



Lab-Grown “Mini-Brains” Suggest COVID-19 Virus Can Infect Human Brain Cells

A Johns Hopkins collaboration has demonstrated that the novel coronavirus, SARS-CoV-2, can infect and replicate within a human mini-brain model

Link to paper: doi:10.14573/altex.2006111

A multidisciplinary team from two Johns Hopkins University institutions, including neurotoxicologists and virologists from the Bloomberg School of Public Health and infectious disease specialists from the School of Medicine, has found that organoids (tiny tissue cultures that simulate whole organs) made from human cells (known as “mini-brains”) can be infected by the SARS-CoV-2 virus that causes COVID-19. The results are published online in *ALTEX* (doi: 10.14573/altex.2006111).

Early reports from Wuhan, China, have suggested that 36% of COVID-19 patients show neurological symptoms, but until now it was not clear whether the virus infects human brain cells. The Johns Hopkins researchers have now demonstrated that certain human neurons express a receptor, ACE2, that the SARS-CoV-2 virus uses for entering the lungs – and possibly the brain.

When the researchers introduced SARS-CoV-2 virus particles into a human “mini-brain” model, the team found – for what is believed to be the first time – evidence of infection by and replication of the pathogen.

The human brain is well-shielded against many viruses, bacteria and chem-

ical agents by the blood-brain barrier, which in turn, often prevents infections of the brain. “Whether or not the SARS-CoV-2 virus passes this barrier has yet to be shown,” notes author Thomas Hartung, MD, PhD, chair for evidence-based toxicology at the Bloomberg School of Public Health. “However, it is known that severe inflammations, such as those observed in COVID-19 patients, make the barrier disintegrate.” The impermeability of the blood-brain barrier, he adds, also can present a problem for drug developers targeting the brain.

The impact of SARS-CoV-2 on the developing brain is another concern raised by the study. Other research from Paris Saclay University has shown that the virus crosses the placenta, and embryos lack the blood-brain barrier during early development. “To be very clear,” Hartung says, “we have no evidence that the virus produces developmental disorders.” However, the mini-brains – which model the developing human brain – contain from the very earliest stages the same ACE2 receptor that allows the virus to enter lung tissues. Therefore, Hartung says, the findings suggest that extra caution should be taken during pregnancy.

“This study is another important step in our understanding of how infection leads to symptoms, and where we might tackle the COVID-19 disease with drug treatment,” says William Bishai, MD, PhD, professor of medicine at the Johns Hopkins University School of Medicine, and leader of the infectious disease team for the study.

“There is no doubt that the virus infects neurons and multiplies” Hartung adds, “and

now we have to find out what this means for patients and public health.”

The human stem cell-derived “mini-brain” models – known as BrainSpheres – were developed at the Bloomberg School of Public Health four years ago. They were the first mass-produced, highly standardized organoids of their kind, and have been used to model a number of diseases, including infections by viruses such as Zika, dengue, and HIV.

Select Press Coverage

Financial Times (paywall):

<https://www.ft.com/content/e5f20455-4422-4eea-9c51-b083040a0878>

JHU Hub: <https://hub.jhu.edu/2020/07/01/covid-19-mini-brain-models/>

The Washington Times:

<https://www.washingtontimes.com/news/2020/jul/1/coronavirus-could-infect-brain-cells-johns-hopkins/>

Invitation to Join a Proposal Toward a World Conference on Microphysiological Systems

We call on eminent individuals and organizations to join an effort to organize a series of three World Conferences for Microphysiological Systems (WC-MPS I, II & III) in 2021-2023

The emergence of organotypic cell culture technology, which models aspects of organ architecture and function *in vitro* – collectively defined here as MPS – represents a powerful set of tools for the life sciences. With the goal of creating a long-term,



self-sustained series of international conferences and an international society, we are calling on stakeholders to team up for this effort.

As a first step, we invite you to join us in applying for a substantial sponsorship of this series of events by NIH NCATS. At this stage, we are inviting organizations to join a steering group and individuals to join our advisory board.

Please contact Camila Sgrignoli Januario at: cjanuar1@jhu.edu

Video: Thomas Hartung on “The Role of Non-Animal Approaches in COVID-19 Related Research” Webinar (European Parliament)

Thomas Hartung’s talk for the European Parliament, presented by the Intergroup on the Welfare & Conservation of Animals, is now available on the CAAT YouTube channel.

Watch Now (YouTube): <https://www.youtube.com/watch?v=tH07tyVMU9w&t=19s>

CAAT’s COVID-19 Grants (Update)

Over 60 applications were submitted for our *Fast Track Grant for Research on Non-Animal Approaches to Investigate Mechanisms, Medicines, and Vaccines for Coronaviruses*. All were of high quality, and we are currently evaluating and prioritizing all submissions.

We would like to thank AnimalFree Research and Humane Society International/ Humane Society of the U.S. for their generous donations to support this grant (see next item).

Humane Society International and Humane Society of the U.S. Support CAAT’s Grant to Fast-Track Non-Animal Approaches for COVID-19 Mechanisms, Medicines, and Vaccine Research

Humane Society International and the Humane Society of the United States have joined forces with the Johns Hopkins Cen-

ter for Alternatives to Animal Testing at the Johns Hopkins Bloomberg School of Public Health to fund a fast-track research grant for non-animal approaches to investigate mechanisms, medicines, and vaccines for the novel coronavirus, SARS-CoV-2. The organizations believe that understanding the biological mechanisms that make humans especially susceptible to COVID-19 is urgently needed to inform the development and evaluation of effective countermeasures.

Laboratory investigations of human disease often attempt to artificially reproduce a condition in animals. Since the COVID-19 pandemic was declared, a flood of studies from across the globe have described infecting mice, hamsters, ferrets, monkeys and other animals with COVID-19, yet most report that the animals used were either immune to the new virus, or manifest symptoms that differ substantially from the human condition, including in the most severe clinical outcomes. Even genetically engineered mice experience fairly mild symptoms compared to most human patients. In addition, the animal-based approach is limited in its ability to predict the impact of comorbidities – the presence of two chronic diseases – in COVID-19 patients, or how the various treatments could impact or worsen the infection.

“We believe in standing together in times of global crisis, and are pleased to support the Johns Hopkins Center for Alternatives to Animal Testing in funding research with the potential to spare humans, as well as animals in laboratories, from suffering caused by COVID-19,” says Kitty Block, president of HSUS and CEO of HSI.

The Humane Society family of organizations’ support for the CAAT grant program aims to stimulate innovative and inherently human-relevant solutions for COVID-19. Models based on human biology – from cell and tissue cultures to complex organoids, organs-on-a-chip and computational tools – can help scientists understand the mechanisms of disease progression and rapidly identify interventions that are effective and safe in a human biological environment.

The Humane Society family previously released a multi-pronged policy plan for preventing another global health crisis like COVID-19.

Thomas Hartung on Rush to Publish COVID-19 Papers (*Financial Times*)

Excerpt from *Financial Times* (paywall):

A particular issue is the peer review process, in which journals send out papers before publication to scientists in the same field, who are asked to assess the quality of the research, spot errors and suggest improvements. “I get about 10 requests a day to review articles – and all I can actually do is four per month,” said Thomas Hartung, toxicology professor at Johns Hopkins University. He said he had four promised reviews to complete “but getting my own paper out has to take precedence.”

Full article at *Financial Times* (paywall): <https://www.ft.com/content/61287181-2beb-4356-8de0-06eed906071>

Thomas Hartung interviewed by *Times of India* on “Why Rhesus Monkeys Are Used for Most Vaccine Trials”

Researchers across the world have gone into overdrive to find a vaccine for COVID-19, as the disease continues to affect millions. In this interview, Thomas Hartung explains various aspects of animal testing of vaccines in the developmental stage.

Read more at *Times of India*: http://timesofindia.indiatimes.com/articleshow/75802583.cms?utm_source=contentofinterest&utm_medium=text&utm_campaign=cppst

Thomas Hartung: Toxicity and Disease (Podcast)

Thomas Hartung delivers an insightful overview of his work studying toxicity testing improvements, organoids, and advancing technologies. Hartung discusses his background, and the road he has taken to arrive at his current place as a leading voice in the discussions concerning toxicity and animal testing. He explains that the technological opportunities have advanced significantly in the last few years and discusses “organ-on-a-chip” technologies and other advanced tissue work.

Watch the Podcast on YouTube: <https://youtu.be/0V5fglnjvY>



Alexandra Maertens on Science Podcast: Why Some Diseases Come and Go with the Seasons, and How to Develop Smarter, Safer Chemicals

At this year's AAAS annual meeting in Seattle, host Meagan Cantwell spoke with Alexandra Maertens, director of the Green Toxicology initiative at CAAT, about the importance of incorporating nonanimal testing methods to study the adverse effects of chemicals.

Listen now: <https://www.sciencemag.org/podcast/why-some-diseases-come-and-go-seasons-and-how-develop-smarter-safer-chemicals>

Thomas Hartung Discusses Artificial Intelligence & Big Data for Safety Testing (ToxTalk Podcast)

CAAT director Thomas Hartung discusses some of his innovative contributions to the field of toxicology including developing advanced computer modeling systems using big data analyzed by artificial intelligence used to predict toxicity of novel compounds. Hartung gives a general overview of these concepts, and his perspective on the future of predictive modeling to supplement current toxicology research strategies. He also discusses the potential for predictive modeling to expedite drug development decisions and challenges faced with introducing these technologies to regulated industries.

Listen to the Podcast: <https://www.actox.org/meetCourses/audio/ACT-Hartung-Aartificlia.mp3>

How NAM Can Speed Up COVID-19 Drug Discovery

Researchers from the University of Konstanz and Johns Hopkins University draw attention to the fact that novel animal-free testing methods could help accelerate the discovery and development of COVID-19 drugs and vaccines and advise European Parliament on NAM-based drug development and safety assessment.

"SARS-CoV-2, the novel coronavirus responsible for the global COVID-19 out-

break, is likely to remain a threat to human health unless efficient drugs or vaccines become available," states Marcel Leist, co-director and co-founder of CAAT-Europe (alongside Thomas Hartung from Johns Hopkins University) and Chair of In-Vitro Toxicology and Biomedicine at the University of Konstanz. "In this situation, using new animal-free approach methods for drug development, safety and efficacy as well as quality evaluation could speed up this process."

While animal-based testing is lengthy and likely to fail when a pathogen is specific to man or if the desired drug is based on specific features of human biology, NAM are species-specific (humans) and produce faster outcomes. For instance, NAM have already been successfully applied to predict genotoxicity (a major aspect in the formation of tumors) within days. Recent organ-on-a-chip technologies are allowing researchers to model different compartments, such as the lung and the immune system, and thus to generate data in a timely manner. Also, human antibodies targeting virus epitopes (the part of an antigen that is recognized by the immune system) can be generated in molecular biology laboratories within days without requiring animals. "We strongly believe that, with regard to drug discovery strategies, diversification, specifically the use of NAM, could prove key to global COVID-19 responses," state Marcel Leist and Thomas Hartung.

Full Press Release: <https://www.uni-konstanz.de/en/university/news-and-media/current-announcements/news-in-detail/how-nam-can-speed-up-covid-19-drug-discovery/>

Kathrin Herrmann on Beyond the 3Rs at German Bad Boll Conference

This year, the Bad Boll Annual Animal Welfare Conference (March 6-8, 2020 in Germany) focused on animal experimentation and Germany's stance on funding and furthering the application of the 3Rs and working towards the final goal of the EU Directive 2010/63 to fully replace the use of live animals. Kathrin Herrmann, Director of CAAT's Refinement Program, delivered the keynote lecture on the past 60 years of the 3Rs and why it is time to move

beyond them and focus on animal-free, human-relevant methods.

Lena Smirnova Selected as Innovator Award Finalist

Lena Smirnova's abstract, Studying Gene-Environmental Interactions in Autism with iPSC-derived BrainSpheres: microRNA and Metabolic Biomarkers of the Synergy, has been selected for presentation in the Innovator Award Finalists Platform Session 4 and for consideration in the Innovator Award competition at the annual meeting of the Society for Birth Defects Research and Prevention. The award was announced during the Annual Business Meeting on July 1, 2020.

Vy Tran Presents Award-winning Research at ASCCT

CAAT PhD student Vy Tran, who last year received the ASCCT Student Award, recently presented her award-winning work at the 8th annual meeting of the ASCCT. Tran's research compares the Michigan Cancer Foundation-7 (MCF-7) cells, a human breast adenocarcinoma cell line that is commonly used for *in vitro* cancer research, with human breast cancer tissue from the Cancer Genome Atlas, which has over 1000 breast cancer tissue samples.

Since large data sets with gene expression studies were available for both, she examined the extent to which gene networks were conserved – finding that not only was there very little overlap between the two data sets but, most critically, several drug targets were present in the human-derived samples that were not in the MCF-7 data set. As part of the Mapping the Human Toxome project, CAAT demonstrated that even MCF-7 cells obtained from the same cell batch at the same cell bank can display cellular and phenotypic heterogeneity, which affects reproducibility of experiments using this cell line. This work also demonstrates that the larger data sets likely have similar heterogeneity and should be used with caution.

Vy Tran's webinar is available here: <https://www.ascctox.org/webinars/eVmBMbpCMAg5/63>



CAAT's Coursera Courses Pass 5,000 Learner Mark

CAAT's highly rated Coursera offerings, *Toxicology 21: Scientific Applications* and *Evidence-based Toxicology* have each hit new milestones; over 3,400 active learners for Tox21 and over 1,598 active learners for EBT.

CAAT Article Among Top 100 Downloaded in Scientific Reports

A paper co-written by CAAT Director Thomas Hartung and David Pamies (formerly of CAAT), "A Human iPSC-derived 3D platform using primary brain cancer cells to study drug development and personalized medicine," received over 4,000 article downloads in 2019, placing it as number 15 of the top 100 downloaded cancer papers for *Scientific Reports* in 2019.

Scientific Reports published more than 1,024 cancer papers in 2019.

Paper by EBTC's Katya Tsaion Among Top 10% Downloaded

Quantitative Systems Pharmacology for Neuroscience Drug Discovery and Development: Current Status, Opportunities, and Challenges, co-authored by EBTC's Katya Tsaion, was among the top 10% downloaded papers from *CPT: Pharmacometrics & Systems Pharmacology* between January 2018 and December 2019.

You can read the paper here: <https://tinyurl.com/y7dv248v>

Next Generation Humane Science Award

Deadline Extended to August 1st, 2020

The Next Generation Humane Science Award is available annually to young scientists to acknowledge and encourage researchers who focus on replacing the use of animals in experiments. The 2020 award will be a prize of up to \$5,000 to recognize the work of one young scientist; this may be shared among two or more young scientists.

Details and application: <https://caat.jhsph.edu/programs/awards/HumaneScience.html>

CAAT's Newest Research Associate: Carolina Romero

The main focus of Carolina Romero's research is developing a testing strategy for developmental neurotoxicity (DNT). This strategy will be based on a human 3D iPSC-derived brain model with knocked-in fluorescent tags for neural markers, where six key events of neurodevelopment and their perturbations will be assessed in one assay. The main techniques involved in this project are CRISPR/Cas9 gene-editing technology, high-content imaging, and electrophysiological recording. This new *in vitro* assay, relevant for the assessment of human toxicity, will reduce the costs and accelerate the prioritization and testing of environmental chemicals, hazard identification and characterization within a risk assessment context.

Recent Events

Jamie DeRita Memorial Animal Protection Symposium

July 9, 2020

This online symposium honoring the life of Jamie DeRita, who passed away in June, was held online July 9. Jamie was CAAT's event coordinator from 2012-2018, and was known to many of our friends and colleagues for her tireless work on our many meetings, conferences, and symposia over the years.

Jamie was famous throughout the Maryland shelter and animal welfare communities as someone who could not say no to helping any animal that needed a home. She was known to regularly pick up animals she saw in her daily travels, and to work non-stop to find them perfect homes. Her CAAT family honored her with a series of presentations about our relationship with animals and adoption option, with brief testimonials from family and friends interspersed throughout the event. Her family has also started a fundraiser to secure the future of her four children. Please consider donating any amount here: <https://tinyurl.com/ya4xwxxz>

Guest Speakers included:

- Aysha Akhtar (Center for Contemporary Sciences): *Our shared destiny with animals*

- Stacy M. Lopresti-Goodman (Marymount University): *From "lab dog" to "lap dog": Why dogs released from research make great companions*
- Kathleen (Katie) Conlee (Humane Society of the U.S.): *Advocating for dogs in laboratories*

Toxicology for the 21st Century: What is in the Toolbox for Excipients?

July 8, 2020

(Note: This talk was originally planned as a keynote at the Excipient World Conference on May 12.)

The limitations of animal-based toxicology to predict human health threats are widely recognized. We also are discovering more shortcomings of traditional (human) cell culture such as cell identity, differentiation, genetic stability and mycoplasma infection as well as non-homeostatic and non-physiological culture conditions. The increasing pace of technological developments of modern cell culture and their integration leads to what is called "disruptive technologies" that can lead to the development of alternatives to traditional approaches for product development and safety assessment.

Such technological advances promise to be real "game-changers." Combined with an increased mechanistic base of reasoning (e.g., Adverse Outcome Pathway concepts), Integrated Testing Strategies, and evidence-based methods of data evaluation and integration, a revolutionary change for how we assess the biological effects of substances has been set into motion.

Thomas Hartung was the featured speaker.

Summer School on Innovative Approaches in Science

June 22-26, 2020

Online

The Physicians Committee for Responsible Medicine and CAAT presented the virtual Summer School on Innovative Approaches in Science. The Summer School, modeled after and in cooperation with the European Union's Joint Research Centre Summer School on Nonanimal Approaches in Sci-

ence, was intended to provide students and early career scientists with information on *in vitro*, *in silico*, and human-based methods and approaches for toxicology and biomedical research.

The fully online program featured lectures, in-depth training, virtual laboratory tours, e-poster presentations, and virtual engagement with speakers and attendees. Featured speakers included experts from Harvard University, Johns Hopkins University, the National Institutes of Health, the Environmental Protection Agency, the Physicians Committee for Responsible Medicine, and more.

Hundreds of people attended and the event received excellent reviews.

Helena Hogberg on Platforms for Neurological Drug Discovery and Toxicology Screening

June 30, 2020

Sponsored by AxoSim

Helena Hogberg and Lowry Curley were featured speakers for this interactive webinar aimed at sharing information about innovative platforms for drug discovery mimicking the PNS and CNS. Attendees learned about recent studies recapitulating peripheral neuropathy in four common chemotherapeutics and cutting edge research from CAAT with implications for preclinical neurodegenerative diseases including ALS and Parkinson's.

Environmental Neuroscience: Advancing the Understanding of How Chemical Exposures Impact Brain Health and Disease

June 25, 2020

Online

On June 25, 2020, the Forum on Neuroscience and Nervous System Disorders, in collaboration with the Board on Environmental Studies and Toxicology, hosted a virtual public workshop that brought together experts and key stakeholders in neuroscience and environmental health science to explore the current knowledge landscape and future opportunities.

CAAT deputy director Helena Hogberg presented.

OpenTox Webinar: Harnessing the Power of Novel Animal-free Test Methods for the Development of COVID-19 Drugs and Vaccines

June 11, 2020

This virtual meeting included a perspective by Thomas Hartung followed by a discussion session.

The COVID-19-inducing virus, SARS-CoV2, is likely to remain a threat to human health unless efficient drugs or vaccines become available. Given the extent of the current pandemic and its disastrous effect on world economy (associated with limitations of human rights), speedy drug discovery is critical. In this situation, past investments into the development of new (animal-free) approach methods (NAM) for drug safety, efficacy, and quality evaluation can be leveraged.

Watch Now (YouTube): <https://www.youtube.com/watch?v=-lv14XvII0c&feature=youtu.be>

7th Annual 3Rs Symposium: Practical Solutions and Success Stories

June 4-5, 2020

Online

The 7th Annual 3Rs symposium, organized by Johns Hopkins Center for Alternatives to Animal Testing (CAAT) Refinement Program in collaboration with the USDA Animal Welfare Information Center (AWIC), NIH Office of Laboratory Animal Welfare (OLAW), and the Johns Hopkins Department of Molecular and Comparative Pathobiology, was held online this year. The goal of the symposium was to bring together experts in replacement, reduction, and refinement of animal experimentation to exchange information with scientists, IACUC members, veterinarians, and animal care technicians about practical solutions and recent success stories to reduce the use of animals in research and improve the welfare of the animals who are still deemed necessary.

The format included two days of lectures and panel discussions, including Q&A. These lectures gave participants a strong foundation in the relevant research underlying breakthroughs in the 3Rs, while the Q&A sessions allowed participants to re-

ceive feedback specific to their own facilities from the expert speakers and fellow participants.

SOT Webinar: RT-01: Mechanistic Read-Across of Chemical Toxicants Based on Big Data

June 2, 2020

Chairs: Hao Zhu, Rutgers, The State University of New Jersey; and Thomas Hartung, Johns Hopkins University.

In 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act became the first US legislation to advance chemical safety evaluations by utilizing novel testing approaches that reduce the testing of vertebrate animals. Central to this mission is the advancement of computational toxicology and artificial intelligence approaches to implementing innovative testing methods. In the current "big data" era, the volume (amount of data), velocity (growth of data), and variety (diversity of sources) are critical considerations when characterizing the currently available chemical, *in vitro*, and *in vivo* data for toxicity modeling purposes. Furthermore, as suggested by various scientists, the variability (internal consistency or lack thereof) of publicly available data pools, such as PubChem, also presents significant computational challenges.

The development of novel artificial intelligence approaches based on massive public toxicity data is urgently needed to generate new predictive models for chemical toxicity evaluations and establish scientific confidence in the developed models as alternatives for evaluating untested compounds. In this procedure, traditional approaches (e.g., QSAR) purely based on chemical structures have been replaced by newly designed data-driven and mechanism-driven modeling. The resulting models realize the concept of adverse outcome pathways (AOP), which can not only directly evaluate toxicity potentials of new compounds but also illustrate relevant toxicity mechanisms. The recent advancements of computational toxicology in the big data era are paving the road to future toxicity testing and will have significant impacts on public health.



Thomas Hartung on “The Role of Non-Animal Approaches in COVID-19 Related Research” Webinar (European Parliament)

May 20, 2020

Public Webinar via European Parliament

Thomas Hartung spoke on “Harnessing the power of novel animal-free test methods for the development of COVID-19 medicines and vaccines.” A video of Hartung’s talk may be viewed here: <https://www.youtube.com/watch?v=tH07tyVMU9w&t=19s>

SaferWorldByDesign Online Seminar: How to Submit a Successful Read-Across Justification Report

May 5, 2020

This virtual meeting included a perspective by Costanza Rovida (Scientific Officer and Senior Regulatory Specialist, CAAT) followed by a discussion session. It was pre-

sented by a partner of the recently established EU-ToxRisk Commercial Platform for New Approach Methods (NAMs) in Safety Assessment.

Watch now: <https://www.youtube.com/watch?v=usfi0nNpiIU&feature=youtu.be>

Upcoming Events

Pan-American Conference for Alternative Methods

May 12-14, 2021

Windsor, Ontario, Canada

Recent Publications

Ball, N., Madden, J., Paini, A. et al. (2020).

Key read across framework components and biology based improvements *Mutat Res* 853, 503172. doi:10.1016/j.mrgentox.2020.503172

Busquet, F., Hartung, T., Pallocca, G. et

al. (2020). Harnessing the power of novel animal-free test methods for the development of COVID-19 drugs and vaccines. *Arch Toxicol* 94, 2263-2272. doi:10.1007/s00204-020-02787-2

Flanagan, E., Lamport, D., Brennan, L. et al. (2020). Nutrition and the ageing brain: Moving towards clinical applications. *Ageing Res Rev* 62, 101079. doi:10.1016/j.arr.2020.101079

Maertens, A., Tran, V. H., Maertens, M. et al. (2020). Functionally enigmatic genes in cancer: Using TCGA data to map the limitations of annotations. *Sci Rep* 10, 4106. doi:10.1038/s41598-020-60456-x

Troger, F., Delp, J., Funke, M. et al. (2020). Identification of mitochondrial toxicants by combined in silico and in vitro studies – A structure-based view on the adverse outcome pathway. *Comput Toxicol* 14, 100123. doi:10.1016/j.comtox.2020.100123



Number of animals used in EU chemical tests doubles

In June, the European Chemicals Agency (ECHA) released its fourth report on the use of animals and alternatives under the REACH chemicals regulation¹. According to Article 117, the report must be published every three years; to date there have been reports in 2011, 2014 and 2017.

Using the data provided in the report, it appears that the total number of animals used in REACH testing has doubled since the previous report from 1,119,283

to 2,395,056 animals. The increase is explained by the final registration of lower tonnage substances in 2018, many of which had new reproductive screening studies (an increase from 975 tests to 2,318 tests using a total of over 1 million animals), and the conduct of other higher tier animal tests following testing proposal decisions (including a doubling of the number of developmental toxicity studies from 367 to 743, using a total of 668,700 animals). The total is now greater than the Commission’s “best case” estimate of 1.9 million and just in excess of their “average” estimate. A further

164,080 animals are expected to be used in tests proposed since June 2018, which do not figure in the report.

Cruelty Free International have raised concerns that the number of animal tests will continue to rise as ECHA conducts more compliance checks and substance evaluations. And, the number is at risk of rising even further if plans to extend the data requirements for endocrine disruptors, polymers and lower production volume substances are realized.

Cruelty Free International have complained that the report shows that ECHA

¹ The use of alternatives to testing on animals for the REACH Regulation Fourth report under Article 117(3) of the REACH Regulation. June 2020. https://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2020_en.pdf/b9af7cf7-4ce0-f3a1-1bcb-8de3fd84a1fb

is failing to ensure that animal testing is a last resort. Even ECHA concede in the report that there have been “relatively few changes in the use of alternatives since the last report in 2017.” The use of read-across remains relatively high; it is currently used in 25% of cases. However, the use of weight of evidence (4% of cases) and QSARs (3%) is still relatively low. Cruelty Free International are particularly concerned about the lack of information about why tests such as skin irritation in rabbits are still happening when *in vitro* methods are available.

Calls for human-relevant technologies in search for COVID-19 vaccine

There has undoubtedly been an increase in animal testing in the rush to develop vaccines and treatments for COVID-19. However, to date, no animal model has been able to recapitulate all features of the human infection; even monkeys do not develop the most severe symptoms that COVID-19 causes in humans.

In April, Cruelty Free International issued a joint statement² with other leading animal groups, calling on the World Health Organization (WHO) to coordinate effective human-relevant research and avoid the duplication of animal tests in the search for a COVID-19 vaccine. And, in May, Cruelty Free International joined nearly 100 other experts and scientists worldwide in another open letter directed at the WHO calling for non-animal research methods to be prioritized to help the discovery of effective vaccines and treatments for COVID-19³.

New study questions the use of two animal species in human drug tests

In June, the UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) published their conclusions from a project be-

gun in 2016 with industry to look at the use of dogs and monkeys in drug testing. The organization worked with 18 pharmaceutical and biotechnology companies to evaluate whether data from just one animal species could be used to report on drug safety instead of the two species typically used.

The final report (Prior et al., 2020) revealed that two-thirds of the 172 drugs studied, in hindsight, could have been progressed to human clinical trials with long-term tests from just one, instead of two, species. The report also highlighted a lack of data-sharing between companies, which could lead to more unnecessary animal tests. Cruelty Free International has written to regulatory bodies around the world to draw their attention to this report and urge them to take decisive action.

Cruelty Free International helps save over 80,000 animals from chemical tests

Since the beginning of the EU's REACH chemical safety program, the European Chemicals Agency (ECHA) has published over 1,500 animal test proposals for chemical substances, giving third parties the chance to offer scientific information that could avoid the testing proposed. As part of a collaboration with other European animal groups, Cruelty Free International committed to commenting on as many of these proposals as possible. Our initial findings were presented in 2014 (Taylor et al., 2014).

We have now been able to evaluate our overall success in providing comments and have found that we believe we were influential in 76 cases, helping to save over 80,000 animals from unnecessary tests. Overall, our comments were adopted by ECHA or the company registering the substance in only about 14% of cases, including tests for repeated dose toxicity, reproductive toxicity, and long-term toxicity and mutagenicity, typically involving mice, rats, rabbits,

birds and fish. This suggests that there were still instances where testing that was either scientifically or legally unnecessary was being proposed and, had we not intervened, may have occurred.

For example, a proposal for a 90-day repeated-dose study of 2,4,6-trinitrotoluene (TNT) via inhalation was withdrawn following arguments we made that the substance is an explosive and already considered harmful to humans so testing was dangerous, difficult and unnecessary. In another example, a company had proposed to test slimes and sludges from blast furnace and steelmaking in a two-generation reproductive test. We pointed out that despite its name, the substance was of low toxicity and was not registered at a tonnage level at which the test was necessary. The company agreed and withdrew the testing proposal, saving 2,200 rats.

Latin America moves towards cruelty free cosmetics

In 2017, Guatemala became the first country in Latin America to take legislative action to end the testing of cosmetics on animals. Now Mexico and Columbia are following suit.

In March, Mexico's Senate pledged to outlaw the practice of animal testing for cosmetics, as well as the manufacture, import or marketing of cosmetics tested on animals anywhere in the world after the law comes into force. The bill will now move to the next stage of the legislative process in the Chamber of Deputies.

In June, a bill to restrict the testing of cosmetics on animals and the sale and import of animal-tested cosmetics passed its final vote in the Colombian Senate. Bill 120/2018 will enter into force in four years' time and will restrict reliance on animal testing for ingredients in cosmetics. The bill also includes a commitment to incentives to strengthen the capacity of laboratories and research institutes in Colombia to develop and apply non-animal testing methods.

² Joint statement from Cruelty Free Europe, Cruelty Free International, Eurogroup for Animals and People for the Ethical Treatment of Animals Foundation on 'COVID-19 and animal experiments', 16 April 2020: <https://www.crueltyfreeinternational.org/sites/default/files/COVID-19%20Joint%20groups%20statement%20.pdf>

³ A shift in focus is needed to tackle COVID-19, 14 May 2020: https://www.ad-international.org/admin/downloads/adi_aa465255de851a533279b-f8e1b053287.pdf



International webinar on “Safer cosmetics through animal free testing”

On May 7, Cruelty Free International held a joint webinar with XCellR8, a UK-based laboratory exclusively devoted to animal-free safety testing, for companies, researchers and regulators on how modern animal-free testing methods can now meet the demand for safe and cruelty free cosmetics.

Monica Engebretson, Head of Public Affairs for North America at Cruelty Free In-

ternational provided an overview of testing regulations around the world and explained how the US Humane Cosmetics Act would harmonize state and international laws on this issue. Dr Carol Treasure, co-founder & CEO of XCellR8, explained how non-animal methods are not only more ethical but also more reliable than the animal tests they replace. The webinar was attended by US politicians and also interested parties from Canada, the UK, Italy, France, China, Japan, South Korea and South Africa.

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EUSAAT

European Society for
Alternatives to Animal Testing

EUSAAT Annual General Assembly (AGA) 2020

In the past, EUSAAT has usually held its AGA during the EUSAAT Congresses in Linz, Austria or during the World Congresses. As WC11 has been postponed due to the COVID-19 pandemic to August 2021, the EUSAAT Board is considering to hold the AGA 2020 as a “virtual meeting” later this year in September or October, since this is now technically feasible and ESTIV, one of our befriended European societies, successfully held its AGA 2020 virtually including elections last month. The EUSAAT Board will discuss the issue, draft an agenda and circulate it among EUSAAT members this summer.

EUSAAT will sponsor a workshop at WC11 in 2021 in Maastricht

EUSAAT has confirmed to the organizers of WC11 that it will sponsor Work-

shop (S200), which is entitled *3Rs-Centers Around the World and Their Role in Fostering the Implementation of 3Rs in Academia*.

An increasing number of 3Rs centers have recently been established around the world with different focuses on replacement, reduction and refinement of animal use for scientific purposes. The session aims at providing an overview of their diversity and the challenges they may face within their countries. In addition, we will discuss possible synergies and collaborative activities that can help further the implementation of the 3Rs at different levels such as research, education, and dissemination.

Co-chairs:

Charu Chandrasekera (CCAAM, CA) & Horst Spielmann (EUSAAT, EU/DE)

European 3Rs Centers:

Winfried Neuhaus (EUSAAT & AIT, AT): The EUSAAT initiative to establish a European Network of 3Rs Centers

Adrian Smith (Norecopa, NO): Norecopa: A hub of international 3R resources
Monika Schaefer-Korting (BB3R & FU Berlin, DE): The Berlin-Brandenburg Research Platform BB3R – Research and Graduate Education since 2014

International 3Rs Centers:

Charu Chandrasekera (CCAAM, CA): The Canadian Centre for Alternatives to Animal Methods (CCAAM)
Hajime Kojima (JaCVAM, JP): The Japanese Society for Alternatives to Animal Experiments (JSAAE)
Shujun Cheng (CCARE, Shanghai Jiaotong U, CN): The Consensus Center of Alternatives Research and Evaluation (CCARE)

EUSAAT 2021 Annual Congress on Alternatives

In the past, EUSAAT has held its annual EUSAAT Congresses on Alternatives in

those years in which no World Congress was scheduled. Since WC11 was planned for this year, the next EUSAAT Congresses had been scheduled for 2021 and 2022.

As participation in WC11 in 2021 has the highest priority for all scientists working on the 3Rs, an additional EUSAAT Congress in 2021 is not feasible. The EUSAAT Board is therefore considering holding a one-day congress in Maastricht immediately after WC11 focusing on EUSAAT-specific top-

ics that will not be addressed during WC11. In view of the fact that EUSAAT is helping to build a network of European 3R centers, the EUSAAT Congress 2021 shall focus on this topic, which was highly appreciated by the participants of the EUSAAT Congresses 2018 and 2019. Furthermore, as listed in the previous section, the WC11 Scientific Committee selected only two European centers for oral presentations at the WC11 meeting on 3Rs centers. In addition,

the EUSAAT Board is examining the possibility of organizing a virtual congress in autumn 2021.

Therefore, the EUSAAT Board is planning a one-day meeting to discuss the progress of the 3Rs centers of the European Network immediately after WC11 in Maastricht. The organization of a virtual congress in autumn 2021 is under evaluation. The next “regular” EUSAAT Congress will take place in 2022.



During the historic pandemic related to the SARS-CoV-2 virus, this quarterly update cannot avoid touching this topic. Three issues have been important. First, EU-ToxRisk researchers have faced severe limitations due to the lockdown applied in most countries. This has significantly decelerated the pace of wet-lab research. Second, many project partners used *in silico* methods and other non-experimental approaches to maintain overall consortium productivity. This has resulted in several submitted manuscripts and in publications authored by the partners. Third, partners of EU-ToxRisk feel that SARS-CoV-2, the novel coronavirus responsible for the global COVID-19 outbreak, will remain a threat to human health until efficient drugs or vaccines become available. Therefore, they stressed the importance, in this situation, of diversification in research and the application of new animal-free approach methods (NAM) for drug development. Hence, they compiled information suggesting that NAM-based efficacy, safety, and quality evaluation could speed up the drug discovery process (Busquet et al., 2020).

COVID-19 research can now benefit from the investments made into the development of NAM over the past 20 years. The

European toxicological flagship program, EU-ToxRisk, harbors one of the largest collections of NAM available in Europe and can make them available, now or in the future, for drug safety evaluations. The technologies developed and applied by the consortium – *in vitro* and *in silico* models and high-throughput screening methods – can play a crucial role in this context, having proven to be human-relevant and effective, and allowing safe progression to clinical testing in a shorter time span compared to traditional animal testing.

EU-ToxRisk publications

Exciting novel models have been established in the area of neurotoxicity and developmental neurotoxicity. In one case, hiPSC-based 3D *in vitro* neurospheres were applied to effectively identify (developmental) neurotoxins. This model aims to replace or complement the use of animal models in various basic research and pharmaceutical applications (Kobolav et al., 2020). In Brüll et al. (2020), another test model was introduced. The authors effectively assembled 3D cultured human dopaminergic neurons (LUHMES) together

with human stem cell-derived astrocytes and microglia. Such organoids were successfully applied to quantify toxicant effects on organoids by standard technology and high throughput analysis.

In Troger et al. (2020), researchers from the EU-ToxRisk project developed a model to predict mitochondrial toxicity. Drugs that modulate mitochondrial function can cause severe adverse effects in humans that may be missed in animal models. The authors combined structure-based methods with machine learning to address human mitochondrial respiratory complex I (CI) inhibition. The approach was used for virtual screening of DrugBank and the ChEMBL library: the top-ranked compounds were selected for experimental testing in three *in vitro* assays/NAM. This screening campaign led to the identification of novel CI inhibitors.

Mitochondrial toxicity is also the endpoint of a publication authored by Hemmerich et al. (2020). In this study, *in silico* approaches were applied to indicate hazards early in the drug development pipeline. By combining multiple endpoints, the authors derived the largest so far published dataset on mitochondrial toxicity. The combination of machine learning and structural



alerts also proved the suitability for *in silico* risk assessment of mitochondrial toxicity.

Another fundamental study was published on the application of *in silico* methods to toxicology. To better extrapolate the developmental toxicity effects of chemicals in zebrafish (*Danio rerio*) embryos to humans, the authors developed a physiologically-based pharmacokinetic (PBPK) model designed to predict organ concentrations of neutral or ionizable chemicals up to 120 h post-fertilization. Valproic acid analogues were tested to assess the model's applicability to developmental toxicity (Simeon et al., 2020).

The read-across approach, as used extensively in the submission of dossiers to ECHA (European Chemical Agency), could play a significant role in reducing animal use. Increasing the application domain and the trust in this toxicological approach represents a key mission of the EU-ToxRisk project (Escher et al., 2020). Relevant publications on this topic were recently released. In Rovida et al. (2020), the authors reported the outcome of the t⁴ think tank meeting organized by the project on the state of the art of the acceptance of read-across as a tool for risk assessment for regulatory purposes. In this review, major issues limiting the regulatory acceptance rate of the read-across procedure were identified. The use of NAM was discussed as one of the most important innovations to improve the acceptability of read-across.

Finally, in Gadaleta et al. (2020), the authors introduced an automated procedure for the selection of analogues for data gap-filling, solving the issue of relying on human expert judgement. Results confirmed the

suitability of the procedure as a source of data to support regulatory decision-making.

Outlook

The recently launched EU-ToxRisk Testing Commercialization Platform has initiated, under the umbrella of the SaferWorld-byDesign framework, a series of webinars introducing key partners and key tools of the platform. Videos can be streamed via the dedicated YouTube® channel (<https://www.youtube.com/user/DouglasConnect/videos>).

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