Corners

CAAT Co-directors Thomas Hartung and Marcel Leist Receive Ursula M. Händel Animal Welfare Prize

Thomas Hartung, MD, PhD, Director of the Center for Alternatives to Animal Testing (CAAT) at the Johns Hopkins Bloomberg School of Public Health, and Marcel Leist, PhD, co-director with Hartung of CAAT-Europe and Chair of the department of In Vitro Toxicology and Biomedicine at the University of Konstanz, have been awarded the Ursula M. Händel Animal Welfare Prize by 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.

The two winners will use the prize money to enable early career scientists to perform research in the 3Rs.

The Ursula M. Händel Animal Welfare Prize goes back to the initiative of the founder of that name. A resident of Düsseldorf, Ursula M. Händel (1915-2011) championed animal welfare over several decades. Dedicated to animal welfare in science and research, Händel provided the DFG with the financial backing for the animal welfare prize. The prize is awarded every two years.

Information about the prize, its founder Ursula M. Händel, and the prizewinners can be found in the official press release from DFG: https://idw-online.de/de/news751862

Invitation to Join GCCP 2.0 Scientific Advisory Committee

The third World Congress on Alternatives and Animal Use in the Life Sciences (Bologna,1999) discussed Good Cell Culture Practice (GCCP), i.e., the challenges in the performance of reliable *in vitro* studies using cells and tissues, which led to the Bologna declaration on GCCP.

The European Centre for the Validation of Alternative Methods (ECVAM) of the European Commission then established a taskforce to generate a Good Cell Culture Practice guidance document that would address the key principles required to assure reproducibility and quality of *in vitro* (cell-based) assays in 2002 and 2005. The GCCP documents formed a major basis for a GLP advisory document for *in vitro* studies published by the OECD (2005).

Under the leadership of CAAT, two workshops were held in 2015 in Baltimore, USA, and Konstanz, Germany, as part of the transatlantic think tank for toxicology (t^4). These workshop reports were utilized by a CAAT-initiated expert drafting group to produce a revised version of GCCP called GCCP 2.0, which is available now as supplement to the article recently published in *ALTEX* (doi:10.14573/altex.2007091) to initiate an open public consultation prior to final publication. Over the last few years, a Scientific Advisory Committee (SAC GCCP 2.0) has been formed. We invite all interested stakeholders to join the SAC. Applicants are expected to be *bona fide* cell culture practitioners. Please send an email to: caat@jhu.edu

All members of the SAC will have the opportunity to suggest revisions of the text until November 2020.

CAAT's Fast-Track Grants for Non-Animal Approaches to COVID-19 Research: Grantees Announced

In response to the global COVID-19 pandemic, CAAT redirected a portion of the Alan and Helene Goldberg In Vitro Toxicology Grants to development of tools to address the emerging health threats. This new initiative is our Fast-track grant for research on non-animal approaches to investigate mechanisms, medicines, and vaccines for coronaviruses.

Animalfree Research, Humane Society International, and Humane Society of the

United States provided generous financial support of these grants.

CAAT received 60+ applications – if further funding should become available, the center may consider awarding additional applicants. The awardees are listed below:

Christine Bear, *Senior Scientist, Programme in Molecular Medicine, Hospital for Sick Children*
Development of a platform for SARS-CoV-2 therapy testing and development using primary nasal epithelial cultures

Parastoo Khoshakhlagh, *Co-founder, President and CEOGC Therapeutics, Inc*.
Investigating the effects of hypertension drugs on the infectivity of SARS-CoV-2 in synthetically accelerated vascularized type II pneumocyte-containing pulmonary organoids

Thomas Hartung Interview on NBCLX News (Video)

How "Mini-Brains" Grown in the Lab Are Helping Researchers Study the Coronavirus
"Mini-brains" are minuscule organoids made from stem cells that mimic the behavior of the human brain. A team at Johns Hopkins University is using "mini-brains" to study the effects of the coronavirus on the brain without using animal research. Thomas Hartung joined LX News to talk about what his team is learning from this research.

Watch Now (NBC LX News): https://www.lx.com/science-tech/how-mini-brains-grown-in-the-lab-are-helping-researchers-study-the-coronavirus/16218/

VIDEO: Thomas Hartung at ESOF2020: COVID-19: A Brain Disease?

Interviewed by *Financial Times* Science Editor Clive Cookson, the results of Johns Hopkins University's breakthrough research on the profound neurological impacts of COVID-19 are presented here for the first time. Thomas Hartung, describes how lab-grown "mini-brains" (tiny tissue cultures that simulate whole organs made from human cells) can be infected with SARS-CoV-2. The virus infects neurons in the mini-brains via the ACE2 human protein that is known to be an important entry point for SARS-CoV-2. The virus then multiplies within the neurons. Within three days the number of copies increases at least a hundredfold.

Patients exhibit symptoms ranging from inflammation, dizziness, headache and delirium to seizures, nerve damage, and stroke. Numbness, weakness, and memory problems can persist long after the virus has gone. Subtle brain damage might only become apparent in years to come. A special concern is that brain development of the embryo in pregnant patients could be affected.

Watch Now (YouTube): https://youtu.be/rNzpahkQcmY

VIDEO: Demonstrating that Lab-grown "Mini-brains" can be Infected with COVID-19

Thomas Hartung describes how lab-grown "mini-brains" (tiny tissue cultures that simulate whole organs made from human cells) can be infected with SARS-CoV-2.

Watch Now (YouTube): https://www.youtube.com/watch?v=cUyxyc2Xup8&feature=youtu.be

2019 Science-Based Refinement Awardee: Costança Carvalho

In 2020, CAAT presented the 2019 Science-Based Refinement Award to Constança Carvalho (University of Lisbon), for her project "Rat use in Major Depressive Disorder research. Assessing the past to improve the future." Please join us in congratulating Constança!

Carvalho, C., Peste, F., Marques, T. A. et al. (2020). The contribution of rat studies to current knowledge of major depressive disorder: Results from citation analysis. *Front Psychol 11*, 1486. doi:10.3389/fpsyg.2020.01486

More about CAAT's Science-Based Refinement Award: https://caat.jhsph.edu/programs/awards/AWE/index.html

Thomas Hartung on Kuhn's *The Structure of Scientific Revolutions*

CAAT director Thomas Hartung led a discussion of excerpts from *The Structure of Scientific Revolutions*, the groundbreaking and influential book by Thomas Kuhn.

Watch now on CAAT's YouTube Channel: https://youtu.be/lqBbWBlXxwQ

VIDEO: EBTC COSTER Webinar Now Available

While growth in publication of systematic reviews in toxicology and environmental health research (EH) is exponential, their quality is very uneven. This is at least partly because current guidance on how to conduct SRs is either focused on healthcare contexts or, if specific to EH research, collectively inconsistent. In response to this situation, a cross-sector group of stakeholders has developed COSTER, intended as a reference-point for good practice in conduct of SRs of environmental health and toxicological research.

This webinar describes how COSTER was developed, gives an overview of its recommendations, and discusses how COSTER should be used.

Watch the full presentation video: https://www.youtube.com/watch?v=gpmrDBVmuWA&t=27s

Thomas Hartung on Putin Foe Navalny Poisoning (CBS News)

Thomas Hartung was interviewed by CBS News about the poisoning of Putin foe Alexei Navalny. Hartung noted that Novichok, the Soviet-era nerve agent used to poison former Russian spy Sergei Skripal and his daughter in Britain – and the purported toxin used against Navalny – is a cholinesterase inhibitor. Hartung predicted that "we will know soon which substance was used."

Full Article (CBS News): https://www.cbsnews.com/news/alexei-navalny-news-poisoning-claim-cannot-be-true-russian-president-putin-spokesman-says-2020-08-26/

CAAT Co-Sponsors Webinar Series on Animals, Climate Change, and Global Health

A series of six webinars with leading experts from around the world, with the aim of inspiring an in-depth conversation – and actions – at the nexus animals x climate change x global health.

Full details and registration: https://animalsclimatehealth.com

Session 1: Animals, Pandemics and Global Health
September 18, 2020
Watch now: https://animalsclimatehealth.com/session-1/

Session 2: COVID-19 Research: With or Without Animals?
October 16, 2020

Session 3: Animals in Crises
November 11, 2020

Session 4: Animals Affected by Climate Change
November 20, 2020

Session 5: Animals as Drivers of Climate Change
December 9, 2020

Summer School Presentations and Videos Now Available

This first US Summer School on innovative, animal-free approaches in science was co-hosted by CAAT, the Physicians Committee for Responsible Medicine, and the European Commission Joint Research Centre, building on two past summer schools held at the European Commission Joint Research Centre in Ispra, Italy. The program was designed to offer students a chance to learn more about cutting-edge science from experts from Johns Hopkins University, Harvard University, the National Institutes of Health, the Environmental Protection Agency, the Physicians Committee for Responsible Medicine, and more.

Over 600 people attended, and the program was given very high marks by many of the participants. Presentation slides and recordings are available here: https://pcrm.widencollective.com/portals/tgkpbij2/SummerSchool2020

WATCH NOW: Thomas Hartung on "COVID-19: What is in the Box of Alternative Methods?"

The 11th World Congress on Alternatives and Animal Use in the Life Sciences held two 1.5-hour webinars on the 3Rs in COVID-19 research. Thomas Hartung spoke on "COVID-19 – What is in the Box of Alternative Methods?".

Watch the presentation on YouTube (Thomas Hartung discussion begins at 4:45 mark): https://youtu.be/kFUDo0HNc2A

EBTC GRADE Webinar Videos: Watch Now

The video recordings of the Evidence-based Toxicology Collaboration's (EBTC) GRADE Pre-Meeting Webinars, which took place June 15, 2020, are now available for viewing: https://www.youtube.com/channel/UCrTXH6Yh-djmbmoluzgI_2w

Thomas Hartung on the Discovery of Active Properties of "Inactive" Drug Ingredients (*The Scientist*)

Excerpt:
The success of a drug often depends not simply on the active ingredients it contains, but on how it is formulated, explains Thomas Hartung, a pharmacologist at Johns Hopkins University who also did not participate in the research. The inactive components may stabilize the drug, prevent contamination, control the drug's metabolism, or improve its taste or identification. But, Hartung continues, there is a sort of "toxicological ignorance" about these substances in part because they are largely considered safe and because to screen them is an "enormous burden" and costly.

The new study, which was an academia-industry collaboration funded in part by the US Food and Drug Administration (FDA), has made great inroads to addressing this "silent area," Hartung says, and has shown "that among very many innocent substances there can be a black sheep" that we should "have an eye on."

Activities Discovered for Some Inactive Drug Ingredients (*The Scientist*): https://www.the-scientist.com/news-opinion/activities-discovered-for-some-inactive-drug-ingredients-67764

Jamie DeRita Memorial Animal Protection Symposium (Watch Now)

The Jamie Derita Memorial Animal Protection Symposium was held online (via Zoom) on July 9, 2020. This symposium honored the life of Jamie DeRita, who passed away in June 2020.

Guest Speakers included Aysha Akhtar (Our Shared Destiny with Animals), Stacy M. Lopresti-Goodman (From "lab dog" to "lap dog": Why Dogs Released from Research Make Great Companions) and Kathleen (Katie) Conlee, (Advocating for Dogs in Laboratories).

Jamie DeRita Memorial Animal Protection Symposium (YouTube): https://youtu.be/rrIv6_kr2-s

CAAT's Coursera Courses Pass 8,000 Learner Mark

CAAT's highly rated Coursera offerings, Toxicology 21: Scientific Applications and Evidence-based Toxicology have each hit new milestones; over 5,000 active learners for Tox21 and over 3,000 active learners for EBT.

Upcoming Events

SAVE THE DATE!
World Summit on Microphysiological Systems (MPS-WS-1)
December 13-16, 2021
New Orleans, LA

Co-hosts Suzanne Fitzpatrick (FDA), Thomas Hartung (Johns Hopkins CAAT), and Donald Ingber (Wyss Institute, Harvard University)

The planned series of Microphysiological Systems (MPS) conferences will bring together a broad audience, including institutions (government, health foundations, charities), the academic research commu-

nity (universities, research institutes), environmental and human toxicity experts, pharmaceutical and other industries (cosmetics, chemical, and food industries), medical centers and practitioners, patient associations, policy makers, and testing centers from across the globe to create a series of global conferences and a road map for MPS technology and to raise awareness while building a network for MPS technologies.

This will be a first step in establishing an international MPS society to facilitate stakeholder communication to promote international standardization and harmonization of MPS and establish a global training environment.

Several other organizations to be confirmed. Please send a letter of motivation of your organization to join this alliance. Individuals are invited to express their interest to join the Scientific Advisory Committee.

If you have questions about the proposed World Conference on MPS, or would like to join us in this effort, please contact Camila Sgrignoli Januario at: cjanuar1@jhu.edu

New Publications

Calina, D., Hartung, T., Docea, A. O. et al. (2020). COVID-19 vaccines: Ethical framework concerning human challenge studies. *DARU J Pharm Sci*, Online ahead of print. doi:10.1007/s40199-020-00371-8

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

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

Cruelty Free INTERNATIONAL
Ending animal experiments worldwide

UK publishes 2019 animal testing statistics

According to the latest Home Office annual report, published on July 16, a total of 3.4 million animal procedures were completed in the UK in 2019. This represents a decrease of just 3% since 2018.

Approximately 1.67 million procedures (49%) were related to the "creation" and breeding of genetically altered animals while the remaining 1.73 million (51%) were actual experiments on animals for various purposes including basic and applied research and regulatory testing.

Notably, there were some significant decreases in regulatory tests where there are validated non-animal alternatives. There was a 60% decrease in eye irritation tests on rabbits, a 29% decrease in skin irritation tests, and a 95% increase in skin sensitization tests. No rabbit pyrogen tests were reported at all in 2019, compared to 638 tests in 2018.

72% of EU citizens want a phase-out plan for animal tests

Almost three quarters (72%) of EU citizens think Europe should set targets and deadlines to phase out animal testing, according to a new opinion poll commissioned by Cruelty Free International.

In the poll, carried out by Savanta ComRes in June, 70% of adults across 12 EU member states agree that replacing animal tests with non-animal methods should be an EU priority while 66% think that the EU should immediately end all animal tests.

The poll also revealed that at least three quarters of adults in Portugal (85%), Croatia (84%), Poland (80%), Romania (80%), Italy (79%), Germany (76%) and France (75%) agree that the EU should invest more in alternative methods to animal testing.

76% of adults in EU member states agree that animal tests for household cleaning products should be banned in the EU while 74% agree that animal tests for cosmetics and ingredients are unacceptable in all circumstances.

Savanta ComRes interviewed 5,653 adults (aged 18+) from 12 EU member states online from June 9-19, 2020. Data were weighted to be representative of the population size of the 12 countries and demographically representative by age, gender and region in each country. https://comresglobal.com/polls/cruelty-free-europe-animal-testing-in-the-eu/

European Parliament Motion for Resolution supports non-animal methods

The European Parliament supported a motion for resolution on July 14 on the proposed EU chemicals strategy for sustainability. The strategy is a key part of the EU Commission's Green Deal to achieve zero pollution and a toxic-free environment.

The resolution "*calls on the Commission*

to come up with a comprehensive Chemicals Strategy for Sustainability to bring about the necessary paradigm shift to implement the zero-pollution ambition for a toxic-free environment, ensuring a high level of protection of human health, animal health and the environment, minimising exposure to hazardous chemicals, with particular regard to the precautionary principle and the effective protection of workers, minimising the use of animal testing, preserving and restoring ecosystems and biodiversity, and fostering innovation in sustainable chemicals".

Members of the European Parliament voted strongly in favor of more support in the resolution for non-animal methods, including:

- Reiterating the need to minimize and progressively replace animal testing through an expanded use of new approach methodologies and intelligent testing strategies,
- Regretting the fact that there is insufficient funding for the research and development of non-animal methods; requesting that action be taken to remedy this situation,
- Calling on the European Chemicals Agency to dedicate resources to promote non-animal testing methods,
- And requesting that the bans on testing on animals set by the Cosmetics Regulation must not be compromised by testing conducted under other legislation such as REACH.

The finalized chemicals strategy is expected to be published soon, followed by an action plan in 2021.

Build Back Better Online workshop September 10

On September 10, Cruelty Free Europe hosted an online workshop called "Building Back Better – A roadmap to human relevant research in a post-COVID-19 world" to discuss the replacement of animal tests with humane and human relevant alternatives.

A broad range of stakeholders from the EU and the US, including decision-makers, academics, scientists, campaigners and industry representatives agreed that the EU should develop and adopt an effective strategy with fixed milestones and deadlines, like those seen in other EU sectors such as climate change and pollution, to replace animal testing.

The first of the day's three workshops examined examples of pro-active replacement and reduction plans for animal research. In the second workshop, participants looked at the call for the EU to take the lead in sustainable science and solutions to reduce the suffering of animals in laboratories, while the final workshop examined what the EU's bans on cosmetics animal testing have achieved since coming into final effect in 2013 and what the future of the ban looks like.

Participants agreed that increased collaboration between stakeholders, increased public and political pressure, and increased education and funding for non-animal technologies are key to ending animal tests in Europe. Global harmonization, timely updates, and changes to legislation and stronger enforcement were also considered essential.

Progress and updates on key US legislation for animals

The Humane and Existing Alternative in Research and Testing Sciences (HEARTS) Act, introduced earlier this year by Representatives Lucille Roybal-Allard (D-CA) and Ken Calvert (R-CA) and supported by Cruelty Free International has recently gained 16 cosponsors. In July, language pulled from the HEARTS Act was included in the House Fiscal Year 2021 Labor-HHS-Education Appropriation Bill – providing a pathway to achieve some of the changes sought in the HEARTS Act within the next year. The bill asks the National Institutes of Health (NIH) to assemble a panel to make recommendations to incentivize the use of non-animal methods in NIH-funded research and to provide a report on the panel's findings by June 2022.

In August, Congressman Tony Cárdenas (D-CA) introduced H.R. 8001, the Companion Animal Release from Experiments (CARE) Act, backed by Cruelty Free International. The bill would require that research facilities in receipt of funding from the NIH develop and implement adoption policies for dogs, cats and rabbits no longer used in research. The bill also requires that information about the adoption policy and the success of the program (e.g., number of animals used, adopted or destroyed) be made available on the facilities website.

The new House and Senate version of the Humane Cosmetics Act that Cruelty Free International welcomed back in November 2019 has continued to gather bipartisan cosponsors in both chambers (18 in the Senate and 158 in the House) but did not receive a hearing this year. State laws in California, Nevada and Illinois went into effect on January 1 of this year, and bills in Hawaii, Maryland, Virginia and New York were introduced and debated but did not pass this year as some state legislature adjourned early due to Covid-19.

REACH Board of Appeal cosmetic cases spark outrage

On August 18, 2020, the European Chemicals Agency's Board of Appeal adopted two decisions that relate to the links between EU chemicals legislation, REACH, and the Cosmetics Regulation and the requirement for testing on animals (https://echa.europa.eu/about-us/who-we-are/board-of-appeal)

The appeals were brought by Symrise AG, a major German producer of flavors and fragrances, after ECHA had instructed them to carry out animal tests on two UV filters (homosalate and 2-ethylhexyl salicylate) that are used solely in cosmetic products.

The Board followed the position of the European Commission and ECHA that REACH covers worker safety and the Cosmetics Regulation covers consumer safety. They agreed that if tests on animals are needed for worker safety under REACH, this would not violate the animal testing bans under the Cosmetics Regulation. Even for ingredients that are only used in cosmetics, animal testing may be required – despite the fact that the tests for consumer and

worker safety are identical and data from worker safety tests cannot be ignored for consumer safety.

Andrew Fasey, Technically Qualified Member of the Board of Appeal and rapporteur for the cases, admitted that, "I don't expect that everyone will agree entirely with these decisions". And, not surprisingly, the decision has sparked outrage by "cruelty free" cosmetics companies and animal protection groups. In effect, they say, there is very little left now of the European cosmetic testing bans and it is all but impossible for cosmetics companies to market products that do not contain an ingredient that has been or will be tested for REACH. It is hoped that Symrise will appeal to the European Court.

EUSAAT

European Society for Alternatives to Animal Testing

The EUSAAT initiative to establish a European Network of 3Rs Centers (EU3Rnet)

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

After an initial meeting of representatives of 3Rs-Centres and societies at the EUSAAT conference in September 2018 in Linz (Austria) and three follow-up meetings in 2019, future initiatives were decided and agreed on. One of the results is a consensus statement of the platform EU3Rnet published in ALTEX (doi:10.14573/altex.2010061).

The network is an entirely independent, open and free community, which is very much dependent upon initiatives of its protagonists and personal efforts. It is based on a bottom-up approach, and every 3Rs centre, institute or society is welcome to join.

EUSAAT Annual General Assembly (AGA) on 12.11.2020 as virtual meeting

In the past, the EUSAAT has usually held its AGA during the EUSAAT Congresses or during the World Congresses. As WC11 was postponed due to the COVID-19 pandemic, the EUSAAT Board has decided to hold the AGA 2020 as a virtual meeting on November 12. The agenda will be distributed among the EUSAAT members in October.

Announcement: EUSAAT Webinar Series

As the next EUSAAT Congress will only take place in 2022, the EUSAAT board has decided to organize a webinar series as an alternative for the 3Rs community. It is planned that this series will allow top researchers and stakeholders as well as young researchers to present their topics and results. The topics will cover current 3R topics typically dealt with at EUSAAT conferences including regulatory, ethical, educational, 3Rs center and certainly scientific news, discussions and developments. In addition to the Webinar series committee, everyone is welcome to suggest topics and lecture titles. We especially aim to promote and encourage young scientists to present their projects in the webinar series. Please contact us if you are interested in presenting (winfried.neuhaus@ait.ac.at).

As the final year approaches, EU-ToxRisk has started to take stock of the experience and general learnings from this flagship project and to assess its most relevant results and remaining challenges. The project is working hard to build a legacy that should provide a platform to jump-start new efforts in next-generation risk assessment (NGRA).

All EU-ToxRisk new approach methods (NAM)-enhanced read-across (RAx) case studies have been presented and discussed on several occasions. Feedback has been collected from the regulatory community (Moné et al., 2020). For instance, the case studies (CSs) were included in the OECD CS portfolio for the Integrated Approach to Testing and Assessment (IATA) project. The final endorsement (foreseen in fall 2020) will allow for the publication of the related reports by the OECD, representing a major milestone for our consortium and toward regulatory recognition of NAM-based RAx. Based on this success, a new set of EU-ToxRisk case studies has been designed and initiated. These new studies address challenging regulatory and scientific questions of broad impact: testing of chemicals with little or no observed adverse effects; testing of chemicals inducing multi-target organ toxicity; testing of metabolism-activated toxicants. The first results will be discussed at the 4th EU-ToxRisk Virtual Open Symposium on February 22-23, 2021.

In parallel, the EU-ToxRisk Testing Commercialization Platform has been further developed. This major sustainability initiative of the project will provide a one-stop-shop in safety assessment solutions. More information can be found on the initiative's website (https://saferworldbydesign.com/eu-toxrisk/).

EU-ToxRisk publications

A short project summary was just published to explain the learnings, gaps, and unresolved issues of the regulatory implementation of NAMs, with a particular focus on NAM-enhanced RAx. This communication is intended to facilitate new efforts in NGRA and thus to advance the field of safety assessment by more mechanistically driven and animal-free approaches (Moné et al., 2020).

One of the major learnings was the need for a thorough definition of the above strategy aspects, ideally in form of a study pre-registration, to allow adequate interpretation of the data and to ensure overall scientific and toxicological validity. This was extensively detailed in the publication by Krebs et al. (2020), where a unified strategy for such collaborative testing was presented. In this study, a strategy to provide valid regulatory data was exemplified by using a panel of > 20 assays (with > 50 individual endpoints), each exposed to 19 well-known test compounds. The publication details all procedures required to allow test information to be used for integrated hazard assessment, strategic project decisions, and/or for regulatory purposes.

A key message from this experience is that correct detailed documentation of test method systems is crucial to ensure correct handling of test systems and data. Some examples of the implication of such procedures have been detected in recent EU-ToxRisk publications.

The handling of cells has a strong impact on the testing outcome. In Boon et al. (2020), the authors demonstrated that energy substrates can actively influence the cellular maturation of hepatocytes. Hepatocytes are among the metabolically most active cells, and their overall phenotype is determined also by their metabolic demands and activities. The study highlighted how nutrients can be used as a potential tool to guide hepatic maturation in several cell models. The described optimization creates significantly improved and long-term stable models for identification of liver toxicants.

In cell biology, pharmacology, and toxicology, dose-response and concentration-response curves are frequently fitted to data with statistical methods. In Kappenberg et al. (2020), the authors described how the negative control data sometimes deviate from the values measured for low (ineffective) test compound concentrations. Different strategies to tackle the problem were proposed by the authors, including recommendations on how to handle deviating controls.

In Gupta et al. (2020), the authors introduce a novel *in silico* tool for biological data interpretation. The tool FuSe was presented to improve RNA-Seq analyses by grouping the transcripts based on their similar functions. Typical RNA-Seq analyses are performed either at the gene or transcript level. As a consequence, functional changes are not well illustrated. FuSe was developed to predict functional similarities using the primary and secondary structure of proteins.

In parallel, the consortium has continued to develop and apply novel systems for repeated-dose toxicity (RDT) and developmental and reproductive toxicology (DART) studies. In van der Stel et al. (2020), the authors systematically investigated the effect of electron transport chain (ETC) inhibitors on multiple mitochondrial-related parameters in two human cell

types, HepG2 and RPTEC/TERT1. The study includes examples of a mitochondrial assessment workflow and establishes measurable key events of ETC inhibition by agrochemicals.

In the developmental neurotoxicity (DNT) field, different test systems were recently applied to support epidemiological evidence. In Klima et al. (2020), microcystins, a group of cyanobacterial toxins, were tested in human central and peripheral neurons to support epidemiological studies suggesting effects on the nervous system via drinking water or food. In Zhong et al. (2020), selective serotonin reuptake inhibitors (SSRIs), frequently used to treat depression during pregnancy, were tested in an organotypic human induced pluripotent stem cell (iPSC)-derived brain model (BrainSpheres) to solve contradictory evidence regarding effects on human brain development.

These studies show that NAM could be applied in novel regulatory frameworks to support risk assessment decisions. This issue was discussed in Paparella et al. (2020), where the authors provided a guide to the development of new alternative methods for IATA with diverse applications and support decision-making for their regulatory acceptance. Increased use of NAM in IATAs represents a unique occasion to improve current standard animal testing in DNT.

Outlook

The 4th EU-ToxRisk Open Symposium will take place on February 22-23, 2021. Due to the pandemic, the sessions will be held virtually. More information will be made available on the project website in the upcoming weeks (www.eu-toxrisk.eu).

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