Corners

CAAT feed

Database Analysis More Reliable than Animal Testing for Toxic Chemicals; Study Shows that Computer Algorithms Could Replace Standard Toxicology Tests on Animals

Advanced algorithms working from large chemical databases can predict a new chemical's toxicity better than standard animal tests, suggests a study led by scientists at Johns Hopkins Bloomberg School of Public Health.

The groundbreaking paper by Thomas Luechtefeld, Dan Marsh, Craig Rowlands, and Thomas Hartung, which received extensive international press coverage, is now online in its entirety in the latest issue of *Toxicological Sciences* (Luechtefeld et al., 2018).

Press release from Johns Hopkins Bloomberg School of Public Health: https://bit.ly/2EhEnso

Software Beats Animal Tests at Predicting Toxicity of Chemicals (*Nature*): https://go.nature.com/2uo9KcG

New Digital Chemical Screening Tool Could Help Eliminate Animal Testing (*Science*): https://bit.ly/2KNl8K5

Thomas Hartung German Radio Interview

Thomas Hartung was interviewed about alternatives to animal testing by German radio station SWR2. You can listen to the interview (in German) here: https://bit.ly/2IVaexX

Thomas Hartung on Tomorrow Today – the Science Magazine

Thomas Hartung discusses advances in alternatives on the German television program on Deutsche Welle. Click on individual video segments to play them. Link: https://bit.ly/2yC2FYa

EBTC Project Tests if Fish Embryos Can Simplify Pre-natal Developmental Toxicity (Chemical Watch)

Excerpted from Chemical Watch:
US Scientists are investigating whether pre-natal developmental toxicity (PNDT) testing for regulatory hazard assessment could be radically simplified through use of fish embryos. The hope is that a zebrafish embryo test (ZET) can be used to meet existing requirements for PNDT data for a second species, leading to significant reductions in cost, time and vertebrate animal use.

Zebrafish are model vertebrate organisms but tests on embryos are widely considered a replacement for animal experiments. The embryos do not fall under regulatory frameworks dealing with animal experimentation, largely because tests are done before independent feeding begins. The ZET also has the benefit of being rapid to perform and relatively cheap.

Coordinated by the Evidence-based Toxicology Collaboration (EBTC) at Johns Hopkins Bloomberg School of Public Health in the US, the team has worked on the review protocol and study since 2012. It originally ran a pilot study on several chemicals with ZET data, including thalidomide, before it refined its review protocol.

Dr Tsaioun awaits the results with an open mind. "If the animal tests are useful in protecting human health and there is nothing else available, they should be used. But if there are other technologies that are cheaper, more predictive, faster, and pointing to specific mechanisms of human toxicity which the animal tests do not detect, then they should be incorporated into the regulatory paradigm," she says.

Full Article at Chemical Watch: https://bit.ly/2RCo38l

Recent Events

CAAT Presents Mini-Brain Research on Capitol Hill

Physicians Committee for Responsible Medicine (PCRM) had a briefing at Capitol Hill Visiting Center on September 27, 2018 titled "How Innovation Can Conquer Alzheimer's Disease – A Briefing on the Promise of Human-Based Research." Helena Hogberg, Deputy Director of CAAT, was there to present the center's mini-brain work in her talk entitled "3D Human Brain Models as an Alternative to Animal Studies for Toxicity and Disease."

Thomas Hartung Appointed Expert Advisor to Chinese National Institutes for Food and Drug Control

Thomas Hartung was appointed as advisor to the Chinese NIFDC at the "2nd International Conference on Cosmetics Alternative Methods in NIFDC" in Beijing on September 20-21, 2018.

Marcel Leist and Thomas Hartung at EFSA Conference in Parma

Marcel Leist and Thomas Hartung attended the EFSA conference in Parma on September 18-21 (1400 attendees) to inform participants about the use of new approach methods (NAM) in risk assessment. They were part of an extensive podium discussion on issues in current health risk assessment. Leist, Hartung, and the participants discussed better integration of NAM and epidemiology, not only to provide data for risk assessors, but to help the public understand a more realistic risk perception. Thomas Hartung's talk was titled "Software beats animal testing at predicting toxicity of chemicals".

Workshop: Using the Monocyte Activation Test as a Standalone Release Test for Medical Devices

September 18-19, 2018
National Institutes of Health,
Bethesda, Maryland

Thomas Hartung gave an overview of the MAT and comparison to the rabbit pyrogen test and other pyrogen detection tests, including the mechanism by which the MAT detects pyrogens, applicability of the MAT to detect endotoxins and non-endotoxin pyrogens in medical devices and extracts, MAT patent status, and limits or gaps in mechanisms of detection.

The PETA International Science Consortium and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) co-organized this workshop to discuss non-animal approaches for medical device pyrogen testing.

2nd Pan-American Conference for Alternative Methods in Brazil Sells Out

The 2nd Pan-American Conference for Alternative Methods in Brazil was deemed a rousing success by both the organizers and attendees. The sold-out conference, which brought together researchers, academics, members of the animal welfare community, industry representatives, and regulators to further alternative methods and build scientific collaborations, took place August 23 and 24 in Rio de Janeiro. Over 260 attendees came from across the Americas, the UK, Europe, and India.

We look forward to the 3rd Pan-American Conference, which will take place in Canada. Stay tuned for details.

Meeting on Internationalization of Read-across as Validated New Approach Method for Regulatory Toxicology

This workshop, co-organized by CAAT-Europe and EU-ToxRisk, was held on July 16-18 in Ranco, Italy. More than 20 international experts on read-across from regulatory agencies (e.g., EURL-ECVAM, EFSA, NIH, OECD), industry, and academia met to discuss the steps necessary to make read-across more consistent and standardized and the road-map to follow for expanding the use and acceptance of read-across to international regulatory agencies.

Upcoming Events

Book Launch Animal Experimentation: Working Towards a Paradigm Change

Friday, November 30th, 2018
The Johns Hopkins Bloomberg School of Public Health
615 N. Wolfe St.
Baltimore, MD 21205

Russell and Burch introduced the principles of replacement, reduction, and refinement of animal experimentation in 1959 in their groundbreaking book, *The Principles of Humane Experimental Technique* (Russell and Burch, 1959). Their highest goal was to avoid the use of animals wherever possible, and – in cases where animals were still deemed indispensable – to significantly enhance their treatment while also improving the quality of research and testing. There is growing recognition that a focus on human-relevant data is needed for the understanding and possible treatment of chronic, complex diseases, many of which are not well-understood and, thus, cannot be readily modeled in other animals. The technology revolution has greatly changed the field of life sciences and now provides us with tools enabling a shift away from animal experimentation. The 51 experts who contributed to *Animal Experimentation: Working Towards a Paradigm Change* review current animal use in science, present new and innovative non-animal approaches to address urgent scientific questions, and offer a roadmap towards the continuing replacement and eventual elimination of animals used in science as envisioned by Russell and Burch almost 60 years ago.

CAAT's Assistant Scientist and Veterinarian Kathrin Herrmann is one of the book's editors. Thomas Hartung contributed the concluding chapter.

At this book launch event, several of our mostly North America-based authors will give talks based on their book chapters.

Awards and Calls for Proposals

Next Generation Humane Science Award

This award is available annually to young scientists working in the U.S. to acknowledge and encourage researchers who focus on replacing animal experiments. The 2018 award will provide a prize of up to $9,000 recognizing the work of one young scientist, or may be shared among two or more young scientists.

The deadline for applications is December 31, 2018.

Details: http://caat.jhsph.edu/humanescienceaward.html

Call for Proposals: 2019 Science-based Refinement Awards

Attention veterinarians, animal care technicians, researchers, and those who care for the well-being of animals used in science: The

Johns Hopkins Center for Alternatives to Animal Testing (CAAT) is now accepting proposals for the 2019 Science-Based Refinement Awards.

These awards focus on research projects to enhance the housing, handling, and/or experimental procedures for laboratory animals or that can reduce animal use by (for example) identifying areas of research and testing where animal models lack reproducibility and translational value. Hence, the small grants are intended for those who work hands-on with animals, such as animal welfare scientists, veterinarians, and animal care technicians, as well as for researchers who conduct systematic reviews and meta-analyses of animal studies.

For 2019, we will offer two awards of $5,000 each. There are no facilities and administrative costs allowed on these awards.

Studies with animals must be non-invasive, with the possible exception of obtaining blood for biochemical measurements (and, in this case, animals should be trained to cooperate during venipuncture). Preference will be given to studies that have broad applicability.

Deadline for applications is December 31, 2018. Details: https://bit.ly/2pRnFG8

New Publications

Fritsche, E., Grandjean, P. and Crofton, K. M. (2018). Consensus statement on the need for innovation, transition and implementation of developmental neurotoxicity (DNT) testing for regulatory purposes. *Toxicol Appl Pharmacol 354*, 3-6. doi:10.1016/j.taap.2018.02.004

Gutbier, S., May, P., Berthelot, S. et al. (2018). Major changes of cell function and toxicant sensitivity in cultured cells undergoing mild, quasi-natural genetic drift. *Arch Toxicol*, Epub ahead of print. doi:10.1007/s00204-018-2326-5

Harris, G., Eschment, M., Orozco, S. P. et al. (2018). Toxicity, recovery, and resilience in a 3D dopaminergic neuronal in vitro model exposed to rotenone. *Arch Toxicol 92*, 2587-2606. doi:10.1007/s00204-018-2250-8

Luechtefeld, T., Marsh, D., Rowlands, C. and Hartung, T. (2018). Machine learning of toxicological big data enables read-across structure activity relationships (RASAR) outperforming animal test reproducibility. *Toxicol Sci 165*, 198-212. doi:10.1093/toxsci/kfy152

Cruelty Free INTERNATIONAL
Ending animal experiments worldwide

Cruelty Free International (www.crueltyfreeinternational.org) is the leading organisation working to create a world where nobody wants or believes we need to experiment on animals. Its dedicated team are experts in their fields, combining award-winning campaigning, political lobbying, pioneering undercover investigations, scientific and legal expertise and corporate responsibility. Educating, challenging and inspiring others across the globe to respect and protect animals, it investigates and exposes the reality of life for animals in laboratories, challenges decision-makers to make a positive difference for animals, and champions better science and cruelty free living.

Widely respected as an authority on animal testing issues, it is frequently called on by governments, corporations and official bodies for advice or expert opinion. Building relationships with politicians, business leaders and officials, analysing legislation and challenging decision-making panels around the globe, it acts as the voice for animals in laboratories.

Over 8 million signatures brought to UN for a global end to cosmetics animal testing

On October 4, World Animal Day, Cruelty Free International and The Body Shop took a record-breaking 8.3 million signatures from supporters all over the world to the United Nations (UN) Headquarters in New York to call for a global end to cosmetics testing in animals.

The petition, which reached its total in just 15 months, calls on the countries of the UN to create a global framework to end cosmetic animal testing internationally and permanently.

Despite the availability of approved non-animal testing methods and existing ingredients safe for human use, there are still no laws banning animal tests for cosmetics productions and ingredients in 80% of the world. Cruelty Free International estimates that over half a million animals are still used annually in worldwide cosmetics testing.

European Parliament discusses the use of non-human primates in neuroscience research

An EU Parliament meeting to discuss the use of non-human primates in neuroscience research was held in Strasbourg on September 12, 2018. The event was part of the monthly Intergroup on the Welfare and Conservation of Animals where MEPs meet to discuss animal welfare issues.

The meeting opened with the screening of recent footage from Animal Defenders International, which showed monkeys being prepared for experiments at the Biomedical Primate Research Centre (BPRC) in the Netherlands. Presentations were then given by Francesca Pistollato from the Joint Research Centre of the European Commission and Dr Katy Taylor from the European Coalition to End Animal Experiments (ECEAE, of which Cruelty Free International is a member), who called for an end to the use of non-human primates in

neuroscience research on both ethical and scientific grounds.

Almost 9,000 non-human primates are used in research and testing across Europe each year and several hundred of these are used in neuroscience experiments, which involve cruel practices such as restraining the animals by their heads for hours at a time, brain surgeries to implant recording devices on their skulls and deprivation of food or water for long periods to encourage them to co-operate.

A 2016 review published by Cruelty Free International showed that neuroscience experiments on non-human primates are flawed, misleading and unnecessary and that ethical studies involving humans are of much greater value. In 2007, the European Parliament called for a phasing out of the use of non-human primates in experiments, but little progress has been made and this commitment seems to have been forgotten. MEPs present at the Strasbourg meeting agreed that this initiative must be revived. A weblink to the event can be found here: http://www.animalwelfareintergroup.eu/meetings/

A six-point plan for a more humane chemicals regulation

June 1, 2018 was the final day for chemicals produced and sold within the EU to be registered with the European Chemicals Agency (ECHA), a deadline which marks 10 years under the EU's REACH legislation.

Cruelty Free International urged the EU to use the passing of the final REACH registration deadline as an opportunity to review and revise the chemicals legislation to eliminate animal testing. As part of this call, a six-point plan was published to encourage the use of better and more human-relevant non-animal alternatives in chemical testing. The plan was proposed to members of the European Parliament's Petitions Committee for consideration at their July meeting. The full plan can be found here: https://crueltyfreeinternational.org/what-we-do/breaking-news/read-our-6-point-plan-more-humane-chemicals-regulation.

Cruelty Free International, along with its partners at ECEAE, also called on ECHA's Board of Appeal (BoA) to provide clarity on their legal position on the testing of cosmetic ingredients under REACH, an issue which they continue to sidestep. The latest example of this came in August when ECHA's BoA dismissed a long-running case submitted by Symrise on their registered substance climbazole. Due to the substance's exclusive use as a cosmetic ingredient, we argued that ECHA should not have asked for animal tests under REACH because of the ban on animal testing under the Cosmetics Regulation. However, instead of addressing these arguments, the case was dismissed on a procedural matter. Unfortunately, this is the second time that the BoA has missed an opportunity to provide guidance on this issue. In a similar previous appeal case submitted by BASF, the BoA Decision also focused on other arguments.

Ipsen's cell-based test for botox approved

On September 3, French pharmaceutical company Ipsen announced that they have adopted a new non-animal method to test their botulinum toxin products sold in Europe. The cell-based test is a welcome replacement of the controversial LD50 (Lethal Dose) test which kills hundreds of thousands of mice every year.

Ipsen are now the third botulinum toxin manufacturer to have implemented the cell based assay for batch testing, following Allergan and Merz. Cruelty Free International together with their partners at ECEAE have campaigned against the use of animals in botulinum toxin tests for several years. We have recently reviewed these developments in the use of animals to test botulinum toxin products in Europe (Taylor et al., 2018)

Reference

Taylor, K., Gericke, C. and Rego Alvarez, L. (2018). Botulinum toxin testing on animals is still a Europe-wide issue. *ALTEX*, Epub ahead of print. doi:10.14573/altex.1807101

EU-ToxRisk

The EU-ToxRisk summer was studded with interesting meetings, courses and publications.

The season started with the organization of the t[4] think tank on "*Internationalization of read-across as validated new approach method (NAM) for regulatory toxicology*". Read-across application to risk assessment is a core topic of the project, as most of the case studies aim to develop and make available NAMs as biological support for a read-across approach. The think tank meeting hosted more than 20 international read-across experts from regulatory agencies, industry and academia. The participants met in July 2018 to discuss the steps that would be necessary to boost the standardization of the read-across methods and to expand their use and acceptance among international regulatory organizations. The debate tackled the urgent need for harmonization of the methodology among the different national and international agencies. The discussion stressed the necessity to build confidence in the method among the regulators. A published meeting report will compile the final recommendations.

Another core topic of the EU-ToxRisk project is the development of high content imag-

ing (HCI) and high throughput transcriptomics methods for hazard screening and identification (Delp et al., 2018; Wink et al., 2018). To discuss these novel approaches, a new EU-ToxRisk webinar series has started as a result of the strong joint interest in these topics of EU-ToxRisk project members and their US partners from Tox21. The first three webinars described HCI methods applied to developmental neurotoxicity and drug-induced liver injury assessment.

Early in September, an EU-ToxRisk delegation organized a public information session at the EUROTOX 2018 conference in Brussels (Belgium). The advancements of the EU-ToxRisk project were also presented at the EFSA conference in Parma (Italy). The conference, focused on the "*contextualization of read-across assessment*", attracted more than 1000 participants including researchers, risk assessors, risk managers, and stakeholders. During the "*Advancing risk assessment science- human health*" session, Prof. van de Water, coordinator of the project, explained the integration of various NAMs into some of the EU-ToxRisk case studies and their usefulness for the assessment of the overall applicability domain of these NAMs in chemical hazard and ultimate risk assessment.

EU-ToxRisk publications again addressed a broad range of topics; the impact of EU-ToxRisk in mixture risk assessment was discussed in the publication by Bopp et al. (2018); although EU-ToxRisk does not directly address mixture effects, the tools and the approaches developed in the project could strongly support the hazard assessment of mixtures.

An example for novel tools was described by Coll et al. (2018). They reported a novel differentiation protocol to obtain hepatic stellate cell (HSC)-like cells from induced pluripotent stem cells (iPSC). Such iPSC-HSCs closely resemble primary human HSCs at the transcriptional, cellular, and functional levels. This approach would provide a robust *in vitro* system to study HSC development, model liver fibrosis, and for drug toxicity screening.

The use of gene expression profiling for toxicity assessment applied to kidney and liver toxicity models was also described by Limonciel et al. (2018). Transcriptomic alterations were evaluated in differentiated kidney (RPTEC/TERT1) and liver (HepaRG) cells and compared to non-transcriptomic label-free sensitive endpoints of chemical-induced disturbances. The results showed that utilizing a gene panel of about 3000 probes, it is possible to discriminate basal tissue-specific signatures, generate dose-response relationships and to discriminate compound-specific and cell type-specific responses. This study also confirmed previous findings that chemical-induced transcriptomic alterations occur at non-cytotoxic exposure, and that a transcriptomics approach provides in-depth mechanistic information on the effects of chemicals on cellular transcriptional responses (Waldmann et al., 2014; Rempel et al., 2015; Shinde et al., 2016; Wolters et al., 2018).

Finally, Luechtefeld et al. (2018) described a novel *in silico* approach called RASAR (read-across structure activity relationship), which uses novel computational tools to define chemical similarity. Simple RASAR models tested in cross-validation achieve 70%-80% balanced accuracies. By combining RASAR models across toxicological domains (e.g., skin sensitization and skin irritation), balanced accuracies of 80%-95% were reached.

Outlook

The first set of read-across report studies will be prepared for (mock) regulatory submissions and reviewed by regulatory professionals. This will constitute the first litmus test of the EU-ToxRisk strategy, and the feedback will give important input to fine-tune the project direction.

References

Bopp, S. K., Barouki, R., Brack, W. et al. (2018). Current EU research activities on combined exposure to multiple chemicals. *Environ Int 120*, 544-562. doi:10.1016/j.envint.2018.07.037

Coll, M., Perea, L., Boon, R. et al. (2018). Generation of hepatic stellate cells from human pluripotent stem cells enables in vitro modeling of liver fibrosis. *Cell Stem Cell 23*, 101-113.e7. doi:10.1016/j.stem.2018.05.027

Delp, J., Gutbier, S., Klima, S. et al. (2018). A high-throughput approach to identify specific neurotoxicants/ developmental toxicants in human neuronal cell function assays. *ALTEX 35*, 235-253. doi:10.14573/altex.1712182

Limonciel, A., Ates, G., Carta, G. et al. (2018). Comparison of base-line and chemical-induced transcriptomic responses in HepaRG and RPTEC/TERT1 cells using TempO-Seq. *Arch Toxicol 92*, 2517-2531. doi:10.1007/s00204-018-2256-2

Luechtefeld, T., Marsh, D., Rowlands, C. and Hartung, T. (2018). Machine learning of toxicological big data enables read-across structure activity relationships (RASAR) outperforming animal test reproducibility. *Toxicol Sci 165*, 198-212. doi:10.1093/toxsci/kfy152

Rempel, E., Hoelting, L., Waldmann, T. et al. (2015). A transcriptome-based classifier to identify developmental toxicants by stem cell testing: Design, validation and optimization for histone deacetylase inhibitors. *Arch Toxicol 89*, 1599-1618. doi:10.1007/s00204-015-1573-y

Shinde, V., Perumal Srinivasan, S., Henry, M. et al. (2016). Comparison of a teratogenic transcriptome-based predictive test based on human embryonic versus inducible pluripotent stem cells. *Stem Cell Res Ther 7*, 190. doi:10.1186/s13287-016-0449-2

Waldmann, T., Rempel, E., Balmer, N. V. et al. (2014). Design principles of concentration-dependent transcriptome deviations in drug-exposed differentiating stem cells. *Chem Res Toxicol 27*, 408-420. doi:10.1021/tx400402j

Wink, S., Hiemstra, S. W., Huppelschoten, S. et al. (2018). Dynamic imaging of adaptive stress response pathway activation for prediction of drug induced liver injury. *Arch Toxicol 92*, 1797-1814. doi:10.1007/s00204-018-2178-z

Wolters, J. E. J., van Breda, S. G. J., Grossmann, J. et al. (2018). Integrated 'omics analysis reveals new drug-induced mitochondrial perturbations in human hepatocytes. *Toxicol Lett 289*, 1-13. doi:10.1016/j.toxlet.2018.02.026

Giorgia Pallocca and Marcel Leist