had resulted in a treatment in clinical use and even then, with significant caveats. Twenty were classified as failures with no direct or indirect human benefit found, three were inconclusive, and three were partially successful.

The review reveals the degree of exaggeration by the media of the relevance and benefits of animal studies for humans, drawing from claims made by the researchers themselves and calls for a more honest discussion about the true value of animal research.

Bailey, J. and Balls, M. (2020). Clinical impact of high-profile animal-based research reported in the UK national press. *BMJ Open Science 4*, e100039.

## Cruelty Free Europe calls for immediate end to animal tests where alternatives already exist

On October 2, World Animal Day, Cruelty Free Europe launched the RAT (Replace Animal Tests) campaign, which calls on regulators to immediately end experiments on animals where valid humane alternatives already exist.

The RAT list highlights ten animal tests that could end today with no impact on human or animal health, saving the lives of an estimated three quarters of a million laboratory animals every year in Europe alone.

Despite the acceptance by the European Union (EU) of replacement non-animal methods for animal tests such as skin and eye irritation, skin sensitisation, various batch safety tests, and antibody production, these tests are still being conducted in Europe to meet legal requirements.

Not only are the non-animal methods usually cheaper, faster, and more accurate than the animal tests they replace, but EU laws on animal experiments demand that countries do not authorise the conduct of an animal test if another method or testing strategy for obtaining the result sought, not entailing the use of a live animal, is recognised under EU rules. In many cases, the problem lies with lack of enforcement by the authorities and the absence of a joined-up approach to acceptance of non-animal tests around the world.

The complete RAT list can be found here: www.crueltyfreeeurope.org/RATlist

## New update on recently approved alternative methods for regulatory testing

In October, Cruelty Free International produced its third annual update on alternative approaches that have been approved within the last year in the fields of chemicals and pharmaceutical regulatory testing.

The update describes the 3Rs implications of new and revised guidelines and guidance documents produced by the Organisation for Economic Cooperation and Development (OECD), the EU Test Methods Regulation (EC/440/2008), the European Chemicals Agency (ECHA), the European Directorate for the Quality of Medicines & Healthcare (EDQM), the European Medicines Agency (EMA), the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), and the International Conference on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH).

The European Commission has already shared this important update with all member states and stakeholders to promote the use of alternatives. To obtain a copy, please e-mail laura.alvarez@crueltyfreeinternational.org

## The Geoffrey Deckers Award is launched

In December, Cruelty Free Europe announced the launch of the Geoffrey Deckers Award, a prize open to small and medium-sized European groups, coalitions and organisations opposed to animal experiments, for projects related to ending animal tests in Europe.

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.

Geoffrey was a dedicated animal protection campaigner who co-founded Dutch animal protection group, Diervriendelijk Nederland, in 1998. He went on to lead many successful campaigns in the Netherlands, across Europe and beyond. Most notable was the ending use of chimpanzees in biomedical research in the Netherlands, which saw the last chimps kept in European laboratories transferred to a sanctuary in 2006.

The €6,000 annual award will be offered to successful applicants on January 13 each year to mark Geoffrey's birthday. The award will go 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.

Application letters must be sent to info@crueltyfreeeurope.org by December 31.

### Open letter calls on EU to uphold cosmetics testing bans

Cruelty Free Europe has joined other animal protection organisations and over 450 cruelty-free cosmetic brands, including The Body Shop, Dove and Simple, in sending an open letter calling on European authorities to uphold the EU Cosmetics Regulation's animal testing and marketing bans.

A series of testing decisions by ECHA made with the backing of the European Commission, along with recent decisions by the ECHA Board of Appeal, have challenged a basic principle of interpreting EU legislation by disregarding the clear intention of legislators and thereby seriously undermining the cosmetics testing ban. In addition, the European Commission's Chemicals Strategy for Sustainability is set to re-open the Cosmetics Regulation, with the potential to introduce new testing requirements at the expense of many more animals' lives.

The letter urges European authorities to honour the efforts of European citizens and their representatives in European Parliament who fought hard for the cosmetics testing bans and demands that they be upheld as intended so that animals do not suffer for cosmetics in Europe.

The letter – also supported by PETA, Humane Society International and Eurogroup for Animals – can be found here: https://www.crueltyfreeinternational.org/what-we-do/latest-news-and-updates/europes-leading-animal-protection-groups-unite-defend-eu

### EU chemical and pharmaceutical strategies fail to prioritise non-animal methods

The European Commission has published its "EU Chemicals Strategy for Sustainability" and "Pharmaceutical Strategy for Europe" in October and November, respectively.

Despite calls from Cruelty Free Europe to put innovation in humane and human-relevant safety at the heart of the two strategies, both documents fail to prioritise non-animal methods and instead promote continued reliance on outdated and unreliable animal tests.

While the chemicals strategy acknowledges the commitment in EU law to make full replacement of animal tests the ultimate goal, it makes no concrete plans to change this beyond the need to "foster multidisciplinary research and digital innovations for advanced tools, methods and models, and data analysis capacities to move away from animal testing". There is no mention of how this will be achieved in the Action Plan annexed to the strategy, contrary to the wishes of the European Parliament in their motion for a resolution.

Similarly, while the pharmaceutical strategy states that it supports the advancement of new technologies, including artificial intelligence and computer-based modelling, there is no specific action to facilitate the uptake of non-animal approaches in the plan. This is at odds with the European Medicines Agency's own strategic plan to 2025, which has several action points related to replacing animal experiments.

A Pharmaceutical Strategy for Europe: https://ec.europa.eu/health/human-use/strategy_en

Chemicals Strategy for Sustainability Towards a Toxic-Free Environment: https://ec.europa.eu/environment/pdf/chemicals/2020/10/Strategy.pdf

Motion for a resolution on the Chemicals Strategy for Sustainability: https://www.europarl.europa.eu/doceo/document/B-9-2020-0222_EN.html

EMA Regulatory Science to 2025: https://www.ema.europa.eu/en/about-us/how-we-work/regulatory-science-strategy#regulatory-science-strategy-to-2025-section

EUSAAT

*European Society for Alternatives to Animal Testing*

### EUSAAT Virtual Annual General Assembly (AGA)

In the past, EUSAAT has usually held its AGA during the EUSAAT Congresses in Linz, Austria or during the World Congresses. However, as you know, WC11 was postponed due to the Covid-19 pandemic and will now be held in Maastricht/NL in August 2021.

Therefore, this year's AGA was successfully held as a virtual meeting on 12.11.2020. The virtual format enabled several members to participate from all over the world. After the official summaries and reports from the president and general secretary, lively discussions about past and coming activities of EUSAAT took place, resulting in an excellent roadmap for the future path of EUSAAT. One of the initiatives is the planned EUSAAT webinar series, which aims to provide the 3Rs field with a lecture-based communication platform.

### Announcing EUSAAT webinar series

The EUSAAT board has decided to organize a webinar series to allow top researchers and stakeholders as well as young re-

searchers to present their topics and results. The topics will cover current 3Rs topics typically dealt with at EUSAAT conferences including regulatory, ethical, educational, 3Rs center and scientific news, discussions and developments. In addition to the webinar series committee, everyone is welcome to suggest topics and lecture titles.

*Key information on the EUSAAT webinar series:*

- It will take place every second week on a fixed day of the week and time.
- EUSAAT aims to present the diversity of 3R research in Europe.
- During every webinar EUSAAT wants to feature one senior scientist and one early career scientist.
- The maximum duration of each webinar will be 1-1.5 h.

*Call for action*

We are seeking experts and early career scientists who are willing to present their research during one of these webinars! Please contact us if you want to give a presentation during this series or have any recommendations for suitable speakers by sending an e-mail with the name, topic/title, information on career stage, and contact information/affiliation to annemarie.lang@charite.de!

We strongly encourage you to share this information/possibility with early career scientists in your lab or network – it will be a marvelous experience for them and us.

## News from the European Network of 3Rs Centers (EU3Rnet)

The purpose of the EU3Rnet network is to bring European 3R centers, institutes and societies together to share best practices, enhance communication, support the exchange of information, and prepare the ground for common initiatives.

The recently published consensus statement of EU3Rnet can be found in this issue (doi:10.14573/altex.2010061). Further, it is a pleasure to share the information that the Austrian government has decided to financially support the establishment of an Austrian 3R center. This endeavor will be organized by the Austrian 3Rs society RepRefRed (https://www.reprefred.eu/EN).

## EUSAAT insists on the EU Cosmetics Regulation animal testing ban

Since March 2013, the EU Cosmetics Regulation animal testing and marketing bans are the gold standard around the world – setting the precedent for cosmetics products and ingredients to be used safely without subjecting animals to cruel and unnecessary tests. These bans have now been stopped following a series of regulatory decisions made by the European Chemicals Agency (ECHA), with support from the European Commission and ECHA's own Board of Appeal.

There is, quite simply, no reason to test cosmetics products, or the ingredients used in them, on animals. Cosmetic ingredients, which are the target of ECHA's testing decisions, have a long history of safe use by consumers, and have been handled safely in factories for many years, thanks to effective, exposure-based assessments and controls by the manufacturers.

In line with EU animal protection legislation, scientists should use the established non-animal safety assessment approaches and develop new ones. Therefore, the EU Commission and ECHA must ensure that the advanced technologies of the 21st century are applied to comply with animal testing bans under the EU Cosmetics Regulation including the chemicals regulation REACH.

Therefore, EUSAAT is joining the major European animal welfare NGOs and cosmetic companies in their protest against the decision of ECHA and the EU Commission in an open letter:

Cosmetics Animal Testing Ban Effectively Shredded *to David Maria Sassoli, President, European Parliament; Charles Michel, President, European Council; Ursula von der Leyen, President, European Commission:* https://crueltyfreeeurope.org/sites/default/files/Joint statement - against the use of animals to test cosmetics ingredients under REACH.pdf



Although the project is reaching its end, the EU-ToxRisk consortium has been experiencing a new (scientific) spring. The successful conclusion of the first phase of the project has been transformed into a strong tailwind for the flagship project's sails. Contributing to this is the positive feedback on the EU-ToxRisk approach from the OECD and the publication of the EU-ToxRisk case study (CS) reports within the Integrated Approach to Testing and Assessment (IATA) Case Studies Project. A summary report for the final CS[1] is available. The studies focused on read-across (RAx) approaches for different pesticides and fungicides (deguelin[2], strobilirubin[3]), drugs (2-ethylbutyric acid[4]), and food additives (methyl hexanoic acid[5]).

The scientific and regulatory learnings from this phase, as summarized by Moné et al. (2020), are now being used in the second round of case studies. These new subprojects were all started in 2020 with the purpose of addressing a set of toxicological questions of broad regulatory and scientific impact. For instance, they deal with: (i) identifying a strategy to define a chemical as having no adverse effects; (ii) testing of chemicals inducing multi-target organ toxicity; (iii) testing of metabolism-activated toxicants; (iv) testing of chemicals that may trigger developmental neurotoxicity.

The consortium and its advisory boards have recently met virtually to discuss the progress of these research lines. Despite the pandemic situation, much data of great interest has been generated that promises to provide exciting CS conclusions to be expected by the end of the project.

## EU-ToxRisk publications

The EU-ToxRisk toolbox approach has been further expanded and documented in publications.

The possible adverse effects of bisphenol A (BPA) in humans have long been debated. More recently, bisphenol F (BPF) and S (BPS) have been suggested as "safer" alternatives. However, these structurally similar compounds can also induce estrogenic effects. Lucendo-Villarin et al. (2020) used human PSC-derived hepatocyte-like cells (HLCs) to assess the effects of these chemicals on cell viability, inhibition of cytochrome P450 activity, and genome-wide RNA profiles. No major differences were observed on these endpoints among the tested chemicals. Additionally, RNA profiling showed a bisphenol-derived effect on the pathways associated with cellular differentiation and embryonic development. The data thus indicate a similar toxicological hazard of BPA and its potential substitutes.

In another study, EU-ToxRisk researchers, together with a team of experts, asked whether long-term experiments, which are particularly difficult to model *in vitro*, are really required. For this, they used legacy data on tested food constituents and compared the data of 90-day studies to data of chronic studies. Relatively little difference was found. In conclusion, the long-term studies may be dropped, and NAM research can focus on replacement of studies of up to 90 days (Guth et al., 2020).

EU-ToxRisk research also covered toxicants such as acrylamide (ACR). The known neurotoxicant can be found both as a food component (deriving from heat treatment) and an environmental pollutant (used for water management and cosmetic products). In Attoff et al. (2020), the researchers aimed to elucidate whether non-cytotoxic concentrations of ACR can alter neuronal differentiation in a human neuroblastoma SH-SY5Y cell model. The results revealed that micromolar concentrations of ACR sustain proliferation, decrease neurite outgrowth, and interfere with signaling pathways involved in neuronal differentiation. Thus, the study showed that ACR may trigger developmental neurotoxicity.

Non-invasive measurements of test chemical concentrations in complex models (organoids and microphysiological systems (MPS)) are currently difficult due to a lack of sensitivity on the nanoliter (nL) scale. Project partners asked how MPS could be better analyzed in the future. In Grisi et al. (2020), the authors presented a novel analytical method for non-invasive spectroscopy. The researchers used a microsystem, which combines CMOS technology with 3D microfabrication, enabling a platform tool for non-invasive spectroscopy of microorganisms, organoids, and microtissues. As proof-of-principle, the technology was successfully applied for the detection of lipids in liver microtissue models.

Two other studies that are important for liver toxicology have been published recently. Gupta et al. (2020) asked how well *in vitro* models represent the human liver. RNA-sequencing was used to compare a group of human *in vitro* liver cell models. Microtissues and primary human he-

[1] www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2020)24&doclanguage=en
[2] www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2020)22&docLanguage=en
[3] www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2020)23&doclanguage=en
[4] www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2020)20&docLanguage=en
[5] www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2020)21&docLanguage=en

patocytes showed higher similarity to human *in vivo* liver tissue, while HepG2, although widely used in hepatotoxicity studies, ranked last, showing a low degree of similarity.

Currently, the prediction of drug-induced liver injury (DILI) based on gene expression data from *in vitro* assays remains a challenge. In Aguayo-Orozco et al. (2020), the authors developed a novel computational tool to predict drug-induced liver injury (DILI) based on gene expression data from *in vitro* assays. The Qualitative Gene expression Activity Relationship (QGexAR) approach was applied to assess the risk of DILI using classification models based on gene expression profiles of 276 chemicals tested on breast and prostate cancer cell lines. Genes and pathways involved in liver metabolism contributed most to the performance of the models, giving some indication of the mechanisms of action leading to DILI.

Some studies on sophisticated *in vitro* test systems were also published in the last months. In Loser et al. (2020), the researchers developed a system allowing assessment of complex signaling in both individual human neurons and on the network level. The human cell-based *in vitro* method was initially characterized in relation to the expression pattern of many neuronal signaling components. Additionally, a quantitative characterization of agonist and antagonist responses on classical $Na^+$ channels, classical ionotropic neurotransmitter receptors, and neurotransmitter transporters was performed. The authors could show how this new test system allows multiple types of neuronal signaling, within and between cells, to be assessed, quantified, and characterized for their potential disturbance. The question how the fish embryo acute toxicity test (FET) could be used to support RAx studies was addressed by von Hellfeld et al. (2020). The FET has been established as an alternative to the OECD acute fish toxicity test (AFT). With the growing need to understand the developmental toxicity of compounds found in the environment, the FET protocol has repeatedly been extended to a multitude of additional morphological endpoints. To contribute to the harmonization of observations and terminology, this study provided a catalogue of morphological alterations in zebrafish embryos after exposure to chemicals.

## Outlook

Discussion and active interaction with EU-ToxRisk and international experts will be possible at the upcoming fourth EU-ToxRisk Open Symposium. The meeting will be held virtually on February 23-24, 2021[6].

In March, EU-ToxRisk partners will contribute to the scientific program of the SOT annual meeting. A dedicated symposium focusing on the "Regulatory learnings from the EU flagship non-animal toxicology project EU-ToxRisk" will be held on March 25, 2021. Additionally, a joint workshop between EU-ToxRisk and the US Tox21 program will be held on "Applications of Novel High-Throughput Approaches for Mechanism-based Chemical Safety Assessment" (March 22, 2021). In this session, the use of transcriptomics, metabolomics, and phenomics-based approaches for characterization and/or predictions of mechanisms of action will be demonstrated for potential use in chemical safety assessment.

## References


Aguayo-Orozco, A., Brunak, S. and Taboureau, O. (2020). Extrapolation of drug induced liver injury responses from cancer cell lines using machine learning approaches. *Comput Toxicol 17*, 100147. doi:10.1016/j.comtox.2020.100147

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[6] https://www.eu-toxrisk.eu/media/EU-ToxRisk-Symposium-2021_Summary%20Agenda_v3.pdf

LUSH PRIZE

SUPPORTING ANIMAL-FREE TESTING

## Lush Prize Conference 2020

The first ever virtual Lush Prize Conference was held over two days in November. With the theme "Can Big Data Replace Animal Testing?", the conference explored computational toxicology, one of the three key fields at the centre of Lush Prize's focus.

Our new strategy, developed in 2019, is based on projects most likely to lead to practical non-animal tests as part of the shift towards new approach methodologies (NAMs) which could be accepted by regulators. The other two areas – organ-on-a-chip and adverse outcome pathways – were the themes of previous Lush Prize Conferences in 2018 and 2015.

One of the challenges of moving away from animal models to a whole new paradigm (the goal of the Lush Prize) is that complex validations are often required against animal tests with a scientific basis which is, to some degree, flawed (a mouse is not a human). The big data approach has the potential to finesse this catch-22 situation, at least in some circumstances.

Holding the conference over two days, and for just three hours per day, allowed for people from varying time zones to participate. Hundreds of people from South America to the Middle East joined us, including industry and academic scientists, students, regulators, campaigners, and members of the public. We are delighted to see the growth in interest in the Lush Prize Conference since our first one back in 2013.

This year's prize winners included three computational toxicology projects. Dr Tim Allen of the MIE Atlas Team at Cambridge University won the £50,000 Science Prize and also started off the conference with his presentation "In Silico Models to Predict Human Molecular Initiating Events".

The two other computational toxicology winners were both Young Researchers from Italy: Dr Domenico Gadaleta and Edoardo Carnesecchi.

The sessions held on day one gave an introduction to the conference theme as well as the developing role of big data in COVID-19 research. Rebecca Ram of the Safer Medicines Trust introduced the work of the new international CIAO Project (COVID-19 Adverse Outcome Pathway), and Professor Thomas Hartung of CAAT gave a very informative presentation covering issues such as artificial intelligence and data mining.

The first panel on day two covered the role of big data in Next Generation Risk Assessment. Dr Chloé Raffali, Toxicologist at Lush Cosmetics, presented the work of the Advancing Animal-free Safety Assessment Collaboration, and we were delighted to hear from a previous Young Researcher winner, Dr Vinicius Alves, now at the National Institute of Environmental Health Sciences, on the STopTox *in silico* platform as an alternative to animal testing for acute systemic and topical toxicity.

The final panel session on regulatory acceptance helped bring together everything discussed so far into strategizing for continued progress. Clemens Wittwehr of the JRC and Dr Nicole Kleinstreuer, Acting Director of NICEATM (a Young Researcher winner in 2016), were joined by Kristie Sullivan of Physicians Committee for Responsible Medicine, giving a broad input into regulatory issues.

As several speakers noted, many companies are using computational modelling and data-driven predictive models for internal decision-making and product assessment but then doing animal tests to meet regulatory requirements. So, there is still work to do on persuading regulators to accept these non-animal methods in place of animal tests. Kristie Sullivan, who is also a Lush Prize judge and helps advise us on our strategies, did a fantastic job of explaining what regulatory acceptance means and where our efforts should be focused.

As the conference was particularly heavy on science, and the Lush Prize audience is much broader, the science presentations were broken up by a series of informal "fireside chat" interviews with some of this year's winners: Public Awareness winner SOKO Tierschutz, Lobbying winner Environment and Animal Society Taiwan, and Training winner TPI Helpathon. These proved to be very popular, with much discussion on social media about the work of these organisations.

The Awards Ceremony was held on the evening of the first day of the conference, where we officially announced the winners of the £250,000 Lush Prize. Rather than doing an entirely pre-recorded event, the regular approach during this pandemic, we decided to run a mostly live event – not an easy task when bringing winners in via internet from so many countries. Fortunately, this was a great success with no technical problems, and we hopefully provided the winners, and our global live-stream audience, with a better feeling of excitement than a pre-recorded ceremony. It wasn't quite the same as having everyone in the same room eating and chatting together, and hopefully we can do that again with the next ceremony.

Recordings of the Conference and Awards Ceremony can be watched on the Lush Prize website www.lushprize.org

*Craig Redmond, Lush Prize*