Groundbreaking CAAT Research Shows Antidepressant Harms Baby Neurons in Lab-Grown "Mini-Brains" – *Stem-cell-derived model of developing brain could allow rapid testing of drugs, chemicals for developmental neurotoxicity*

Researchers at CAAT have demonstrated the use of stem cell-derived "mini-brains" to detect harmful side effects of a common drug on the developing brain. Mini-brains are miniature human brain models, developed with human cells and barely visible to the human eye, whose cellular mechanisms mimic those of the developing human brain.

The scientists, who published their findings on February 21 in *Frontiers of Cellular Neuroscience*, used the mini-brains to determine that the common antidepressant paroxetine suppresses the growth of synapses, or connection points between neurons, and leads to significant decreases in an important support cell population. Paroxetine is sold under the brand names Paxil and Seroxat, among others.

Paroxetine, which can cross the placenta in pregnant women, currently comes with a warning against use in early pregnancy, largely due to a known risk of heart and lung defects. Some epidemiological studies also have suggested that paroxetine raises the risk of autism. The new findings are likely to heighten concerns about the effects of this drug, and others in its class, on the developing brain.

The study authors say that the findings suggest that lab-grown mini-brains, which they call BrainSpheres, are a good alternative to traditional animal testing. In particular, they can reveal drugs and other chemicals that are harmful to young brains.

"There's a growing concern that we have an epidemic of neurodevelopmental disorders, including autism, and that these might be caused by exposures to common drugs or other chemicals. However, since traditional animal testing is so expensive, we haven't been able to properly investigate this question," says co-senior author Thomas Hartung, MD, the Doerenkamp-Zbinden Chair and Professor in the Department of Environmental Health and Engineering and director of CAAT at the Bloomberg School.

Hartung and colleagues developed the mini-brains to model early brain development. The tiny clumps of brain tissue are made by taking cells from adult humans, often from their skin, and transforming them into stem cells, and then biochemically nudging the stem cells to develop into young brain cells. The mini-brains form a rudimentary brain-like organization over a period of a few months. Because they are made of human cells, they may be more likely to predict effects on the human brain – and because they can be mass-produced in the lab, they are much cheaper to work with than animals.

A set of animal toxicology tests for a single chemical costs about $1.4 million on average, the authors note, which explains why the vast majority of chemicals used in drugs and other consumer products have never been tested for toxicity. In contrast, toxicity testing using mini-brains costs only a few thousand dollars.

In the new study, the scientists used mini-brains to test for neurodevelopmental effects of paroxetine. It and other antidepressants in its class, known as SSRIs or selective serotonin reuptake inhibitors, are among the world's most commonly prescribed drugs, accounting for at least hundreds of millions of prescriptions annually.

The research team exposed mini-brains to two different concentrations of paroxetine over eight weeks as the clumps of tissue developed. Both concentrations were within the therapeutic range for blood levels of the drug in humans. In the experiments, the researchers also used two different sets of mini-brains, each derived from a different stem cell.

The scientists found that while paroxetine did not seem to have a significant neuron-killing effect, at the higher concentration it reduced levels of a protein called synaptophysin, a key component and marker of synapses, by up to 80%. Paroxetine reduced levels of two other synapse-related markers as well. Similarly, the team observed that paroxetine reduced the normal outgrowth of structures called neurites, which eventually develop into the output stalks and root-like input branches of mature neurons. Finally, the researchers noted that paroxetine-exposed mini-brains developed with up to 75% fewer oligodendrocytes, the

support cells that are crucial for the proper "wiring" of the brain, than controls.

These effects suggest that the drug might hinder the normal formation of interconnections among developing neurons – a result that could conceivably underlie autism or other disorders.

The study also shows the broader potential of mini-brain-based testing to detect adverse effects of drugs on the developing brain.

"In this report, we were able to show that testing with mini-brains can reveal relatively subtle neurodevelopmental effects, not just obvious effects, of a chemical," Hartung says. "Whether paroxetine causes autism has been a decade-long debate, which could not be settled with animal tests or epidemiological analyses. So, we see mini-brains as technology for broader assessment of the risks of common drugs and chemicals, including those that might be contributing to the autism epidemic."

Hartung and colleagues recently received a grant from the U.S. Environmental Protection Agency to develop their technology as an alternative to animal testing. David Pamies, who was corresponding author of the paper, worked at the Center for Alternatives to Animal Testing during the study and is now at the University of Lausanne.

The study was supported by funding from the European Union's Horizon 2020 research and innovation program (grant No. 487 681002).

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

Australian Government Includes UL Chemoinformatics Tool Kit (Based on CAAT Research) as Appropriate *In Silico* Model

The Australian Government Department of Health has included the UL Cheminformatics Tool Kit as appropriate *in silico* models for seven human health hazard endpoints in the AICIS Categorisation Guidelines. The Industrial Chemicals Act 2019 or AICIS (Australian Industrial Chemicals Introductions Scheme) will replace NICNAS effective July 1, 2020. This law creates a new regulatory scheme for the importation and manufacture of industrial chemicals in Australia. The final draft of the new Guidelines now has added the Cheminformatics Tool Kit as appropriate *in silico* models for acute toxicity, skin irritation/corrosion, eye damage/irritation, and genetic toxicity.

Thomas Hartung is one of the developers of the technology underlying UL's Chemoinformatics Tool Kit, along with Craig Rowlands (UL) and Tom Luechtefeld (ToxTrack, formerly CAAT). More about the project may be found here: https://msc.ul.com/en/products/cheminformatics/meet-our-team/

Announcing: The Alan and Helene Goldberg In Vitro Toxicology Grants

2021-2022 Call for Pre-proposals
Now Open

The Johns Hopkins Center for Alternatives to Animal Testing (CAAT) is pleased to announce the establishment of the *Alan and Helene Goldberg In Vitro Toxicology Grants* program (formerly CAAT Grants Program). The program was renamed in 2019 to honor the Founding Director (Emeritus) of the center, Alan M. Goldberg, and his wife, Helene Goldberg (BSPH, MPH 1981). Alan is a professor of toxicology at the Bloomberg School of Public Health and was director of CAAT from its founding in 1981 until 2008. He then served as a Pew Commissioner on the study of the Impact of Industrial (US) Farm Animal Production on issues of public health, environment, animal welfare, and social justice, and was a coauthor of the Pew report *Putting Meat on the Table: Industrial Farm Animal Production in America*. He is currently principal of the Global Food Ethics Project at Johns Hopkins University, where he is developing a framework for ethical food systems. His new book, *Feeding The World Well: A Framework for Ethical Food Systems*, will be released this July from Johns Hopkins University Press.

The grants program is a centerpiece of our work, providing initial funding for scientists to develop alternatives to the use of animals in biomedical research and product safety testing. To date, the center has funded over 300 grants (including renewals) for a total of more than $6 million.

The Johns Hopkins Center for Alternatives to Animal Testing (CAAT) is soliciting projects that focus on the implementation of the NAS Report: *Toxicity Testing in the 21st Century: A Vision and a Strategy* in the following areas:

- Proposals Relating to Toxicology: Maximum grant amount is $40,000. The objective should be to significantly reduce or replace laboratory animals. Examples of acceptable projects could include: providing mechanistic understanding of *in vitro* responses to toxicants in human cells, development of AOPs, or conducting systematic reviews. Consideration should be given to the translation of this new method to evaluate/predict health outcomes.

Although relatively small individually, these grants offer critical seed money that allows researchers to demonstrate the value of a particular area of study so they can gain support from the NIH and other sources.

Full details and pre-proposal application: https://caat.jhsph.edu/programs/grants/index.html

2020 Next Generation Humane Science Award: Application Deadline May 31, 2020

This award is available annually to young scientists to acknowledge and encourage researchers who focus on replacing animal experiments. The 2020 award will provide a prize of up to $5,000 recognizing the work of one young scientist, or may be shared among two or more young scientists.

Qualification Criteria

The work must be focused on the replacement of animals used in experimentation and exhibit excellence of research outcome as demonstrated by publications and presentations at scientific meetings. The review committee will also take into account:

- The significance of the potential to replace animal experiments
- Inspiration to others (fellow students, members of the research group) and outreach to wider audiences

- The potential for the replacement methodologies to be used in a regulatory context

Eligibility Criteria

- The candidate must be a citizen or permanent resident of the United States working at a US-based institution.
- The candidate should not have received a PhD or similar degree earlier than 2010.
- Current and former employees (or their family members) of the Center of Alternatives to Animal Testing at Johns Hopkins University may not apply.

Apply Now! https://caat.jhsph.edu/humanescienceaward.html

CAAT and EBTC at the American Association for the Advancement of Science (AAAS) Annual Meeting

Members of CAAT and EBTC spoke at the American Association for the Advancement of Science annual meeting, which took place February 13-16 in Seattle. Thomas Hartung presented on *AI in Environmental Health: From Data Poverty to Factfulness and Evidence* as part of the session on Bionformatics and AI, while EBTC's Paul Whaley discussed *Knowledge Systems for the Anthropocene* in the same session. Alexandra Maertens spoke about *Known Unknowns and Unknown Unknowns: Ending Regrettable Substitutions* and Thomas Hartung spoke on *Green Toxicology: From Science to Solutions* as part of the Green Toxicology session.

Following the session on Green Toxicology, Alexandra Maertens and Andre Kleensang were interviewed about how Green Toxicology can improve our understanding of chemical hazards while using fewer animals.

Watch the interview here: https://youtu.be/JgDzVQDDkgU

Kathrin Herrmann Speaks on Animal Ethics at XIV International Seminar on the UNESCO Universal Declaration on Bioethics and Human Rights

Kathrin Herrmann spoke about animal ethics and issues surrounding animal experimentation at the XIV International Seminar on the UNESCO Universal Declaration on Bioethics and Human Rights, which was held February 13 at the University of Barcelona.

The conference analyzed articles of the Universal Declaration of Bioethics and Human Rights, and Herrmann's talk was entitled "The 3Rs in Practice: Are we doing what we can?" She discussed the scientific necessity of moving away from animal models, the shortcomings in the applications of the 3Rs, and the biggest hurdles that need to be taken in order to move away from animal use in science.

CAAT's Alexandra Maertens in Bioinformatics Ebook

An article by CAAT researcher Alexandra Maertens on the molecular mechanisms of bisphenol A toxicity is included in the upcoming ebook *Emerging Bioinformatic Tools in Toxicogenomics*. Dr Maertens and her team used computational tools to analyze the gene expression patterns of MCF-7 cells exposed to a dose response curve. The analysis pointed to candidates other than estrogen receptor activation as the likely molecular mechanisms – an important consideration for trying to design alternatives to bisphenol A.

Download the ebook (Epub format) here: https://tinyurl.com/wzgymgl

CAAT on LinkedIn

Please visit and follow CAAT's new page on LinkedIn. Just search for the center, or visit: https://tinyurl.com/ulhlzkv

Publications

These two publications exemplify drug discovery in the cancer field based on mechanistic assays using human cells.

Marques, L. B., Ottoni, F. M., Xavier Pinto, M. C. et al. (2020). Lapachol acetylglycosylation enhances its cytotoxic and pro-apoptotic activities in HL60 cells. *Toxicol In Vitro 65*, 104772. Epub ahead of print. doi:10.1016/j.tiv.2020.104772

Ripani, P., Delp, J., Bode, K. et al. (2019). Thiazolides promote G1 cell cycle arrest in colorectal cancer cells by targeting the mitochondrial respiratory chain. *Oncogene 39*, 2345-2357. doi:10.1038/s41388-019-1142-6

Upcoming Events

Thomas Hartung Keynote at Excipient World Conference and Expo

May 11-13, 2020
Kissimmee, FL

Excipient World showcases formulation and delivery system advances, regulatory and compliance initiatives, innovative product applications, manufacturing technologies and equipment, supply chain solutions, testing, contract manufacturing, and other manufacturing services year round, enabling professionals in pharmaceutical, biologic, veterinary medicine, combination medical device, and consumer healthcare manufacturing to meet consumer demands and remain competitive.

Thomas Hartung will be giving a keynote talk: Toxicology for the 21st Century: What is in the Box for Excipients? at 8am on Tuesday, May 12th.

Details and Registration: https://excipientworld.org/

7th Annual Symposium: 3Rs: Practical Solutions and Success Stories (formerly Social Housing Symposium)

June 4-5, 2020
USDA National Agricultural Library
Beltsville, MD

The 7th Annual 3Rs symposium, co-hosted by the USDA Animal Welfare Information Center (AWIC), NIH Office of Laboratory Animal Welfare (OLAW), the Johns Hopkins Department of Molecular and Comparative Pathobiology, and the Johns Hopkins Center for Alternatives to Animal Testing (CAAT), will be held June 4-5, 2020 in Beltsville, Maryland. The goal of this year's symposium is 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 their welfare.

The format includes 1.5 days of lectures and panel discussions with interactive breakout sessions in the afternoon on day two. These lectures give participants a strong foundation in the relevant research underlying breakthroughs in the 3Rs, while the breakout sessions allow participants to receive feedback specific to their own facilities from experts and colleagues. A half-day tour of labs and research centers at the Agricultural Research Service is planned for June 3, 2020 as an optional pre-symposium event.

https://www.eventbrite.com/e/7th-annual-symposium-3rs-practical-solutions-and-success-stories-tickets-89264060207

Summer School on Innovative Approaches in Science

June 22-25, 2020
Johns Hopkins University School of Public Health
Baltimore, MD

Hosted by CAAT, Physicians Committee for Responsible Medicine, and The European Commission Joint Research Centre

Calling all students and early-career scientists interested in innovative approaches in toxicology and biomedical sciences

In response to a growing need to be conversant in innovative approaches in toxicology and biomedical sciences, this Summer School will share knowledge and experience with a new generation of scientists on research and testing methods. The program will highlight modern alternatives to the use of animals – including *in vitro* and computational modeling – in toxicology and biomedical sciences. The state of scientific research will be explored through lectures and discussion.

The Summer School will be separated into two tracks: toxicology and biomedical science. The program will combine lectures from experts in the field of toxicology and biomedical science with interactive sessions to encourage discussion and facilitate networking among participants.

This program will offer:

- Information about modern alternatives to the use of animals in toxicology and biomedical sciences
- Diverse presentations from all major stakeholders
- Opportunity to present your work at a poster session
- Welcome and networking reception dinners
- Laboratory visits
- Travel awards and more

Who can participate? This Summer School is geared towards students and early-career scientists who are interested in working with non-animal approaches and their application in various fields such as toxicology and biomedical science.

The program will incorporate a variety of sessions including joint and track specific lectures. Students and early-career researchers can choose between the toxicology or the biomedical science track.

In general, the toxicology track is designed for post-graduate students and early-career scientists and the biomedical science track may be of interest for undergraduate students through early-career scientists.

Costs: No registration fee will be charged. Participants will need to cover the costs of their travel, accommodation, and daily subsistence.

Full Details: https://www.ascctox.org/innovativescience2020

Cruelty Free INTERNATIONAL
Ending animal experiments worldwide

EU Commission publishes new animal testing statistics

On February 7, 2020, the European Commission published a new report detailing the total number of animal experiments that took place in the European Union (EU) between 2015 and 2017 including a summary of the number of experiments conducted in each member state.

Between 2015 and 2017, the total number of experiments on animals in European laboratories decreased by 2% from 9.78 million in 2015 to 9.58 million in 2017. However, there was an increase to 10.03 million in 2016. This means that almost 30 million animal experiments were conducted over a 3-year period in the EU.

The United Kingdom has emerged as the country that conducts the highest number of animal experiments in Europe (2.6 million in 2017), replacing Germany. Germany remains the second highest user, completing 2.1 million experiments in 2017, while France is third with 1.9 million experiments that year.

Despite increased public demand for a reduction in animal research, there has been little change since the previous report was published. Cruelty Free International is calling on European governments to listen to public demand and act urgently to make real progress in reducing animal testing numbers.

New estimate of worldwide laboratory animal use published

Cruelty Free International has published the most up-to-date, accurate and comprehensive estimate of the number of animals used in scientific experiments worldwide, ten years after the animal protection organisation's previous, widely cited 2005 estimate.

Based on data from the 37 countries that publish national statistical reports and a prediction model using publication rates to estimate animal use in a further 142 countries, 79.9 million experiments on animals are estimated to have been performed worldwide in 2015, a 37% increase on the 58.3 million animal experiments estimated for 2005. While some of the increase can be explained by the improved accuracy of the prediction model and recent changes to reporting requirements in the European Union, there has also been an increase in animal experiments in some of the reporting countries.

The estimate was further extrapolated to obtain a more comprehensive final global figure for the number of animals used for scientific purposes in 2015, of 192.1 million animals, compared to 115.2 million in 2005. As well as animals used in experiments, this figure also includes animals killed for their tissues, animals used to maintain genetically modified animal strains and animals bred for experiments but not used or killed as surplus.

The findings show that the top ten animal users in 2015 were China (20.5 million experiments), Japan (15.0 million experiments), the United States (14.6 million experiments), Canada (3.6 million experiments), Australia (3.2 million experiments), South Korea (3.1 million experiments), Brazil (2.2 million experiments), the United Kingdom (2.6 million experiments), Germany (2.0 million experiments) and France (1.9 million experiments).

USDA publishes 2018 animal research statistics

According to the latest US Department of Agriculture (USDA) annual report, which was published in January, a total of 780,070 animals were used in US research in 2018, a slight reduction of 1.5% since 2017.

Massachusetts is the top user of animals for research in the US, testing on 82,177 animals in 2018 (9% increase since 2017), followed by California with 68,337 animals (6% increase), and New Jersey with 62,862 animals (2% decrease).

The USDA does not include tests on mice, rats, fish or birds, even though these animals are known to be used in millions of experiments. As a result, the true number of animals used in US laboratories is estimated to be in the tens of millions.

Last year, the US Environmental Protection Agency announced it would prioritize efforts to reduce animal research with the goal of eliminating toxicity tests on mammals by 2035. Cruelty Free International is calling on US authorities to set similar targets and timetables across the board.

HEARTS Act congressional briefing held in Washington DC

On February 13, Cruelty Free International hosted a congressional briefing in the US House of Representatives in Washington DC entitled "Advancing Humane Science in NIH-funded Research" that highlighted the importance of the HEARTS Act (H.R. 1209) to increase the use of non-animal alternatives.

Cruelty Free International's Senior Science Advisor Laura Alvarez was a featured speaker and was joined by Dr Paul Locke of Johns Hopkins Bloomberg School of Public Health. Their presentations outlined some of the modern non-animal research methods that are increasingly available to researchers, highlighted the barriers that can prevent these humane methods from being used, and explained how these challenges could be overcome with help of the HEARTS Act.

The briefing was hosted in conjunction with Representatives Lucille Roybal-Allard (D-CA) and Ken Calvert (R-CA), sponsors of H.R. 1209. Both Representatives Roybal-Allard and Calvert attended the briefing and spoke about the importance of continuing to move away from animal-based research and embracing new non-animal methods.

Review on computer-based approaches to replace animal tests

Cruelty Free International has published a review in *Computational Toxicology* that highlights the accelerating development of computer-based techniques to replace animal tests over the past two decades.

The review focuses on the regulatory and scientific drivers that have encouraged the development of computer-based approaches to replace animal testing for cosmetics and considers key legislative and policy changes in Europe, North America and at the OECD over the last 20 years as contributing factors.

In Europe, public opinion and ethical considerations were considered to be the key drivers of legislative changes, while in North America efforts towards the development of computer-based techniques have been made largely in response to economic and scientific factors, rather than public opinion or ethics.

Poll reveals almost 90% of Canadians support end to cosmetic animal testing

According to a new nationwide poll conducted by SurveyUSA on behalf of Cruelty Free International, 88% of Canadians would support a federal law to end cosmetics tests on animals. Support for a ban crosses party lines and generations.

The poll also showed that 82% of participants feel that Canada should take a global leadership role to help achieve a worldwide end to animal testing in cosmetics, whilst 84% of respondents believe corporate organisations should ensure that they do not test their products on animals.

In 2017, Cruelty Free International and The Body Shop launched a campaign to end the sale and manufacture of animal-tested cosmetics in Canada. This campaign has brought awareness to the Canadian public about the need to end cosmetic animal testing and helped Bill S-214 (The Cruelty-Free Cosmetics Act) get to Second Reading in the House of Commons – the furthest any bill on this issue has ever made it in the legislative process in Canada. Unfortunately, the bill ran out of parliamentary time just before the 2019 election. Cruelty Free International is calling on legislators to bring this bill back and to end cosmetic animal testing with government legislation.

LPT headquarters is denied permission for new animal tests and has husbandry licence removed

Last year, Cruelty Free International and SOKO Tierschutz released the results of a joint investigation into the Laboratory of Pharmacology and Toxicology (LPT) animal testing facility in Mienenbüttel in Germany, which revealed shocking levels of animal suffering and breaches of domestic and European law.

The investigation generated widespread international media coverage and worldwide outrage, resulting in the closure of the Mienenbüttel site in February and the rehoming of the facility's dogs, cats and monkeys.

In an expert hearing on February 7, it was announced that LPT's headquarters in Neugraben would not receive any new permits for animal experiments and that any previous authorizations for animal experiments would be withdrawn.

One week later, Hamburg's Authority for Health and Consumer Protection announced that LPT Neugraben would be joining LPT Mienenbüttel in having its animal holding license removed with immediate effect. They also called on the German government to improve efforts to uphold EU laws governing animals in laboratories.

References

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Taylor, K. and Rego Alvarez, L. (2020). Regulatory drivers in the last 20 years towards the use of in silico techniques as replacements to animal testing for cosmetic-related substances. *Comput Toxicol 13*, 10112. doi:10.1016/j.comtox.2019.100112

EUSAAT

European Society for Alternatives to Animal Testing

EUSAAT activities in 2020

EUSAAT has been hosting the international EUSAAT Congresses in Linz/Austria since 1992. In order not to interfere with the World Congresses on Alternatives and Animal Use in the Life Sciences, EUSAAT does not hold congresses in years in which World Congresses are scheduled. Thus, we held EUSAAT Congresses in 2018 and 2019, and the next one is planned for 2021.

In the meantime, the EUSAAT Board will continue to focus its activities on "*Establishing a European 3Rs-Center Network*" and will actively support WC11 in August 2020 in Maastricht/NL by sponsoring a workshop on "*3Rs centers around the world and their role in fostering the implementation of 3Rs in academia*".

The EUSAAT initiative to establish a European network of 3Rs centers

The purpose of this network is to bring European 3Rs centers 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 centers and societies at the EUSAAT conference in September 2018 in Linz, the first follow-up meeting was hosted by *Freie Universität Berlin* in March 2019.

Major common aims were identified: to further advance the 3Rs, to help implement the aims of Directive 2010/63/EU locally, and to reach out and connect with scientists in basic research. The network could be used as a platform to exchange experience on a variety of topics, for example, how the various 3Rs centers and societies were established, how they organize events, how they secure funding, which teaching strategies and resources they use to implement the 3Rs in education, etc.

During the discussions it became clear that the diversity of the members could be the strength of the network, since they cover many different topics and have experts on refinement, reduction and replacement of animal experiments.

Topics with an urgent need were identified and working groups for these topics were defined. The progress of these working groups was presented at the third meeting held during the FELASA conference in June 2019. In October 2019, at the fourth meeting at the EUSAAT 2019 conference in Linz, future initiatives were decided and agreed upon.

So far, members from over 25 countries have joined and the network is growing. The network is an entirely independent, open and free community, which is very much driven by initiatives of its protagonists and personal efforts. It is based on a bottom-up approach, and every 3Rs center or society is welcome to join.

Coordinator: Winfried Neuhaus
(winfried.neuhaus@ait.ac.at)
AIT – Austrian Institute of Technology GmbH, Center Health and Bioresources, Competence Unit Molecular Diagnostics, Giefinggasse 4, 1210 Vienna, Austria

3Rs centers around the world and their role in fostering the implementation of 3Rs in academia *Workshop (S200) sponsored by EUSAAT at WC11 in Maastricht/NL*

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 the various centers may face within their countries. In addition, we will discuss possible synergies and collaborative activities that can help further the implementation of 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 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)

In February, the yearly core event of the EU-ToxRisk project was again held in Egmond aan Zee. The 3rd EU-ToxRisk Open Symposium was extremely successful. It counted more than 100 participants, who traveled to the Netherlands to discuss relevant issues of New Approach Method (NAM)-based regulatory strategies despite the challenging storm Sabine/Ciara. The meeting has established its role as an effective platform for industry, regulatory and academic stakeholders from different parts of the world and with different regulatory background, who participate to discuss practical issues related to next-generation risk assessment (NGRA).

This year, the program benefited from presentations by external stakeholders coming from industry partners and regulatory agencies. Their contributions significantly enriched the discussion, offering an overview of concrete benefits, gaps, and issues related to real-life examples of the use of RAx justifications in risk assessment. A wide and international perspective on the application of NGRA frameworks was also offered, thanks to the participation of experts from diverse regulatory organizations, i.e., the Japanese NIHS, the US NIEHS and the European EFSA.

On this occasion, EU-ToxRisk experts also presented the regulatory and scientific steps towards the finalization of the EU-ToxRisk Advisory Document on a new approach method (NAM)-enhanced read-across (RAx). The approach detailed in the advisory document has been examined by the OECD with altogether positive feedback. The consortium plans to finalize the advisory document and the annexed EU-ToxRisk *in silico* RAx toolbox early next year.

Finally, interaction and involvement of interested stakeholders with the EU-ToxRisk NAM-based Testing Commercialization Platform was promoted. The platform is based on an EU-ToxRisk partnership aiming to translate the expertise of next-generation predictive toxicology and safety assessment into the premium place for customers to go with their needs and problems in product safety design and management.

EU-ToxRisk publications

The advisory boards and stakeholders were extremely positive about the recent scientific highlight of the project and its progress towards the establishment and validation of NGRA. Some examples of the newly developed tools can be found in the latest EU-ToxRisk publications. In Kranaster et al. (2020), the authors describe a novel approach to quantify synthesis, localization, and pool sizes of carbohydrates during physiological responses and toxicological stress. This unique approach, based on metabolic glycoengineering (MGE), allows the identification of subtle impairments of neurochemical function with very high sensitivity. In Ballester et al. (2020), a strategy was described to generate an unlimited source of homogenously induced hepatocyte-like cells from donors with different genetic backgrounds that perform typical hepatic functions, making them suitable for drug research and other *in vitro* applications. Human fibroblasts were reprogrammed into induced hepatocyte-like cells through the expression of a set of transcription factors via a lentiviral vector. Yang et al. (2020) used microscopy-based quantification and dynamic modeling to detect the molecular actors (ATF6) central to the activation of adaptive stress responses in HepG2 reporter cell lines.

Finally, a consensus report on the application of microphysiological systems (MPS) was recently published by Marx et al. (2020). In June 2019, 46 leading experts from all stakeholders – academia, MPS supplier industry, pharmaceutical, consumer products industries, and leading regulatory agencies met in Berlin to agree on a roadmap for promoting industrial adoption of MPS by end-users. The experts highlighted the potential of MPS-based human disease models to feedback into laboratory animal replacement in basic life science research.

Outlook

The recently launched EU-ToxRisk Testing Commercialization Platform will be promoted at the 21st international congress organized by the European Society of Toxicology in Vitro (ESTIV, June 8-11, 2020, Barcelona). On this occasion, the partners will present the platform and the related offer to all interested customers.

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Marx, U., Akabane, T., Andersson, T. B. et al. (2020). Biology-inspired microphysiological systems to advance patient benefit and animal welfare in drug development. *ALTEX*, Epub ahead of print Feb 28. doi:10.14573/altex.2001241

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Giorgia Pallocca and Marcel Leist