Corners

AxoSim Licenses Revolutionary Mini-Brain Technology from Johns Hopkins University Enabling the Use of Human Data to Speed New Drug Research

AxoSim, Inc., the premier provider of neuroscience discovery platforms that mimic the structure and function of the human nervous system, has announced an exclusive license from Johns Hopkins University to intellectual property underlying the "Mini-Brain" technology, which uses induced pluripotent stem cells (iPSCs) to create functional models of the human brain. Mini-Brain technology enables scientists to study key brain functions, test new therapies, and screen for toxic substances in human rather than animal models at an early stage of research.

Almost 90% of drugs that look promising in animal models fail once they are tested in humans, driving up the average cost and time to develop a new drug to an estimated $2.6 billion and more than 10 years. The problem is especially acute for neurological disorders such as Alzheimer's disease, amyotrophic lateral sclerosis and multiple sclerosis, where animals are notoriously poor predictors of human outcomes and better therapies are urgently needed. The recent costly clinical trial failures of new Alzheimer's drugs highlight the need for better ways to test drug candidates much earlier in development.

The Mini-Brain technology was developed to replace inaccurate animal testing with accurate, predictive models of the human brain. It has the potential to reduce the time and cost of new drug R&D for disorders such as multiple sclerosis, glioblastoma, Alzheimer's disease, infectious diseases, and others, in addition to its applications in toxicology. Related Nerve-on-a-Chip® technology developed at AxoSim has been shown to achieve research milestones at a fraction of the time and cost of conventional animal testing.

In a related development, AxoSim announced that Dr Thomas Hartung, who led the team that invented the Mini-Brain intellectual property, will serve as a Consulting Vice President of Scientific Affairs. Dr Hartung is the Doerenkamp-Zbinden Chair for Evidence-based Toxicology and Director of the Center for Alternatives to Animal Testing at the Johns Hopkins Bloomberg School of Public Health. The company also reported that it has acquired all the assets of Organome, Inc., a company founded by Dr Hartung to commercialize the Mini-Brain technology and other functional organ equivalents.

Full press release: https://us4.campaign-archive.com/?u=066d5d7abe2de2d5e04d214bf&id=98da26686f

CAAT and Hopkins Researchers Identify Neurotransmitter that Helps Cancers Progress

Using human cancer cells, tumor and blood samples from cancer patients, researchers at CAAT and Johns Hopkins Medicine have uncovered the role of a neurotransmitter in the spread of aggressive cancers. Neurotransmitters are chemical messengers that transmit impulses from neurons to other target cells.

The work, described in *Cell Reports* (see below), found that the neurotransmitter N-acetyl-aspartyl-glutamate (NAAG) is more abundant in cancers with a tendency to grow and spread rapidly, i.e., so-called higher grade cancers, than in lower grade tumors, making it a potential marker for tumor progression or regression during cancer therapy. The experiments also demonstrated that NAAG is a source of glutamate in tumors that express the enzyme glutamate carboxypeptidase II (GCPII). Inhibiting GCPII with 2-PMPA to treat human ovarian tumors implanted in ovaries of mice reduced tumor weights and glutamate concentrations. They noted that targeting both GCPII and glutaminase, the enzyme that converts glutamine to glutamate, resulted in a more substantial tumor reduction in patient-derived pancreatic cancer tumors implanted in pancreas of mice, since it attacked the production of glutamate from both glutamine and from NAAG.

CAAT's Thomas Hartung and Andre Kleensang co-authored the paper.

Full press release: https://www.eurekalert.org/pub_releases/2019-04/jhm-hri042319.php

Nonanimal Models for Acute Toxicity Evaluations: Applying Data-Driven Profiling and Read-Across

The use of animals to test the toxicity of chemicals may one day become outdated thanks to a low-cost, high-speed algorithm developed by researchers at Johns Hopkins, Rutgers, and other universities.

Toxicity testing – determining the amount of exposure to a chemical that is unsafe for humans – is vital to the safety of millions of workers in various indus-

tries. But of the 85,000 compounds used in consumer products, the majority have not been comprehensively tested for safety. Animal testing, in addition to its ethical concerns, can be too costly and time consuming to meet this need, according to the study published in *Environmental Health Perspectives*.

"There is an urgent, worldwide need for an accurate, cost-effective and rapid way to test the toxicity of chemicals, in order to ensure the safety of the people who work with them and of the environments in which they are used," Daniel Russo, lead researcher and a doctoral candidate at the Rutgers University-Camden Center for Computational and Integrative Biology, said. "Animal testing alone cannot meet this need."

Previous efforts to solve this problem used computers to compare untested chemicals with structurally similar compounds whose toxicity is already known. But those methods were unable to assess structurally unique chemicals – and were confounded by the fact that some structurally similar chemicals have very different levels of toxicity.

The Rutgers-led group overcame these challenges by developing a first-of-its-kind algorithm that automatically extracts data from PubChem, a National Institutes of Health database of information on millions of chemicals. The algorithm compares chemical fragments from tested compounds with those of untested compounds, and uses multiple mathematical methods to evaluate their similarities and differences in order to predict an untested chemical's toxicity.

Thomas Hartung co-authored the paper, which appeared in *Environmental Health Perspectives*, see below.

AI Beats Animal Testing at Finding Toxic Chemicals (The Scientist)

CAAT Director Thomas Hartung recently penned an editorial for *The Scientist*.

Excerpt:
There are more than 100,000 chemicals in consumer products. For the vast majority, there is very little information about their toxicity. Traditionally, researchers will test chemicals of interest in animals. As an extreme example, a pesticide undergoes about 30 animal tests, costing about $20 million and consuming more than 10,000 mice, rats, rabbits, and dogs over five years. About 20 kilograms of the chemical are needed for this testing; obtaining such a volume can be quite a challenge for a substance not yet on the market. Other substances receive less scrutiny, but even products with lower regulatory standards, such as industrial chemicals, can require $5 million worth of animal testing before entering the marketplace.

Our group, the Center for Alternatives to Animal Testing (CAAT) at Johns Hopkins University, sought a better way. As so many biologists are doing these days, we turned to intelligent computer programs for help. We showed that artificial intelligence (AI) could mine existing data on chemical toxicity and generate new information. In 2016, we compiled a database of 800,000 toxicological studies on more than 10,000 chemicals registered under the European REACH legislation for industrial chemicals, and used it to feed an advanced predictive algorithm that enabled us to predict the toxicity of any chemical without setting foot in the animal lab.

The software takes advantage of the power of big data and transfer learning, a machine learning method that applies information from one task or set of items to another. Similar chemicals have similar properties. Based on that principle, the software builds a map of the chemical universe. Similar chemicals are put close to each other, dissimilar ones more distant. Then, the model can place new chemicals on the map, assess what is known about their neighbors, and from that information surmise their potentially harmful health and environmental effects. The more data are fed into the model, the more powerful it becomes.

Full Article *(The Scientist)*: https://www.the-scientist.com/critic-at-large/opinion--ai-beats-animal-testing-at-finding-toxic-chemicals-65795

Science-Based Refinement Awards

CAAT's Science-Based Refinement Awards provide funding for strictly non-invasive research that helps to refine the housing, handling, and/or experimental situations for laboratory animals or that has the potential to reduce the number of animal subjects used, e.g., by critically appraising animal models and by enhancing transparency in animal experimentation. For 2019, two awards for $5,000 were presented to:

- Constança Carvalho, Centro de Filosofia das Ciências da Universidade de Lisboa, Portugal for *Citation analysis on the contribution of rat models to our current understanding of Major Depressive Disorder (MDD)*
- Steven Chamuleau, Department of Cardiology, UMC Utrecht, The Netherlands for *Promoting transparency in preclinical research through the establishment of a preregistration platform for animal studies*

For more about CAAT's Science-Based Refinement Awards and how to apply, visit: http://caat.jhsph.edu/programs/awards/AWE/index.html

Thomas Hartung Appointed Editor of Frontiers in Artificial Intelligence

Big Data and Artificial Intelligence (AI) are reshaping our world, revolutionizing the way we work and live. Last year, Frontiers launched *Frontiers in Artificial Intelligence*. The journal publishes a broad range of topics in AI by bridging gaps between disciplines. *Frontiers in Artificial Intelligence* will be led by Thomas Hartung, Director of CAAT and professor of toxicology at the Johns Hopkins Bloomberg School of Public Health.

"AI is about making sense of Big Data, which is relevant in all sciences," Hartung explains. "It complements hypothesis-driven research by moving data mining from a fishing expedition to an intelligent query of assembled facts."

"Scientists on the editorial board supporting *Frontiers in Artificial Intelligence* are foremost experts in their disciplines," Hartung continues. "AI is such a cross-cutting technology – it is fascinating how this disruptive and enabling technology fertilizes so many disciplines. Researchers contributing to this area demonstrate true courage. They cross those boundaries to collaborate with each other and find an-

swers to common questions from sharing data and algorithms, to quality assurance, to ethics."

Frontiers in Artificial Intelligence (Journal): https://www.frontiersin.org/journals/artificial-intelligence

Marcel Leist in *Der Spiegel* (German)

CAAT Co-director Marcel Leist was quoted in *Der Spiegel* on how scientists are looking for ways to reduce animal research.

PDF in German: http://caat.jhsph.edu/media/HeileMause.pdf

CAAT Student Vy Tran Receives CERSI Scholar Award

CAAT student Vy Tran has been awarded travel and research funding as a CERSI Scholar at the Center of Excellence in Regulatory Science and Innovation at the Johns Hopkins Bloomberg School of Public Health. CERSI leverages the geographic proximity and extensive relationship between Johns Hopkins and the FDA and uses this as a foundation to promote a programmatic focus on three priority areas for the agency: improving clinical studies and evaluation; strengthening the social and behavioral sciences to support informed decisions; and developing a new prevention-focused food safety system.

Upcoming Events

5th International Conference on Developmental Neurotoxicity (DNT) Testing (DNT5)

April 6-8, 2020
Konstanz, Germany

The effects of chemical exposure on the susceptible developing human nervous system can cause severe lasting neurological deficits.

This conference will bring together diverse stakeholders from around the globe, including research scientists, regulators, industry representatives, academics, and pediatricians to discuss the actions to take for improving the development of time- efficient and human-relevant predictive *in vitro* DNT methods, and boosting their use in the risk assessment regulatory decision-making process.

Details: https://www.uni-konstanz.de/en/dnt5/about-the-event/

Thomas Hartung to Deliver Keynote at American College of Toxicology Annual Meeting

Thomas Hartung will deliver the keynote talk at the 2019 American College of Toxicology Annual Meeting on November 19 in Phoenix, Arizona. Hartung's talk is *From Microphysiological to Micropathophysiological Models*. The meeting takes place from November 17-20.

This November gathering brings together a community of toxicologists at small venues conducive to idea exchange, professional networking, and continuing education. The scientific sessions are member driven, pharma-focused (but not pharma-exclusive), and organized to maximize learning opportunities. The 40th Annual Meeting will include a wide-ranging scientific program, a welcome reception and dinner, the popular traditional poster session reception, an awards ceremony and luncheon, distinguished plenary speakers, and numerous professional networking events.

Recent events

Intersection of Chemistry and Toxicology

Databases, software, machine learning, and online marketplaces are increasingly used by chemists and toxicologists to identify and/or design safer chemicals and materials. This day-long symposium, held April 30, 2019 in Washington, D.C., provided CSW and NCAC-SOT members with updates on advancements in each of these tools, identified what improvements are needed, and discussed strategies for increasing worldwide adoption of such tools. Recognized experts from industry, NGOs, academia, and government presented their perspectives on this topic.

Thomas Hartung presented a talk on *Read-across-based structure activity relationships (RASAR) – a new kid on the block of chemical safety assessment*.

CAAT Presentations at JRC Summer School on Non-Animal Approaches in Science

CAAT Refinement program director Kathrin Herrmann and Francesca Fagiani, a visiting scholar from the University of Pavia, presented at the recent JRC Summer School on Non-Animal Approaches in Science (May 21-24) in Ispra, Italy. Fagiani presented a poster entitled "CHD8 knockout BrainSpheres and chlorpyrifos to study gene environmental interactions in autism" and Herrmann presented CAAT's new Humane Science Course, which covers not only the 3Rs but also teaches students about the limitations of animal use in science and how to critically appraise the validity of animal and non-animal models and methods in order to choose the best means for particular research interests.

Costanza Rovida at Chemical Watch Biocides Symposium

CAAT-Europe's Costanza Rovida discussed "Application of New Approach Methodologies (NAMs) in the definition of endocrine disruptor properties of biocide substances" during the breakout session on ED criteria at the Chemical Watch Symposium in Rome on May 24th, 2019.

Workshop: Assuring the Quality of Systematic Reviews Published in Toxicology and Environmental Health Journals

This workshop, held May 29-30 at Research Triangle Park, brought together editors to develop a joint strategy that will assure the quality of the systematic reviews published in environmental health and toxicological sciences journals. This workshop was funded by t4, the Transatlantic Think Tank for Toxicology, a collaboration of the toxicologically oriented chairs in Baltimore, Konstanz, and Utrecht sponsored by the Doerenkamp-Zbinden Foundation and the Evidence-based Toxicology

Collaboration (EBTC) at Johns Hopkins University Bloomberg School of Public Health (USA).

Public EBTC Workshop: Application of Evidence-based Methods to Construct Mechanistic Frameworks for the Development and Use of Non-animal Toxicity Tests

Systematic evidence synthesis methods and adverse outcome pathways are two relatively recent arrivals in the toxicologist's toolbox. The goal of this workshop, held June 12, 2019 at McMaster University in Ontario, was to explore, via discussion of four related themes, how systematic review methods and AOP concepts could be combined to develop and use non-animal test methods for predicting the toxicity of chemical substances in an evidence-based manner.

CAAT-Europe Information Day on Biology-inspired Microphysiological Systems (MPS) to Advance Medicines for Patients' Benefit

Co-organized with the Centre for Entrepreneurship (CfE) of the Technische Universität Berlin

The Information day, held on June 17, 2019 in Berlin, hosted key international experts from academia, regulatory agencies, and industry. Microfluidic microphysiological systems (also referred to as organs-on-a-chip) are considered an enabling technology for the development of approaches to reliably predict the safety and efficacy of novel drug candidates prior to their use in humans. A transatlantic toxicology think tank involving academia, industries, and regulatory bodies from all over the world reviewed the status quo of MPS in June 2015 in Berlin (Marx et al., 2016, *ALTEX 33*, 272-321). Now, four years later, stakeholders met again in Berlin to update the review and to examine the roadmap for the reduction and replacement of animals by MPS tools for precision benefits for patients.

EBTC's Katya Tsaioun at World Pharma Week

Katya Tsaioun spoke at the World Pharma Week in Boston on June 20, 2019, where she showed data from the EBTC modeling project on predicting drug liver toxicity using systematically reviewed published literature, the public US EPA ToxCast database, and adverse events databases. She highlighted the quality and detail of the data in published literature and other public and proprietary databases.

New Publications

Albrecht, W., Kappenberg, F., Brecklinghaus, T. et al. (2019). Prediction of human drug-induced liver injury (DILI) in relation to oral doses and blood concentrations. *Arch Toxicol*, Epub ahead of print. doi:10.1007/s00204-019-02492-9

Delp, J., Funke, M., Rudolf, F. et al. (2019). Development of a neurotoxicity assay that is tuned to detect mitochondrial toxicants. *Arch Toxicol*, Epub ahead of print. doi: 10.1007/s00204-019-02473-y

Finke, A., Schneider, A. K., Spreng, A. S. et al. (2019). Functionalized DNA hydrogels produced by polymerase-catalyzed incorporation of non-natural nucleotides as a surface coating for cell culture applications. *Adv Healthc Mater 8*, e1900080. doi: 10.1002/adhm.201900080

Gerding, H. R., Karreman, C., Daiber, A. et al. (2019). Reductive modification of genetically encoded 3-nitrotyrosine sites in alpha synuclein expressed in E.coli. *Redox Biol 26*, 101251. doi:10.1016/j.redox.2019.101251.

Leite, P. E. C., Pereira, M. R., Harris, G. et al. (2019). Suitability of 3D human brain spheroid models to distinguish toxic effects of gold and poly-lactic acid nanoparticles to assess biocompatibility for brain drug delivery. *Part Fibre Toxicol 3*, 22. doi:10.1186/s12989-019-0307-3

Nguyen, T., Kirsch, B. J., Asaka, R. et al. (2019). Uncovering the role of N-acetyl-aspartyl-glutamate as a glutamate reservoir in cancer. *Cell Rep 27*, 491-501.e6. doi:10.1016/j.celrep.2019.03.036

Russo, D. P., Strickland, J., Karmaus, A. L. et al. (2019). Nonanimal models for acute toxicity evaluations: Applying data-driven profiling and read-across. *Environ Health Perspect 127*, 047001. doi:10.1289/EHP3614

Wolffe, T. A. M., Whaley, P., Halsall, C. et al. (2019). Systematic evidence maps as a novel tool to support evidence-based decision-making in chemicals policy and risk management. *Environ Int 130*, 104871. doi:10.1016/j.envint.2019.05.065

Cruelty Free INTERNATIONAL
Ending animal experiments worldwide

Concern over additional animal tests requested in US FDA's over-the-counter sunscreen proposal

The US Food and Drug Administration (FDA) has issued a proposal for a final monograph for non-prescription, over-the-counter (OTC) sunscreen drug products. As part of the proposal, the FDA has requested additional data on 12 out of 16 substances that are commonly used in sunscreens to ensure that they are generally recognized as safe and effective (GRASE).

In our opinion there is a disproportionate number of animal safety tests being requested, including several developmental studies in rodents and non-rodents as well as 2-year carcinogenicity studies. These data requests go far beyond what is required in Europe for testing chemicals including cosmetic substances and, indeed, pharmaceuticals in the USA, Japan and EU (according to ICH guidelines). Furthermore, a proportion of the requested data already exists as a consequence of the EU REACH Regulation and is available on the REACH database. In May, Cruelty Free International submitted comments on the proposal, urging the FDA to review the existing data for all 12 sunscreen ingredients and reevaluate the scientific need for additional animal tests, prior to finalizing the monograph.

The Estée Lauder Companies announces plans to go cruelty free

On June 20, Cruelty Free International announced a new collaboration with global cosmetics firm The Estée Lauder Companies, one of the world's leading manufacturers and marketers of skincare, haircare and makeup products, to achieve a worldwide end to animal testing for cosmetics.

The Estée Lauder Companies will support Cruelty Free International's efforts to encourage leaders to embed cruelty-free consumption and production measures in the United Nation's Sustainable Development Goals – a collection of targets set by the UN General Assembly for the year 2030 to achieve a better and more sustainable future.

As a demonstration of its commitment, the Estée Lauder Companies will also begin its journey of Leaping Bunny cruelty free certification, starting with the certification of some of its brands.

Appeal success at ECHA to prevent conduct of unnecessary animal test

Last year, Cruelty Free International, on behalf of the European Coalition to End Animal Experiments (ECEAE), successfully applied to intervene in a Board of Appeal (BoA) case at the European Chemicals Agency (ECHA).

The case related to a request from ECHA to German chemical company Brüggemann to conduct a carcinogenicity test on an industrial substance using at least 400 rats despite an update to the registration dossier demonstrating that there was no strong reason to conduct the test. Because the update was submitted after an arbitrary deadline set by the agency, the company's arguments were ignored by the agency. ECEAE supported the case brought to the BoA, arguing that animal welfare should not be overlooked in favor of bureaucracy. In April, the BoA ruled that ECHA had been unfair in not looking at the updated dossier and had been overly bureaucratic in setting their cut-off. It is now unlikely that the carcinogenicity test will be conducted.

Successes with State Cosmetics Legislation builds momentum for US federal ban

This year, Cruelty Free International backed legislation in multiple states across the US aimed at restricting animal testing for cosmetics.

In June, Nevada became the second state after California to ban the sale of animal-tested cosmetics and a similar bill in Illinois is on the governor's desk awaiting signature. The new law will prohibit the sale of any cosmetic product that has been tested on animals after January 1, 2020.

In addition to Nevada and Illinois, bills aiming to prohibit the sale of new animal tested cosmetics were also introduced (or reintroduced) in New York, Hawaii, Virginia, Maryland, Connecticut and New Jersey.

US history has shown that state activity often leads to change at the federal level. Cruelty Free International's state efforts are now serving as a catalyst to bring stakeholders to the table and is informing a forward for federal legislation, which has failed to gain traction in previous years.

HEARTS Act introduced to prioritize the use of non-animal methods in US NIH funded research

Cruelty Free International is championing the Humane and Existing Alternatives in Research and Testing Sciences Act [The HEARTS Act (H.R. 1209)], introduced by Rep. Lucille Roybal-Allard (D-CA) and Rep. Ken Calvert (R-CA) earlier this year.

While federal regulations and guidelines state that researchers should consider methods that can avoid or minimize animal use, US law does not require that experimenters use available alternatives. Millions of animals are used in research experiments annually in the US and over 12 billion tax-payer dollars are spent in experiments funded by the National Institutes of Health (NIH).

The bill would amend the Health Research Extension Act of 1985 to:

- Provide meaningful incentives for the use of scientifically satisfactory non-animal alternatives in research proposals seeking NIH funding.
- Require that investigators fully evaluate non-animal methods using standardized search guidelines.
- Ensure that research proposals are reviewed

by at least one person with expertise in non-animal research methods and that reviewers have access to a reference librarian with expertise in evaluating the adequacy of the search methods used for alternatives.
– Require harm-benefit analyses of proposed animal studies.

US residents can help advance the HEARTS Act by contacting their US Representative and asking that they become a cosponsor of the bill: https://e-activist.com/page/41727/action/1?ea.tracking.id=web

Letter on the problems associated with CRISPR-mediated gene editing

Trends in Biotechnology has published a letter by Dr Jarrod Bailey, Senior Research Scientist at Cruelty Free International, which summarizes the many scientific and ethical issues with the use of clustered regularly interspaced short palindromic repeats (CRISPR)-mediated genetic modification (GM) (Bailey, 2019).

The CRISPR technique is becoming an increasingly popular tool for gene editing because it is an inexpensive, relatively simple and quick way of producing GM animals. It has also been claimed to not only be more accurate than traditional methods but to actually help reduce animal numbers (because it is so accurate that fewer animals might be wasted). However, there remains substantial evidence to warrant great concern over the poor efficiency of CRISPR. In his Letter, Dr Bailey argues that there is evidence that the efficiency of CRISPR to "knock-in" a particular gene in an animal is less than 4%, while the efficiency to "knock-out" a gene is around 7%. Furthermore, it appears that CRISPR introduces unwanted or "off-target" mutations and while the degree is open to question, these unintended genetic modifications can have serious consequences for animal welfare and wastage.

BCLAS symposium on non-technical summaries

The 43rd annual Symposium of the Belgian Council for Laboratory Animal Science was held in Blankenberge on May 20-21, 2019. The theme of this year's symposium was "Be prepared to PREPARE (guidelines)" and it included several workshops and presentations on issues relating to transparency and animal welfare that were mainly aimed at animal caretakers and technicians.

Dr Katy Taylor was invited to present the findings of the review of EU non-technical summaries (NTS), which was published last year in ALTEX by Cruelty Free International's science team (Taylor et al., 2018). The review focused on the publication speed, accessibility and quality of NTS across all EU countries as well as a more detailed review of the quality of reporting in a selection of NTS from Germany and the UK. The review found that NTS are not being published as efficiently as they should be and that their content is often misleading and incomplete.

The BCLAS session on transparency in animal research featured other speakers from across the sector and was well attended and received. Efforts are being made both nationally and at the EU level to improve the quality and accessibility of the NTS.

References

Bailey, J. (2019). CRISPR-mediated gene editing: scientific and ethical issues. *Trends Biotechnol*, Epub ahead of print. doi:10.1016/j.tibtech.2019.05.002

Taylor, K., Rego, L. and Weber, T. (2018). Recommendations to improve the EU non-technical summaries of animal experiments. *ALTEX 35*, 193-210. doi:10.14573/altex.1708111

EUSAAT

European Society for Alternatives to Animal Testing

Third meeting on the development of a European network of 3Rs centers and 3Rs societies in Prague

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.

On June 10, 2019 representatives of 3Rs centers met during the FELASA Congress 2019 in Prague to discuss the results of the working groups that were formed after the second meeting in Berlin in March. Winfried Neuhaus, President of EUSAAT, served as coordinator of the meeting. The chairs of the groups "Dissemination of 3Rs", "Assessment/Quality of the Implementation of 3Rs", "Education", "Scientific Translatability" and "Ethics" presented their concepts and progress.

In addition, representatives from the European Commission – Directorate General Environment and the Joint Research Centre's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM), joined the meeting. They talked about ongoing relevant projects with the aim to ensure coordination of the current efforts and to prevent duplication of work. Topics discussed included a feasibility study on establishing indicators on the uptake of alternatives to animal testing, a 3Rs teaching strategy and material for high schools, universities and post-graduates, and

the future development and expansion of the ETPLAS platform with novel eLearning tools.

Funding options for the network of 3Rs Centers from the EU Commission were discussed, such as the COST (CO-operation in Science and Technology) Action Program. COST is a pan-European intergovernmental framework dedicated to networking activities for European researchers to jointly develop their own ideas and new initiatives across all scientific disciplines. The COST program promotes and spreads excellence, fostering interdisciplinary research for breakthrough science and empowering and retaining young researchers and innovators. COST has contributed to closing the gap between science, policy-makers and society throughout Europe and beyond since its creation in 1971. Since the scientific approach at 3Rs centers is interdisciplinary and focuses on the training of young scientists, building a European network of 3Rs Centers fits the aims of the COST program perfectly.

It was also very encouraging to listen to new participants of the 3Rs network, who gave short presentations to introduce themselves, their 3Rs aims, work and needs.

The network of 3Rs Centers has continued to grow steadily, and now consists of over 50 participants from 22 countries (Austria, Belgium, Czech Republic, Denmark, Estonia, Germany, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Spain, Sweden, Switzerland and the UK). The Norwegian 3R Centre Norecopa has constructed an overview of European 3R Centres: https://norecopa.no/3REuropeOverview

Since EUSAAT serves as a platform for disseminating information on the 3Rs, *the building of the European 3Rs centres network will be one of the main topics at the EUSAAT 2019 Congress on October 10-13, 2019 in Linz, Austria*.

Current coordinator: Winfried Neuhaus (winfried.neuhaus@ait.ac.at)

Highlights of the Program of the EUSAAT 2019 Congress
which will be held on October 10-13, 2019 in Linz, Austria

Specific 3Rs Challenge in 2019 Two Mouse Genetics Institutes are Closing 60 Years after the 3Rs Concept was Proposed
60 years after Bill Russell and Rex Burch published their groundbreaking book *The Principles of Humane Experimental Technique*, two of the internationally most respected mouse genetics institutes, the Wellcome Sanger Institute near Cambridge and the Harwell Mouse Genetics Institute of the MRC near Oxford, announced in May and June 2019 that they will close their animal facilities. The decisions of the Wellcome Trust and the MRC, which are based on scientific evidence, will trigger world-wide ethical discussions on institutions that continue to maintain animal facilities for genetic studies in mice and other species.

Since this is a highly controversial change of paradigm in the life sciences, we must put it on the agenda in October at the EUSAAT 2019 Congress. We are planning to hold sessions and a round table discussion on the consequences of this important change in the scientific evaluation of mouse genetics.

Awards, Keynote Lectures and Sessions

Award Ceremonies

Björn Ekwall Memorial Fund Award 2019 *for 3Rs Lifetime Achievements*
Jan van der Valk, Utrecht University, NL
October 11, 2019 at 17:30

ALTEX Prize 2019 *for the best article published in ALTEX in 2018*
Fabian Grimm, Texas A&M University, College Station, TX, USA
October 12, 2019 at the Social Event

Keynote Lectures

Christoph Giese (ProBioGen, Berlin, DE)
The Artificial Human Lymph Node

Lucia Lu Lee (Zhejiang Chinese Medical University, Hangzhou, China)
Evaluation of *in vitro* Embryotoxicity Tests for Chinese Herbal Medicines

Susanna Louhimies (EU Commission, Brussels, BE) (to be confirmed)
Implementing EU Dir. 2010/63 for the Protection of Experimental Animals

Jan van der Valk (Utrecht University, NL)
Fetal Bovine Serum (FBS):
Past – Present – Future

Round Table Discussions

Future of Mouse Genetics

Establishing a European 3Rs Centers Network

Biology-inspired Microphysiological Systems (MPS) to Advance Medicines for Patients' Benefits

Sessions – General Topics

Björn Ekwall Memorial Fund Session *"Initiative for implementing serum free media"*
co-chairs Jan van der Valk (Utrecht) & Stina Oredsson (Lund)

Establishing a Network of European 3Rs Centers
coordinators Winfried Neuhaus (EUSAAT) & Arian Smith (Norecopa)

International Progress in 3Rs Research: New Funding Initiatives & Global Cooperation
coordinators Shujun Cheng (CCARE, China) & (Hajime Kojima (JaCVAM, Japan)

Animal Experimentation – Working Towards a Paradigm Change
co-chairs Kathrin Herrmann (CAAT) & Jarrod Bailey (CFI)

YOU EUSAAT 2019 – Young Scientist Events at the EUSAAT Congress 2019
coordinators Annemarie Lang, Christopher Faßbender, Christian Zoschke

Sessions – Specific Topics

LUSH Prize Session – *In Silico* Models: toxicology & efficacy of drugs, chemicals & cosmetics, new approaches for biomedical research
chair Rebecca Ram (LUSH Prize Organization)

Biobarriers
co-chairs Marius Hittinger (Pharmbiotec) & Winfried Neuhaus (EUSAAT)

Ecotoxicology
co-chairs Christopher Faßbender (PETA) & Dominik Ruenzler (EUSAAT)

Education and Academia
co-chairs Chantra Eskes (Swiss3RCC) & Monika Schäfer-Korting (BB3R)

Ethics
co-chairs Christopher Faßbender (PETA), Christa Thöne-Reineke (FU Berlin) & Kristina Wagner (EUSAAT)

Medical Devices
coordinator Helena Kandarova (Centrum.SK)

Vaccines – The IMI VAC2VAC project, an innovative non-animal approach for quality control of established vaccines
coordinator Coenraad Hendriksen (Utrecht U.)

Validation – How to account for uncertainties of reference methods & data for the validation of new approaches?
co-chairs Roman Liska (JRC) & Martin Paparella (U. Graz)

EU-ToxRisk

The EU-ToxRisk summer started with two meeting highlights covering hot topics from the field of *in vitro* chemical safety assessment: (i) the application of new approach method (NAM)-assisted read-across (RAx) and (ii) the use of micro-physiological systems (MPS) in risk assessment decision-making processes.

The RAx approach is one of the most promising new risk assessment strategies. Although frequently chosen in regulatory submission dossiers, this method often fails to be accepted by regulatory agencies, as the associated uncertainties are perceived as being too large. In its first three years, the EU-ToxRisk project has addressed this issue by developing an RAx approach that reduces uncertainties by adding NAM-generated (*in silico* and *in vitro*) data to the available *in vivo* data.

The EU-ToxRisk workshop on "NAM-supported read-across: from case studies to regulatory guidance in safety assessment" took place on May 21-22, 2019 and was organized by EU-ToxRisk, in close collaboration with representatives from several European regulatory agencies (ECHA, EFSA, SCCS), US agencies (NTP, EPA), and other organizations (OECD, Health Canada, NIHS Japan).

On this occasion, a group of more than 60 international experts from industry, academia, and regulatory authorities came to Helsinki/Espoo (Finland) to discuss different case studies developed within EU-ToxRisk, but also at the OECD/IATA, and NIHS Japan programs.

This unique occasion allowed the collection of the scientific and regulatory expert feedback on various RAx scenarios, serving as input to an EU-ToxRisk advisory report for NAM-supported RAx. This advisory document will target the broader toxicology community. It will contain practical instructions on RAx applications in different regulatory contexts to improve the quality of submissions by registrants and to increase the acceptance/success rate of non-animal approaches[1].

Another dimension of new approaches is the application of microphysiological systems (MPS). This comprises the use of 3D organoids and the co-culture of relevant cell types, such as neuronal and glial cells. Moreover, it is directed towards a multi-organ-on-a-chip technology (e.g., developed by the EU-ToxRisk partners TissUse, together with InSphero, Biotalentum, and the Katholieke Universiteit Leuven). The project plans to use four different organs on one chip, all integrated with microfluidics.

This topic was discussed at the t[4] think tank meeting on "Biology-Inspired Microphysiological System Approaches to Solve the Drug Testing Dilemma" which took place on June 18-20 2019, in Berlin (Germany). Almost 40 international experts, including MPS developers, test centers, industry, and regulatory end-users sat together to discuss the application of MPS in the drug safety assessment process. The prime focus was on the issues of industrial adoption and regulatory acceptance, including the validation of MPS-based approaches. An important focus was given to the definition of strategies and the development of recommendations to overcome the identified roadblocks.

EU-ToxRisk publications

In June, an overview of the EU-ToxRisk approach was published in *Current Opinion in Toxicology* by Gräpel et al. (2019). This publication shows how the EU-ToxRisk *in vitro/in silico* toolbox has been applied to different toxicological scenarios. Some interesting applications of these tools have been recently published as a result of the integrative use of novel *in vitro* and *in silico* methods. Schimming et al. (2019) described the application of quantitative image analysis for prediction of cholestasis-inducing toxicants. The authors described the use of high-throughput live-cell visualization of GFP-tagged key proteins of the oxidative stress response/Nrf2 pathway and inflammatory cytokine signaling to follow the temporal responses of individual cells. In another milestone publication, Albrecht et al. (2019) established a novel *in vitro/in silico* method to predict the risk of human drug-induced liver injury (DILI) in relation to oral doses and patient blood concentrations. The performance of the *in vitro* system was optimized by the application of two novel test performance metrics: the toxicity separation index, which quantifies how well a test differentiates between hepatotoxic and non-hepatotoxic compounds; and the toxicity estimation index, which measures how well *in vivo* hepatotoxic blood concentrations can be estimated.

Outlook

The kick-off meeting for the new round of EU-ToxRisk case studies has taken place. The new set of case studies will address different, and relatively challenging, regulatory and scientific questions: testing of chemicals with little or no observed adverse effects or with multi-target organ toxicity. These will utilize the EU-ToxRisk battery of NAMs and assess its applicability in these contexts. In collaboration with the EC-Joint Research Center (JRC), a strong focus will also be on the assessment of chemicals without any prior knowledge of their *in vivo* effects and in the absence of any similar structures with *in vivo* data (so-called *ab initio* case studies).

The chosen focus is in line with the main aim of the project to further advance the field of NAM based hazard assessment. EU-ToxRisk partners will report on the achievements and challenges of the project at the 55th Congress of the European Societies of Toxicology (EUROTOX 2019) in two dedicated sessions.

[1] http://www.eu-toxrisk.eu/media/articles/files/EU-ToxRisk_Press%20Release_FINAL.pdf

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Giorgia Pallocca and Marcel Leist

R2N welcomes over 3000 visitors to the stand at the IdeenExpo

At the IdeenExpo in Hannover, Germany, June 15-23, 2019, representatives of R2N (Replace and Reduce Animal Testing in Lower Saxony, http://r2n.eu) spoke with over 3000 youths (10-18 years old) as well as with parents and teachers, presenting alternative methods to animal testing. The 7th IdeenExpo broke all previous records of attendance. With a focus on interactive experiments, the IdeenExpo aims to reach and teach the public, especially children and teenagers, about opportunities in MINT (Mathematics, Informatics, Natural Sciences and Technology) professions.

The biennial IdeenExpo, now dubbed Europe's biggest youth event for science and technology, welcomed over 395,000 visitors this year to 110,000 m^2 expo area assigned to twelve themes: mobility arena, digital worlds, media lab, daily life, climate zone, production cosmos, mobility miles, life sciences area, energy field, agricultural food park, club future and mission: moon. The visitors were kept busy with over 700 workshops, 650 experiments, the RoboCup Junior championship, Science Slam final competition, live shows on stage and weekend concerts. Over 270 exhibitors, including state and federal government entities, companies, universities, vocational schools and research institutes, generously invested time and funds into this free-entry event to encourage and inspire young people.

R2N was at this year's IdeenExpo to inform youngsters that there are alternatives to animal testing and what these alternatives look like and can accomplish. R2N is a consortium that encompasses 15 research groups within 5 institutions in Lower Saxony: Hannover Medical School, Leibniz University, University of Göttingen, University of Veterinary Medicine Hannover and TWINCORE. It aims to develop scientifically sound alternative methods to minimize the number of animals used in experiments or to fully replace existing animal experiments. The R2N stand was part of the Hannover Medical School's stand. After passing a gateway to a future where there is no longer a need for animal testing, the visitor was introduced to 3 possible alternative approaches: *in vitro, in silico* and body on a chip. The tour continued with four experiments, representing four of the 15 R2N projects: development of a complex 3D-tissue-biofilm model for analyzing oral and intestinal infections (slime-arium experiment), functional liver cells generated from reprogrammed pluripotent stem cells for use in toxicology (animated liver cell race), new analytical methods for safety assessment of gene therapy (gene taxi game), and *in vitro/ex vivo* characterization of lung infections of humans and animals (lung puzzle).

Each visitor received a quiz card with 4 questions pertaining to a respective R2N project and could find the answer by playing a game. The project team leaders were on hand to explain more about their projects: Prof. André Bleich (Institute for Laboratory Animal Science and Central Animal Facility from the Hannover Medical School (MHH), Prof. Meike Stiesch (Clinic for Dental Prosthetics and Biomedical Materials, MHH), Prof. Tobias Cantz (Exzellenzcluster

REBIRTH, Clinic for Gastroenterology, Hepatology and Endocrinology, MHH), Prof. Axel Schambach and Dr Michael Rothe (both of the Institute for Experimental Hematology, MHH) and Prof. Maren von Köckritz-Blickwede (Institute of Physiological Chemistry, University of Veterinary Medicine in Hannover). After all experiments were complete, the visitor was introduced to the R2N Professional Heroes: Bjørn, the biofilmer, Zelda, the cell generator, Galia, the gene navigator and Logan, the lung designer. After the 4 questions were answered correctly, the young future scientist turned in their quiz card and received a token for the machine where they would attempt to win a plush mouse by maneuvering an arm left and right to give the descending hand a chance to scoop up the mouse. Approximately 1,000 plush mice went home with youngsters throughout the IdeenExpo.

R2N was represented on the Career Stage by three people with very different profiles, showcasing the variability of positions involved in R2N projects. Pascal Hoffmann (Institute for Physiology and Cell Biology, University of Veterinary Medicine, Hannover), a PhD student and R2N student representative, spoke on June 15 about what it is like to work in a laboratory and how animal testing is replaced by alternative methods. His PhD thesis is on the development of an *in vitro* model for the investigation of pathogenicity mechanisms of gut diseases caused by zoonotic pathogens. Professor Dr Nils Hoppe (Centre for Ethics and Law in the Life Sciences, Leibniz University Hannover) spoke on June 21 about the ethical and legal ramifications of alternative methods to animal testing. And on June 23, Dr Katja Branitzki-Heinemann (Institute of Physiological Chemistry, University of Veterinary Medicine, Hannover) spoke about the project she leads to generate lung tissue in a Petri dish and investigate bacterial infections in humans. A poetry slammer listened to the talks and at the end of the discussion, she weaved key words into lyrics for the young audience.

The next IdeenExpo will kickoff on July 10, 2021 and R2N looks forward to being there again with our professional heroes, experiments and alternative methods to animal testing on display.

Bobbie Smith and André Bleich

TEDD – Tissue Engineering For Drug Development and Substance Testing

TEDD Annual Meeting 2019: Cell Sources and Stem Cell Generation for Drug Development

This year TEDD will hold its Annual Meeting 2019 on October 24, 2019 under the motto: Cell Sources and Stem Cell Generation for Drug Development. The TEDD Annual Meeting brings together experts from diverse fields with a shared interest in advanced 3D models.

Primary and stem cells are becoming relevant sources for the generation of organotypic tissue models used in many biopharmaceutical applications, regenerative medicine, disease modelling and drug discovery. They promise to revolutionize the drug discovery process at all stages, from target identification through to toxicology studies.

While primary cells by nature represent the native tissue most accurately, they usually have limited capacity to divide. Thus, they need to be freshly isolated for each assay, which limits their application. In contrast, the ability of stem cells to generate physiologically relevant cells in an almost limitless supply makes them an attractive alternative to currently used recombinant cell lines or primary cells. Emerging technologies involve the production of organoids from human pluripotent stem cells (hPSCs) and the use of organ-on-a-chip devices. These approaches are showing great promise for developing more reliable, rapid and cost-effective processes when compared with the current use of animal models. Challenges include routinely directing stem cell differentiation to reproducibly and cost-effectively generate pure specific lineages.

During the TEDD Annual Meeting, we will discuss how stem cells have already been used in the drug discovery process and how novel technologies can be applied to attain widespread adoption of stem cell technology by the pharmaceutical and biotech industry.
Speakers:
- Dr Marisa Jaconi, University of Geneva, Department of Pathology and Immunology, Switzerland
- Prof. Cornelia Kasper, University of Natural Resources and Life Sciences, Austria
- Prof. Phil Stephens, Cardiff University, UK
- Prof. Kenneth Lee, Chinese University of Hong Kong, HK
- Prof. Zhongze Gu, Southeast University, China
- Dr Wing Chan, STEMCELL Technologies, UK
- Dr Parto Toofan, REPROCELL Europe, UK

TEDD invites sponsors and exhibitors to participate in the TEDD Annual Meeting 2019 actively. The famous two-hour long networking lunch will host between 16-18 invited companies at the Greenhouse, where all participants are present. The call for exhibitors is now open. Spaces are allocated on a first-come-first-served basis. Interested companies can contact exhibition manager Simon Stebler at simon.stebler@zhaw.ch.

More info and the conference program can be found at: https://www.zhaw.ch/icbt/tedd
Registration will open in August.

Conference organizer:
Dr Katarzyna Kopanska
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katarzyna.kopanska@zhaw.ch

TEDD Sino-Swiss Workshop on Tissue Engineering

Preceding the Annual Meeting, TEDD will host the first Sino-Swiss Workshop on Tissue Engineering on October 23, 2019 with visiting scientists from China, USA and Hong Kong.

The purpose of this meeting is to develop a strategic academic alliance between the Institute of Biomedical Devices (IBMD), located in Suzhou, China and the School of Biological Science and Medical Engineering (BSME), Southeast University (SEU) in Nanjing, China and specific Swiss academic and industrial research groups active in the field of tissue engineering. Such an alliance would have many benefits such as the exchange of students/researchers between the institutions, the organization of joint scientific events, and options for transnational research projects with complementary expertise of partners.

The one-day TEDD interactive workshop jointly organized by the teams from Switzerland and China will be open to the public.

Topics will include organ-on-chip, 3D cell culture, stem cells, bioprinting, China-Switzerland collaboration opportunities, discussion on funding sources and programs, collaboration with European and international networks such as TERMIS, translation and validation process.

More info: https://www.zhaw.ch/icbt/tedd

TEDD at TERMIS European Chapter Meeting 2019

The theme of this year's TERMIS meeting in Rhodes, Greece was "Tissue Engineering Therapies: From concept to clinical translation and commercialization". The conference is highly crucial to scientists, clinicians and industries interested in tissue engineering and regenerative medicine therapies that aspire to revolutionize healthcare with their reparative capacity. The program of TERMIS EU 2019 included specific workshops and symposia in tissue engineering and regenerative medicine tools, technologies and dis-

coveries; clinical trials; regulatory approval of new devices; scaling up; commercialization; career development of young investigators; women leadership and representation; education; and outreach.

Dr Kopanska represented TEDD at the exhibition booth. Several TEDD member companies presented their products and services as exhibitors. TEDD Chair Prof. Michael Raghunath co-chaired three symposia and gave a presentation. Two of the symposia were TEDD-specific: Industrial Engineering of 3D Tissue Models – Building Physiology for Drug Development and Substance Testing and What Industry Really Wants and Needs.

For more details and impressions, check: https://www.zhaw.ch/de/lsfm/forschung/chemie-und-biotechnologie/competence-centre-tedd/partnership/past-events/

TEDD at Pint of Science with talk about animal use in science

Dr Markus Rimann gave a lecture on the topic "Animal Use in Science" during the "Pint of Science" in Bern together with other specialists in the area. The festival brings science and the general public together in bars and pubs. Researchers give lectures on their research areas and discuss their latest findings, answer questions and connect with the audience. The event takes place at venues around the world – and for the first time in Switzerland in Basel, Bern, Zurich and Lausanne.

Markus Rimann's area of expertise is bioprinting, a tissue engineering method to produce artificial tissues with a 3-D printer, which he pioneered in 2011. With this method, alternative tissue models can be produced to replace or reduce animal testing. He gave an example of an industry project that imitated a two-layer skin model of human cells produced with a bioprinter for testing substances from the cosmetics or pharmaceutical industries.

More info on the event: https://posfrorga.wixsite.com/pintofsciencech/animalusebern

Interview with Markus Rimann:
- Radiostation RaBe: https://rabe.ch/2019/05/22/3d-gewebe-statt-tierversuche/
- Higgs: https://www.higgs.ch/forschen-ohne-tiere/21173/

Markus Rimann on the Board of Animalfree Research

Dr Markus Rimann, who is a member of the TEDD core team at the Zurich University of Applied Sciences, has been elected to the Foundation Board of Animalfree Research. Markus is a group leader of 3D Tissues and Biofabrication at the Institute of Chemistry and Biotechnology (ICBT). Animalfree Research is a foundation supporting and accompanying research projects that promote the replacement or reduction of animal experiments. More info: https://animalfree-research.org

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