Corners

CAAT feed

Breakthrough in Brain Tumor Drug Development: "Mini-brains" Grafted with Brain Cancer Cells May Facilitate New and Personalized Treatments

The Center for Alternatives to Animal Testing (CAAT) pioneered mass-produced standardized human BrainSpheres (or "mini-brains") three years ago. Now, in a paper published in *Nature's* online journal *Scientific Reports* (Plummer et al., 2019), a multidisciplinary team from four institutions has shown how these mini-brains can be used in brain cancer research.

Thomas Hartung, director of CAAT, sees this as a breakthrough in the study of human cell models of disease. "Only recently have stem cell technologies allowed us to reproduce human biology. Now, with the development of 3D organoids like our BrainSpheres, we are using them to model diseases."

Taking a tumor from a patient and culturing it to optimize treatments rarely succeeds. Tumors outside of healthy tissue are not the same, and it is difficult to produce standardized cultures to compare treatments. Often, this requires using mice without an immune system – so-called "nude" mice – but this is costly, time-consuming, and often of limited relevance, as a tumor in mouse tissue is not the same as a tumor in human tissue. Adding a few cells of the tumor to the developing mini-brains, however, allows mass-production of human tumor tissue. Combined with innovative automated histopathology from MicroMatrices in Dundee, Scotland, the University of Dundee, and Perkin Elmer, the reaction of both the tumor and the healthy brain tissue to treatment options can be monitored at the same time.

The collaboration with neurosurgeons at the Mayo Clinic and Johns Hopkins utilized this model to study glioblastoma, a devastating childhood brain tumor found mostly in children but one that sometimes affects adults. "In principle, any tumor and any host tissue can be matched with the mushrooming of new 3D tissue models from stem cells," says Hartung, who initiated the research. His center has been advocating for the use of such alternatives to animal testing for 37 years. Most recently, they showed that their BrainSpheres can be used to model viral infections and neurodevelopmental processes. "These are proofs-of-principle that 21st century human cell-based technologies can succeed where animal models fail."

The successful collaboration of clinicians, pharmacologists, toxicologists, and pathologists is an example of "translational medicine," i.e., the bidirectional collaboration of clinicians and pre-clinical research. In the future, the team hopes that patient-specific decisions can be supported by testing a patient's tumor with some of the more than 200 chemotherapies available.

"If you have only one or two chances, you want the best therapy possible," says Hartung. "It was the personal experience of my sister's goddaughter, who died a few years ago from glioblastoma, which prompted this new use of our mini-brain technology. So, something sad and devastating can bring some light for others."

Save the Date!

6th Symposium on Social Housing of Laboratory Animals

June 3-4, 2019
Beltsville, MD

In collaboration with USDA Animal Welfare Information Center, NIH Office of Laboratory Animal Welfare, and the Johns Hopkins Department of Molecular and Comparative Pathobiology, this symposium brings together experts in animal behavior and welfare to address common issues in trying to achieve the mandate for social housing for social species. Participants will be encouraged to discuss special issues they are facing at their institutions.

Details and registration: http://tinyurl.com/y58tcsuh

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

June 17, 2019
Berlin, Germany
Co-organized with the Centre for Entrepreneurship (CfE) of the Technische Universität Berlin

The Information day on "Biology-inspired Microphysiological Systems (MPS)

to Advance Medicines for Patients' Benefits" will host key international experts from academia, regulatory agencies, and industry.

Microfluidic microphysiological systems (also referred to as tissues-on-a-chip, organ-on-a-chip, multi-organ-chip, human-on-a-chip, body-on-a-chip, or patient-on-a-chip tools) 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. doi:10.14573/altex.1603161). Now, four years later, stakeholders will meet 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.

Upcoming: New Frontiers in 3D Conference

April 25, 2019
Cambridge, MA
Co-sponsored by CAAT

This conference, following the success of its 2016 Baltimore premier, is an exclusive one-day scientific meeting to discuss practical applications for emerging 3D cell technologies for human disease modeling and predictive drug safety testing.

CAAT Director Thomas Hartung will give a keynote presentation on *A World Without Animal Testing: the Future of 3D*. Full details here: https://newfrontiersin3d.com

Helena Hogberg Receives Colgate-Palmolive Grant for Alternative Research

CAAT Deputy Director Helena Hogberg received the Colgate-Palmolive Grant for Alternative Research for her project entitled: Myelination as an Endpoint for Developmental Neurotoxicity (DNT) Testing in a Human 3D *In Vitro* Model.

Thomas Hartung Cited in *Nature Lab Animal*

Excerpt:
"There's a trend for making more and more such data available," said Thomas Hartung, chair for evidence-based toxicology at Johns Hopkins University.

One particularly rich source of information developed after Europe enacted the REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals) regulations in 2006, which required publication of toxicological tests for all candidate chemicals (https://www.echemportal.org/echemportal/propertysearch/index.action). Hartung and his group tapped this source to create a massive chemical topography that can be used to map a new chemical and estimate its toxicological properties.

Hartung's work is looking for regulatory acceptance, which he hopes will be forthcoming in the United States as his group works to get the method validated through The Interagency Coordinating Committee on the Validation of Alternative Methods, a group established in 2000 that includes 16 US regulatory and research agencies.

Full Article: Kling, J. (2019). Toxicology testing steps towards computers. *Lab Anim 48*, 40-42. https://www.nature.com/articles/s41684-018-0227-0

The Scientist Lists CAAT's Breakthrough Machine Learning Research as a Top Technical Advance of 2018

From the article:
Computing power was central to another of this year's methodological innovations, a program that can predict the results of animal-based toxicology screenings. Researchers led by Thomas Hartung of Johns Hopkins University developed the tool using data from thousands of toxicology tests, and found that it accurately predicted the results of such screenings 87 percent of the time. By comparison, repeating the assays themselves only reproduced the original results 81 percent of the time. The authors hope the software can reduce the use of experimental animals. "This won't be the end of all animal testing," Hartung tells *The Scientist*. "But this is an important step to take the bite out of it."

Top Technical Advances in 2018 (*The Scientist*): https://www.the-scientist.com/news-opinion/top-technical-advances-in-2018-65223

CAAT Satellite Meeting at Society of Toxicology Updates on Activities Related to 21st Century Toxicology: Invited Presentations and Open Microphone

A Society of Toxicology (SOT) Satellite Meeting
Organized by CAAT and the Human Toxicology Project Consortium (HTPC)

CAAT and the Human Toxicology Project Consortium (HTPC) held their annual satellite meeting on advancing 21st century toxicology activities at the SOT annual meeting in Baltimore in March, 2019. The satellite meeting provided an informal setting in which interested stakeholders could update each other on this important topic.

The meeting featured a number of invited presentations but also left time for an open microphone segment in which participants were welcomed to make announcements or to comment on germane topics.

CAAT/EU-ToxRisk Symposium at SOT: Strategic Development of Read-Across within the EU-ToxRisk Project and Beyond

EU-ToxRisk attended the SOT 58th Annual Meeting and ToxExpo on March 11, 2019 in Baltimore. The symposium provided a view across the Atlantic. It provided an in-depth overview of the project and demonstrated opportunities for the use of read-across, starting with an EU regulatory perspective and then broadening the scope to the most up-to-date developments. The focus was set on read-across case studies, by which the use of NAMs and mechanistic data was demonstrated. In addition, the symposium addressed au-

tomated read-across (RASAR – read-across-based structure activity relationships) and Good Read-Across Practices.

New Publications

Abreu, C. M., Gama L. and Krasemann, S. (2018). Microglia increase inflammatory responses in iPSC-dervied human BrainSpheres. *Front Microbiol 9*, 2766. doi:10.3389/fmicb.2018.02766

Beger, R. D., Dunn, W. B., Bandukwala, A. et al. (2019). Towards quality assurance and quality control in untargeted metabolomics studies. *Metabolomics 15*, 4. doi:10.1007/s11306-018-1460-7 (Following up on our workshop: Bouhifd, M., Beger, R., Flynn, T. et al. (2015). Quality assurance of metabolomics. *ALTEX 32*, 319-326. doi:10.14573/altex.1509161)

Plummer, S., Wallace, S., Ball, G. et al. (2019). A human iPSC-derived 3D platform using primary brain cancer cells to study drug development and personalized medicine. *Sci Rep 9*, 1407. doi:10.1038/s41598-018-38130-0

Cruelty Free INTERNATIONAL
Ending animal experiments worldwide

Review highlights the lack of correlation between human and animal drug safety data

A critical review of recent efforts to elucidate the scientific validity of animal-based drug tests by Dr Jarrod Bailey, Senior Research Scientist at Cruelty Free International, and Professor Michael Balls, special advisor to Cruelty Free International and former head of EURL ECVAM, has been published in BMC Medical Ethics.

The review discusses the recent analyses of publicly-available toxicity data on the use of animals in testing new drugs, both from pharmaceutical industry scientists and animal welfare organizations. They conclude that, despite decades of use, there remains no published evidence to validate the current regulatory paradigm of animal testing in supporting safe entry to clinical trials. In fact, the data in the recent studies support the contention that tests on rodents, dogs, and monkeys provide next to no evidential weight to the probability of there being a lack of human toxicity, when there is no apparent toxicity in animals.

The review calls on the pharmaceutical industry and its regulators to commission, conduct, and/or facilitate independent studies involving the use of substantial proprietary data.

Bailey, J. and Balls, M. (2019). Recent efforts to elucidate the scientific validity of animal-based drug tests by the pharmaceutical industry, pro-testing lobby groups, and animal welfare organisations. *BMC Med Ethics 20*, 16. doi:10.1186/s12910-019-0352-3

European Ombudsman responds to complaint about slow implementation of alternatives

Last year, Cruelty Free International submitted a complaint to the European Ombudsman regarding the speed with which the European Commission is updating the Test Method Regulation (TMR) to include validated alternatives to animal testing.

The TMR is a list of standardized tests (both animal and non-animal) that can be used to assess the safety of chemicals under EU legislation including REACH. The Commission is mandated by REACH to amend the TMR "*as soon as possible*" in order to "*replace, reduce or refine animal testing*".

According to the complaint submitted by Cruelty Free International, on average, there are currently delays of a minimum of 4 years from validation before inclusion in the regulation, which includes a delay of between 2-3 years following OECD acceptance. In their view, animals are therefore being used unnecessarily to generate the information required by REACH.

While the European Ombudsman has now ruled that there has been no maladministration, she has recommended that the Commission should "*intensify its efforts to simplify and speed up the process for introducing new alternative test methods under the TMR"* and *"ensure, where feasible, that it carries out the other steps necessary for updating the TMR in parallel with the OECD's verification process*".

Petition launched to urge European Chemical Agency to honor cosmetics testing ban

Earlier this year, Cruelty Free International highlighted the fact that the European Chemicals Agency (ECHA) is asking for some chemical substances that are used only in cosmetics to be safety tested under REACH.

ECHA and the European Commission are of the view that they can request animal tests for substances that are used primarily or exclusively in cosmetics if the tests are conducted for the purpose of assessing worker safety rather than consumer safety. Cruelty Free International believe that this is "policy creep", which is a breach of the testing bans in the Cosmetic Regulation as well as REACH, which says that these should take precedence.

In reaction, Cruelty Free International has launched a petition to stop the ECHA "over-REACH": bit.ly/stop-overreach .

Ten years of REACH – an animal protection perspective

A review by Dr Katy Taylor, Director of Science and Regulatory Affairs at Cruelty Free International, on the aims and outcomes of REACH in relation to animal testing and alternatives has been published in *Alternatives to Laboratory Animals* (ATLA).

Two important aspirations of the REACH Regulation were to promote alternative methods and ensure that animal tests are used only as a last resort. While significant progress has been made in the use of data-sharing and read-across to avoid new animal tests, the review concludes that nevertheless over 2.2 million animals have been used to date in new tests for REACH registrations. The use of *in vitro* and (Q)SAR approaches as standalone for animal tests has been relatively low and the level of funding for research into alternative methods is inadequate for the challenge at hand.

The review sets out ten recommendations for better implementation, of these two are key, as well as lessons to be learned for future, similar legislation.

Taylor, K. (2018). Ten years of REACH – An animal protection perspective. *Altern Lab Anim 46*, 347-373.

UK government fails to rule out duplicate chemical tests post-Brexit

With the UK set to leave the EU, Cruelty Free International is putting pressure on the UK government to reach an agreement with the EU to share REACH safety data, which would prevent the need for duplicate chemical tests on animals.

In its report about plans for the creation of a UK regulatory authority to replace the work of ECHA, the Regulatory Policy Committee states that there "*may be a negative impact on animal welfare*" in a scenario where data sharing with ECHA does not happen. It also says that firms "*may be required to duplicate animal testing*" in this transition phase.

So far, the government's response has been disappointing as the Brexit Minister failed to commit to no duplicate animal testing as a result of Brexit. Cruelty Free International has now written to the Parliamentary Under-Secretary of State for Exiting the European Union to reverse this statement.

ICAPPP submits comments on international pediatric medicines guideline

Cruelty Free International, on behalf the International Council on Animal Protection in Pharmaceutical Programs (ICAPPP), has submitted detailed comments to the European Medicines Agency on a new international guideline on pediatric medicines testing.

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) draft S11 guideline on "nonclinical safety testing in support of development of paediatric medicines" recommends tests on juvenile animals to help determine the safe levels of medicines for human children.

ICAPPP argues that adult human data has proven to be more reliable than data from juvenile animal studies and urges against the recommendation of these tests as a "tick-box" exercise or default option for addressing safety concerns.

EUSAAT

European Society for Alternatives to Animal Testing

The European network of 3R centres and societies met in Berlin for the second time

After the initial meeting of representatives of 3Rs centers and societies at the EUSAAT conference in September 2018 in Linz, Austria, the first follow-up meeting was hosted by the Freie Universität Berlin, Germany, and took place on March 14-15, 2019.

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

So far, members from Austria, the Czech Republic, Denmark, Germany, Hungary, Italy, Luxembourg, the Netherlands, Norway, Portugal, Slovakia, Sweden, Switzerland, and the United Kingdom have participated and the network is growing. Institutions from several other countries are in contact with the network and have expressed their wish to join. The meeting began with an introduction of the representatives describing their aims, institutional or societal structure, their tools, and their competencies. 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 include experts on Refinement, Reduction and Replacement of animal experiments.

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

The network is a completely independent, open and free community, which is very much driven by the initiatives of its protagonists and personal efforts. It is based upon a bottom-up approach, and every 3R center or society is welcome to join.

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

22nd European Congress on Alternatives to Animal Testing – Linz 2019 & 19th Annual Congress of EUSAAT – EUSAAT 2019

NEW DATE: 10-13 October 2019, University of Linz, Linz, Austria

There will be many more participants at a major international sports event in Linz than the organizers had expected, so the number of hotel rooms available to our participants might not have been sufficient for our congress had it been held in August as originally announced. Therefore, we have postponed the congress to October.

Call for abstracts

We invite you to submit your abstracts for oral presentations and/or posters. Abstracts may be submitted for all topics:

- Refinement: best practice approaches, animal welfare, avoidance of severe suffering, culture of care
- Reduction: transparency, reproducibility, and translational aspects (species differences)
- Replacement: advanced technologies for implementing the 3Rs: bio-printing, -omics technologies, systems biology approaches, IT and big data
- Banning the 90-day dog study for the safety testing of human drugs and pharmaceuticals
- 3Rs in education and academia
- 3R Centers in Europe & international, national and local centers
- International progress in 3Rs research: New Funding Initiatives & Global Cooperation
- 3D Models & multi-organ-chips (MOC), human-organ-chips (HOC)
- Biological barriers: e.g., lung, gut, kidney & skin epithelia, blood-brain & blood-saliva barriers
- *In Silico* Models: toxicology & efficacy of drugs, chemicals & cosmetics, new a pproaches for biomedical research
- Disease models using HUMAN cells, tissues and organs
- Efficacy and safety testing of drugs, medical devices & biopharmaceutics (incl. vaccines, blood components, allergenics, somatic cells, gene therapies, tissues and living cells used in therapy)
- REACH – the most frequently used alternatives: Read Across, WoE (Weight of Evidence), QSAR
- Advanced safety testing of cosmetics and consumer products (e. g., toys)
- Alternatives to animal testing in food safety, nutrition and efficacy
- Specific Endpoints of Toxicity: repeated-dose toxicity, inhalation, sensitization, reproductive & developmental toxicity (mEST & hEST), carcinogenesis, nanotoxicology
- Neurotoxicity & Developmental Neurotoxicity (DNT)
- Ecotoxicology
- Stem cell models and technology (hIPS, ES, mES, mIPS…)
- Implementing EU Dir 63/2010 – update
- Ethical & legal issues
- Advanced GMO models – CRISPR/Cas *in vivo* & *in vitro*
- Initiative for Implementing Serum Free Culture Media
- Publication policies regarding animal experiments and the 3Rs principles
- An integrated interdisciplinary approach to animal-free nanomaterial and chemical safety assessment: Results of the in3 project
- Vaccines: safety testing & the 3Rs
- "Young Scientists" session
- Free communications

To submit abstracts, please use the online submission system available on the congress website www.eusaat-congress.eu!

Deadline for the submission of abstracts: 14 June 2019

Do you miss a topic? Would you like your topic to be discussed? Are you interested in organizing a session dealing with your topic? Contact us: congress2019@eusaat-congress.eu

Student grants

For this year's congress, two different student grants are offered:

EUSAAT Young Scientist Travel Awards (YSTA 2019) – EUSAAT 2019 YSTA program

To promote the 3R principles of Russel and Burch, EUSAAT is promoting the participation of young scientists from around the world at the EUSAAT 2019 Congress by providing travel support to young scientists via the EUSAAT 2019 YSTA program.

According to the funding provided to the EUSAAT 2019 YSTA program by our sponsors, successful applicants will receive travel support in-kind as

- waiving of the congress fee and
- accommodation in Linz for 3-4 nights
- a diploma.

All abstracts submitted by EUSAAT 2019 YSTA applicants and accepted by the Scientific Committee will be presented as posters. In addition, EUSAAT 2019 will schedule a special "Young Scientists Session", where the best YSTA submissions will be presented as short oral presentations.

Details about the application process are available on the congress website www.eusaat-congress.eu

Deadline for application: 14 June 2019

EPAA 3Rs student grants 2019

The European Partnership for Alternative Approaches to Animal Testing (EPAA) supports students with outstanding work in alternative approaches by funding their attendance at a high-profile scientific event.

For EUSAAT 2019, a lump sum of €1,500 is available. 2 different grants are offered by the EPAA partners: 1 half grant and 1 full grant.

A half grant covers the reimbursement of the event registration fees for the student as

well as travel and accommodation fees, on the basis of the expense receipts up to a maximum of €500.

A full grant covers the reimbursement of the event registration fees for the student as well as travel and accommodation fees, up to a maximum total amount of €1,000, on the basis of the expense receipts.

Details on the application process are available on the congress website www.eusaat-congress.eu

Deadline for the application: 19 August 2019

Registration and hotel accommodation

Our partner holds allotments of rooms at special rates in different categories (****, ***) of hotels for the participants of the congress LINZ 2019 / EUSAAT 2019. The booking of the hotel can be done separately or together with the registration for the congress! To register for the congress and to book your hotel, please use the online registration form for this congress which is available on the congress website www.eusaat-congress.eu.

Don't miss the early bird fees (until 16 August 2019):

- members* of EUSAAT: €186
- general registration fee: €286
- students: €99

* *To become a member of EUSAAT please fill in the application form available on the congress website www.eusaat-congress.eu*

Exhibition and sponsoring

We invite companies, institutes, and other associations to promote their organization, products, and services at the Linz 2019/ EUSAAT 2019 congress. The exhibition space is located next to the poster exhibition and coffee break areas to facilitate your interaction with participants.

We also cordially invite companies, foundations, etc. to actively participate in the "EUSAAT 2019 3Rs Congress Linz" as sponsors. Any contribution is considered an encouragement to progress in the life sciences while applying the ethical principles of the 3Rs of Russell and Burch (1959). To show our appreciation for your financial support, we will offer some privileges to our sponsors. We do hope that among our proposals you will find attractive offers for sponsoring the EUSAAT 3Rs Congress in 2019.

A "Guidance for Sponsors & Exhibitors" with detailed information is available for download on the congress website www.eusaat-congress.eu

Any questions? Please contact the congress office!

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We are looking forward to welcoming you in October at this year's 3Rs congress in Linz!

Horst Spielmann[1], Winfried Neuhaus[2], Dagmar Jírová[3] and Dominik Rünzler[4]

[1]FU-Berlin, DE-Berlin (Head of the Scientific Committee, Secretary General of EUSAAT); [2]AIT Austrian Institute of Technology GmbH, AT-Vienna (Co-Chair, President of EUSAAT); [3]The National Institute of Public Health, CZ-Prague (Co-Chair, Vice-President of EUSAAT); [4]University of Applied Sciences Technikum Wien, AT-Vienna (Co-Chair, Vice-President of EUSAAT)

EUTOXRISK

HORIZON 2020

The EU-ToxRisk project keeps strengthening its international scientific and regulatory network!

For the 3rd time, the partners met at the yearly general assembly in Egmond aan Zee (The Netherlands) this February. The consortium benefited from the candid and well-informed feedback from the members of the scientific advisory board (SAB) and, for the first time since its formalization, the regulatory advisory board (RAB). The input given by the two boards is essential to provide directions and guidance to advance the project into the real regulatory world.

The general assembly was preceded by the 2nd EU-ToxRisk open symposium, an open window for the entire consortium to demonstrate last year's progress to the external stakeholders from industry, regulatory organizations, and academia.

The symposium hosted many interesting presentations and discussions, also offering the stakeholders the opportunity to discuss relevant issues related to NAM characterization and their regulatory acceptance with the project's partners.

The consortium and the stakeholders were also invited to attend the workshop "How can we tackle '*ab initio*' safety assessment of chemicals using non-animal methods?", promoted by the partner Cosmetics Europe, and deepening the burning issue of predicting chemical toxicity of compounds with little prior knowledge.

In March, EU-ToxRisk crossed the Atlantic to attend the 58th Annual SOT Meeting in Baltimore (US). During the project-dedicated session, the partners gave talks with a focus on the "Strategic Development of Read-Across within the EU-ToxRisk Project and Beyond". Besides the specific session, the EU-ToxRisk testing approach and the more specific case studies were presented and promoted to the

congress participants with several additional talks and posters.

Highlights were the closed working meetings with the Tox21c project and with the FDA.

EU-ToxRisk publications

Some of the recent publications highlight the research areas currently explored by the project.

One driving approach is the use of the adverse outcome pathways (AOPs) concept as a promising framework to bridge the gap between molecular level measurements and risk assessment. Martens et al. (2018) explored the strategy to integrate additional tools required for omics-based data analysis and visualization into the AOP Knowledge Base (AOP-KB), a main repository for AOPs, making use of WikiPathways. They showed how this interoperability allows the integration of omics data linked to the molecular pathways with AOPs. Moreover, they demonstrate how this approach will improve risk assessment, because omics data will be linked directly to key events (KEs) and therefore allow the comprehensive understanding and description of AOPs.

As a further step, quantitative AOPs (qAOPs) providing dose-time response predictions would be valuable for risk assessment. In their work, Zgheib at al. (2019) compared three approaches for qAOP building: empirical dose-response modeling, Bayesian network calibration, and systems biology modeling, applying them to the quantification of a simplified oxidative stress induced chronic kidney disease AOP.

Organ toxicity in the kidney was also addressed by Limonciel et al. (2018). In this publication, the relevance of epigenetic regulation was investigated as a putative mechanism of long-lasting effects of chemicals. Nephrocarcinogens were tested on the human proximal tubule cell line RPTEC/TERT1 using high-content mRNA microarrays coupled with miRNA, histone acetylation and DNA methylation arrays, and metabolomics. The integration of omics datasets suggested that the investigated epigenetic mechanisms were not the driving forces in the gene expression changes induced by the chemicals.

Within the project, the basic concepts of the GCCP guidance are applied and disseminated for a more reliable and reproducible testing approach. An important issue addressed in the guidance is the determination of the chemical concentration used in the test methods. Jaffar et al. (2019) highlighted the problem of calculating nominal concentrations, which do not necessarily correspond to local concentrations. Binding of the compound to the plastic of culture vessels or interaction with culture media components, such as lipids and albumin, reduces the free concentrations of the compound to which cells are exposed.

In line with the above, computational approaches for modeling the intracellular concentrations have been established by Fisher et al. (2018) and Toma et al. (2018). Their publications describe *in vitro* distribution models that were developed to predict the freely dissolved concentrations, taking also differential ionization of test compounds between the media and cell cytoplasm into account. These models could improve *in vitro*-to-*in vivo* extrapolation of toxicity endpoints by determining intracellular concentrations for a more accurate translation to *in vivo*.

Outlook

One of the most critical and exciting EU-ToxRisk workshops will take place on May 21-22, 2019 in Espoo (Finland) with a focus on "NAM-supported read-across: from case studies to regulatory guidance in safety assessment". The workshop – co-organized by ECHA, EFSA, NTP, EPA, SCCS, and OECD – will focus on several scientifically advanced project case studies developed within the EU-ToxRisk project, the OECD/IATA program, and NIHS Japan. It will cover different regulatory contexts – both European (e.g., REACH, EU Pesticides) and global (Canada Chemicals Management Plan and Japanese Chemical Substances of Control Law) – with the overall aim of including the outcome of the event in an improved regulatory guidance document for NAM-supported read-across. The EU-ToxRisk team plans to disseminate a refined read-across template for the toxicological community. This will improve the quality of the submission of real read-across cases by registrants, and eventually increase the success rate of non-animal safety approaches.

References

Martens, M., Verbruggen, T., Nymark, P. et al. (2018). Introducing WikiPathways as a data-source to support adverse outcome pathways for regulatory risk assessment of chemicals and nanomaterials. *Front Genet 9*, 661. doi:10.3389/fgene.2018.00661

Fisher, C., Siméon, S., Jamei, M. et al. (2018). VIVD: Virtual in vitro distribution model for the mechanistic prediction of intracellular concentrations of chemicals in in vitro toxicity assays. *Toxicol In Vitro 58*, 42-50. doi:10.1016/j.tiv.2018.12.017

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Limonciel, A., van Breda, S. G., Jiang, X. et al. (2018). Persistence of epigenomic effects after recovery from repeated treatment with two nephrocarcinogens. *Front Genet 9*, 558. doi:10.3389/fgene.2018.00558

Toma, C., Gadaleta, D., Roncaglioni, A. et al. (2018). QSAR development for plasma protein binding: Influence of the ionization state. *Pharm Res 36*, 28. doi:10.1007/s11095-018-2561-8

Zgheib, E., Gao, W., Limonciel, A. et al. (2019). Application of three approaches for quantitative AOP development to renal toxicity. *Comput Toxicol 11*, 1-13. doi:10.1016/j.comtox.2019.02.001

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement n° 681002.

Giorgia Pallocca and Marcel Leist