Corners

In November of 2014 the American Society for Cellular and Computational Toxicology held its 3rd annual scientific and business meeting, Where Chemistry and Biology Meet: AOPs as a Framework for Advancing Toxicology. Plenary lectures were given by Bob Kavlock of the US Environmental Protection Agency, Jennie Larkin and Ajay Pillai, both leaders of the National Institutes of Health Big Data to Knowledge project, and Steve Enoch of Liverpool John Moores University. Each lecture provided a particular facet of the AOP concept, from science to application. A lively and productive panel discussion followed, moderated by ASCCT Board Member Jack Fowle. After lunch, four presentations selected from submitted abstracts were given and provided a diverse set of topics for discussion:

– Predicting Neurotoxicity in Human-Derived iPSC 3D Mini-Brains, David Pamies, Johns Hopkins University
– Predicting Acute Toxicity using In Vitro ToxCast™ HTS Mitochondrial Inhibition Assays, Barun Bhhatarai, Dow Chemical Company
– Comparative Use of the Scientific Confidence Framework for Adverse Outcome Pathways to Assess Potential Applications in Regulatory Decision-Making, Katy Goyak, ExxonMobil
– Activities Aimed at Harmonizing the International Implementation of Test Methods or Approaches that Accomplish the 3Rs, Mei-Chun Lai, Physicians Committee for Responsible Medicine

The Society continued its cooperation with the Japanese Society for Alternatives to Animal Experiments with an invited lecture by Takashi Yamada of the National Institute of Technology and Evaluation, titled HESS: A Tool for Predicting Repeated Dose Toxicity with Toxicological Categories Based on Adverse Outcome Pathways.

The society also heard an overview of the recent 9th World Congress on Alternatives and Animals in the Life Sciences, held in August in Prague from Thomas Hartung. Dr Hartung also provided a look forward towards the 10th World Congress, to be held in Seattle in 2017, and invited members and others to get involved in planning what will be a momentous gathering.

During its business meeting and via e-election, the ASCCT elected new board members and a new president. David Allen of Integrated Laboratory Systems, Inc. is the ASCCT's 2nd president, taking the helm from Rodger Curren. During the meeting the society thanked Dr Curren for his leadership over these past four years. The other existing officers, Treasurer Erin Hill and Secretary Kristie Sullivan were re-elected for another term. The ASCCT board gained one additional new member, and now consists of, in addition to the three officers, Marilyn Aardema, Jack Fowle, Marianna Gaca, Katy Goyak and Thomas Hartung.

A poster session and a reception rounded out the 1-day meeting, with the presentation of the William and Eleanor Cave award, which recognizes achievements in reducing, refining and replacing animals in testing and is awarded by the Alternatives Research and Development Foundation, to Dr Frank Gerberick of Proctor and Gamble.

In 2015 the society is looking forward to offering new webinars and programs for the benefit of its members and the fields of *in vitro* and *in silico* toxicology, and I invite you to join us! Learn more at http://www.ascctox.org.

Kristie Sullivan
ASCCT Secretary

CAAT*feed*

Marcel Leist Receives 2015 SOT Enhancement of Animal Welfare Award

The Society of Toxicology (SOT) – the world's largest and preeminent association representing the field of toxicology – has awarded CAAT-Europe co-director Marcel Leist its 2015 Enhancement of Animal Welfare Award.

The award honors an SOT member for contributions made to the advancement of toxicological science through the development and application of methods that replace, refine, or reduce the need for experimental animals. Dr Leist's laboratory focuses on *in vitro* toxicology, specifically mechanisms and systems related to neurotoxicity.

Marcel Leist was the fourth CAAT representative to win this prestigious award

following Alan Goldberg, Thomas Hartung, and Martin Stephens.

2014 LUSH Prize Awarded to CAAT-Europe

CAAT-Europe's Policy Program was awarded the 2014 LUSH Prize for Lobbying. The Prize honors the work of exceptional individuals, groups, or organizations pushing for change and focusing on policy interventions promoting the use of alternatives. CAAT-Europe, located at the University of Konstanz, is a joint venture between Konstanz and the Johns Hopkins Bloomberg School of Public Health in Baltimore, MD (US). Francois Busquet, Marcel Leist, and Thomas Hartung received the prize for CAAT's commitment to providing scientific information to the European Parliament in Brussels. Busquet, who coordinates the scientific information activities of CAAT-Europe in Brussels, accepted the prize on November 14, 2014 in London.

CAAT-Europe proactively approaches Members of the European Parliament (MEPs) and offers conferences and workshops (more than 100 meetings and ten workshops to date). In 2013, CAAT-Europe was named official external advisor (STOA) of the European Parliament. "We are proud to have carried scientific animal-free testing methods to the level of the institutions of the European Union with our policy program and to have built up CAAT-Europe at the University of Konstanz to a leading institution in this area," commented Marcel Leist, co-director of CAAT-Europe.

Anne Krug, who executed her thesis work at the CAAT laboratories in Konstanz and Baltimore received a young investigator award, as did Róber Bachinski, PhD student of the Postgraduate Program on Science and Biotechnology in the Fluminense Federal University (PPBI/UFF), Brazil, who worked for one year on his PhD at CAAT in Baltimore.

MEP-3Rs Scientists Pairing Scheme (EU Parliament)

The Center for Alternatives to Animal Testing-Europe (CAAT-Europe) has announced a new initiative for better visibility of 3Rs issues in the Brussels policy arena. This initiative pioneers a close and strong relationship of scientists with their elected national members of the European Parliament (MEPs). This initiative will be supported by the MEPs under the title "MEP – 3Rs scientist pairing scheme."

Objectives: MEPs will be paired with 3Rs scientists from their country. The MEP will benefit from the scientists' knowledge, while also enabling support for national 3Rs initiatives and funding schemes, as well as promoting them at the EU level.

This activity will be under the patronage of MEP Pietikainen (Finland). MEPs from France, the Czech Republic, Romania, Italy, Denmark, Germany, Sweden, and The Netherlands have already shown interest in such a network and many more are expected.

Timeline and actions: A launch event for the network is tentatively planned for January 27, 2015 during a reception titled "MEP–3Rs scientist pairing scheme" at the European Parliament in Brussels. The scientists will meet before the reception for a workshop to discuss and prepare a report, which will be published in *ALTEX* and will list the participants as co-authors.

This initiative also provides the opportunity to be introduced to the policy-making process at the European Parliament and key European institutions.

A yearly meeting with the network would then take place to share best practices from each country (e.g., 3Rs communications to lay audience, 3Rs success stories, etc.). Please let us know whether you would be interested in joining by sending us an email to http://caat-eu-policy@uni-konstanz.de

Recent Workshops and Symposia

Progress on Replacement of Animals for Cosmetic Testing and Other Issues and a 75th Birthday Celebration for Alan Goldberg

November 20, 2014
Baltimore, MD

This symposium celebrated the 75th birthday of Alan Goldberg, first by looking at the progress toward the replacement of animals for cosmetic testing since CAAT's inception in 1981, then with a gala dinner reception honoring Alan's distinguished career. Friends and colleagues from around the world gathered to present an overview of the scientific and regulatory achievements made possible – in large part – by the pioneering work of CAAT and its founder. The agenda and list of speakers may be found at: http://caat.jhsph.edu/programs/workshops/alangoldberg75

Workshop: The Emergence of Systematic Review and Related Evidence-based Approaches in Toxicology

November 21, 2014
Baltimore, MD

This workshop, hosted by the Evidence-Based Toxicology Collaboration (EBTC), which CAAT coordinates, showcased not only emerging efforts in evidence-based toxicology but addressed opportunities and challenges to its expanded application. Speakers included representatives of the National Toxicology Program, the Environmental Protection Agency, the European Food Safety Agency, and the EBTC, as well as experts in evidence-based medicine. Several distinguished commentators from industry, academia, federal agencies, and NGOs offered comments on current developments. A training program on systematic reviews for the US FDA is planned for February 18, 2015, and will be available via webcast.

Upcoming Workshops and Symposia

Training Program on Systematic Reviews

February 18, 2015
US FDA (also available via webcast)

Details to be announced via the CAATwalk electronic newsletter, available here: http://eepurl.com/nV845

Updates on Activities Related to 21st Century Toxicology and Evidence-based Toxicology: Invited Presentations and Open Microphone

March 26, 2015
San Diego, CA

A Society of Toxicology (SOT) Satellite Meeting organized by the Center for Alternatives to Animal Testing, The Human Toxome Project, and The Human Toxicology Project Consortium

If you're planning to attend the Society of Toxicology conference in San Diego this March, please join CAAT, The Human Toxome Project Consortium, and The Human Toxicology Project Consortium for our annual satellite meeting on "21st century toxicology activities and related efforts," which this year will feature expanded coverage on evidence-based toxicology and read-across. The satellite meeting provides an informal setting for all interested stakeholders.

The meeting will feature a limited number of invited presentations, but will also leave ample time for an open microphone segment in which participants are welcome to give brief presentations on germane topics, with or without slides.

Symposium on Microphysiological Systems

June 11, 2015
Berlin, Germany

This symposium will be held in conjunction with a workshop on the topic (by invitation only).

Recent Publications

Bouhifd, M., Hogberg, H. T., Kleensang, A. et al. (2014). Mapping the human toxome by systems toxicology. *Basic Clin Pharmacol Toxicol 115*, 24-31. http://dx.doi.org/10.1111/bcpt.12198

Hartung, T. (2014). 3D – a new dimension of in vitro research. *Adv Drug Deliv Rev 69-70*, vi. http://dx.doi.org/10.1016/j.addr.2014.04.003

Hartung, T. and Stephens, M. (2014). Toxicity Testing in the 21st Century, Approaches to Implementation. In P. Wexler, *Encyclopedia of Toxicology* (673-675). 3rd edition. Elsevier Inc., Academic Press.

Hoffmann, S., Stephens, M. and Hartung, T. (2014). Evidence-based Toxicology. In P. Wexler (ed.), *Encyclopedia of Toxicology* (565-567). 3rd edition, volume 2. Elsevier Inc., Academic Press.

Smirnova, L., Block, K., Sittka, A. et al. (2014). MicroRNA profiling as tool for in vitro developmental neurotoxicity testing: The case of sodium valproate. PLoS *ONE 9*, e98892. http://dx.doi.org/10.1371/journal.pone.0098892

Tollefsen, K. E., Scholz, S., Cronin, M. T. et al. (2014). Applying adverse outcome pathways (AOPs) to support integrated approaches to testing and assessment (IATA). *Regul Toxicol Pharmacol*, Epub ahead of print. http://dx.doi.org/10.1016/j.yrtph.2014.09.009

NTP National Toxicology Program
U.S. Department of Health and Human Services

UNITED STATES ICCVAM Advancing Alternatives to Animal Testing

News from NICEATM and ICCVAM

Assessing the biological relevance of *in vitro* data

In a recent case study, high-throughput screening (HTS) methods provided results that were 97% similar to an animal-based screening method, showing promise for the use of HTS to prioritize the testing of large numbers of chemicals. NICEATM director Warren Casey, Ph.D., described the methods in a December 10 presentation to the National Toxicology Program Board of Scientific Counselors.

Using a case study that NICEATM developed in collaboration with the U.S. Environmental Protection Agency (EPA), Casey presented an analysis of 86 chemicals tested in 16 HTS methods measuring the ability of chemicals to interact with the human estrogen receptor. The integrated HTS results were compared to results from the *in vivo* rodent uterotrophic bioassay required by the EPA for assessing estrogen receptor bioactivity in their

Endocrine Disruptor Screening Program, using the same chemicals. The *in vivo* and *in vitro* approaches provided the same result for 97% of the chemicals, with only two chemicals having different results.

"This is an amazing predictive tool for determining whether or not a chemical has the potential to interact with the human estrogen receptor," Casey said, but noted that the HTS approach is only intended as a screen to identify chemicals of concern, and that animal tests would be required to help determine whether or not the chemicals may actually pose a hazard to humans.

ICCVAM to present webinar on reverse toxicokinetic models

ICCVAM is presenting a January 27 webinar on "Reverse Toxicokinetics: Using *In Vitro* Data to Estimate Exposures that Could Be Associated with Adverse Effects *In Vivo*." The webinar will focus on the development and application of reverse toxicokinetic models for extrapolation of high-throughput screening data to *in vivo* dosimetry. John Wambaugh, Ph.D., Physical Scientist at the EPA's National Center for Computational Toxicology (NCCT), will provide an overview of the development of reverse toxicokinetic models. Barbara Wetmore, Ph.D., Senior Research Investigator at the Hamner Institutes for Health Sciences, will discuss the consideration of population variability and sensitive subpopulations in the use of these models. NICEATM and NCCT are organizing the webinar on behalf of ICCVAM.

Information about the webinar and a link to registration are available at http://ntp.niehs.nih.gov/go/ivive-webinar. Registration will be available up until the beginning of the webinar.

Workshop to evaluate alternative tests for pertussis vaccine

The National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) is hosting a workshop that will bring together scientists from government, industry, academia, and research institutions to discuss and evaluate alternative methods to the murine histamine sensitization test (HIST) for monitoring residual pertussis toxin activity in acellular pertussis vaccines. NICEATM is providing administrative support for the workshop.

The workshop, titled "In Search of Acceptable Alternatives to the Murine Histamine Sensitization Test: What Is Possible and Practical?" will be held in London, England, on March 4–5. Workshop participants will review and discuss data from an ongoing study of a cell-based assay proposed as a replacement for HIST, issues associated with alternatives validation and recent relevant scientific advances.

Registration is free but advance registration by February 6 is required. Additional information including a link to registration is available on the NC3Rs website at https://www.nc3rs.org.uk/events/workshop-search-acceptable-alternatives-murine-histamine-sensitization-test-hist-what.

Scoping document on thyroid assays available

NICEATM scientists contributed to the development of the recently issued "New Scoping Document on *In Vitro* and *Ex Vivo* Assays for the Identification of Modulators of Thyroid Hormone Signalling." The document, published by the Organisation for Economic Co-operation and Development (OECD), provides recommendations for development and use of existing *in vitro* and *ex vivo* thyroid assays and identifies data gaps that require development of additional tests. The document was developed under the leadership of the OECD Expert Group on Amphibian Testing and the OECD Validation Management Group on Non-Animal Testing (VMG-NA). NICEATM Director Casey is a member of the VMG-NA.

The scoping document is available, along with other OECD documents on endocrine disruptor testing, on the OECD website at http://www.oecd.org/chemicalsafety/testing/seriesontestingandassessmenttestingforendocrinedisrupters.htm.

NICEATM publications

- NICEATM scientists and collaborators analyzed *in vitro* test data from the Tox21 10K compound collection to identify structural classes that activate the farnesoid X receptor.
  Hsu, C. W., Zhao, J., Huang, R. et al. (2014). Quantitative high-throughput profiling of environmental chemicals and drugs that modulate farnesoid X receptor. *Sci Rep 4*, 6437. http://dx.doi.org/10.1038/srep06437
- NICEATM contractor Nicole Kleinstreuer participated in a 2013 t[4] workshop on integrated testing strategies and is a coauthor on the workshop report in this issue of ALTEX.
  Rovida, C., Alépée, N., Api, A. M. et al. (2014). Integrated Testing Strategies (ITS) for Safety Assessment. *ALTEX 31*, 25-40. http://dx.doi.org/10.14573/altex.1411011

Institute for In Vitro Sciences
Advancing Science & Animal Welfare Together

IIVS News & Views

EPAA and IIVS Collaborate to Produce *In Vitro* Test Method Training Videos

In the fall of 2012, IIVS and the European Partnership for Alternative Approaches to Animal Testing (EPAA) signed a Memorandum of Understanding to coordinate efforts to support the international use of non-animal testing methods. The two organizations agreed to combine resources and collaborate to promote international awareness of and education on these methods, and to provide science-based advocacy to key stakeholders. One of the recently finalized efforts between the two groups was the completion of a training video on the Bovine Corneal Opacity and Permeability (BCOP) assay.

With funding provided by the EPAA, IIVS scientists have produced a 13 minute video that demonstrates how to perform the BCOP assay according to the Test Guidelines set forth for the assay by the Organization for Economic Co-operation and Development (OECD TG 437). The video focuses on steps that are critical to the success of the assay such as handling of the isolated cornea and removal of the test material from the cornea at the conclusion of the exposure time. The completed video, with English subtitles, is available without charge on the EPAA website at http://bit.ly/1xkMMQY. The subtitles are currently being translated into Chinese and Portuguese. Versions of the video with subtitles in these languages should be available on the EPAA website before the end of the year. The next training video effort is focused on the 3T3 Neutral Red Uptake Phototoxicity Assay.

Corrositex® Training Presented in Prague

Hans Raabe, Vice-President of IIVS, presented training on the Corrositex® assay (assay kits generously donated by In Vitro International) during the 9th World Congress on Alternatives and Animal Use in the Life Sciences satellite training course on alternative methods. The course was organized by the European Society of Toxicology In Vitro (ESTIV), The European Society for Alternatives to Animal Testing (EUSAAT), and the Czech Republic National Institute of Public Health (SZU). The focus of the training was *in vitro* skin and eye toxicology. Lectures on skin irritation/corrosion testing using reconstructed human tissue models, the Corrositex® assay, and eye irritation testing using the HET-CAM assay were presented on Thursday August 28th after the conclusion of the World Congress meeting. Hands-on laboratory activities took place the following day. The training was hosted at the National Institute of Public Health (NIPH) campus and was geared toward students and scientists at the beginning of their career in *in vitro* toxicology. This year's post-World Congress training was highly successful with attendees representing many different industries and countries.

IIVS Presentations at Several Industry Events

IIVS was actively involved in the *9th World Congress on Alternatives and Animal Use in the Life Sciences* (WC9) this past August in Prague, Czech Republic. IIVS Vice-President Erin Hill presented a talk on the American Society for Cellular and Computational Toxicology (ASCCT) and its recent work promoting advances in *in vitro* and *in silico* toxicology during the "Global Cooperation, Regulatory Acceptance and Standardization" theme. IIVS President Dr Rodger Curren served as a co-chair and speaker with Nick Jukes of InterNICHE for session 6 of the same theme titled "Breaking Down Barriers and Promoting International Cooperation on 3Rs". In addition to these activities, IIVS scientists presented a number of posters (available as pdfs at http://www.iivs.org) and participated in the post-congress hands-on training. According to the WC9 website, this meeting had the largest number of attendees to date of any World Congress event. The meeting organizers feel that they met their goal of providing an international forum for discussing progress in promoting the 3Rs concept and implementing "Humane Science in the 21st Century". The feedback received from attendees was positive and photographs from the event are available on the WC9 website.

IIVS Study Director Dr Gertrude-Emilia Costin was invited to speak at a two day conference at the end of September in Washington D.C. on US/Global Biocides (Antimicrobials) Regulations. The meeting brought together experts from the US, Asia, and Europe to discuss the latest developments in the regulation of biocides/antimicrobials. The meeting was organized by Chemical Watch and included presentations by representatives from regulatory agencies such as the US EPA and Health Canada, and industry representatives from companies like The Clorox Company and EcoLab. Dr Costin's presentation centered on the new, non-animal based hazard identification strategy for ocular irritation of antimicrobial cleaning products. For information on this topic, or on how you can get involved in the latest efforts to develop a similar strategy for the detection of potential skin irritation of antimicrobial cleaning products, please contact Emilia at ecostin@iivs.org.

Chemical Watch and the PETA International Science Consortium, Ltd. (PISC) co-sponsored a free series of REACH focused webinars. The webinars presented alternative methods and testing strategies that can be used to meet REACH require-

ments. They were intended for industry toxicologists, individuals who may be registering chemicals for the first time, and companies that would like to know more about the validated non-animal tests available and how they are currently used. Dr Costin presented on skin irritation and corrosion. She described the *in vitro* methods that can be used to meet REACH data requirements for skin irritation and corrosion under Annexes VII and VIII. Dr Costanza Rovida, of REACH Mastery and CAAT Europe, explained how the *in vitro* methods can be used in an integrated approach to testing and assessment using specific examples. IIVS Study Director Dr Kimberly Norman presented on eye damage and *in vitro* test methods for determining eye irritation along with João Barroso of EURL ECVAM. They discussed what is required to achieve full replacement of the animal test and how *in vitro* methods can be used alone or in a tiered testing strategy. Recordings of the webinars and others in the series can be found on the Chemical Watch website at http://chemicalwatch.com/peta-webinars.

Tobacco Science Regulatory Conference

IIVS Principal Scientist, Dr Holger Behrsing, presented his work on the Use of Precision-cut Lung Slices to Assess Inflammation, Parenchymal Damage, and Collagen Deposition: Three Markers of Tobacco Exposure-induced Pulmonary Toxicity at the September 28 to October 1 Tobacco Science Regulatory Conference (TSRC) meeting in Charlottesville, VA. This poster is available for viewing on the IIVS website, http://www.iivs.org.

Predicting Eye Stinging using the Novel NociOcular Assay: Investigating Surfactant, Sunscreen, and Ophthalmic Products

The evaluation of ocular irritation potential is of primary importance for the safety assessment of products that are designed to be used in or around the eyes such as cosmetics, sunscreens, and ophthalmic products. In addition, testing for erythema, lacrimation, and the potential to cause stinging is often conducted on baby products such as shampoo and sunscreen to ensure the absence of irritation and pain associated with their use. Although several *in vitro* eye irritation assays are available, none of these assays can effectively predict the stinging potential of products which may come in contact with the eyes. In collaboration with Dr Anna Forsby at Stockholm University and Johnson & Johnson Consumer and Personal Products Worldwide, IIVS has investigated the use of the NociOcular assay for eye sting prediction. Initial investigations, which were focused on eye sting of baby bath products, demonstrated that the NociOcular assay may be used to distinguish stinging from non-stinging baby bath products, and support that the NociOcular assay may serve as a simple bioassay to assess sensory response in the eye. An *in vitro* assay for eye sting prediction may be very beneficial as a pre-clinical screening tool. Also, since *in vitro* testing can be more readily conducted than clinical studies, it may be used as a tool to advance the understanding of the relative contributions to ocular sting of various ingredients within personal care products. With support from collaborators, IIVS has successfully transferred this technology and is now routinely performing the NociOcular assay.

Although the assay has primarily been used to investigate surfactants and surfactant based products, IIVS and its collaborators are currently investigating other product types which may come in contact with the eyes. At the 2014 World Congress on Alternatives and Animal Use in the Life Sciences, IIVS was pleased to present a poster on the assessment of sunscreens, ophthalmic products, and continued assessment of surfactant products in the NociOcular assay. One of the topics featured in the poster was the testing of sunscreens in the assay system. Sunscreen products are often used on the face and near the eyes and many are known to cause a stinging sensation in eyes; this makes them ideal candidates for the NociOcular assay. Furthermore, sunscreens marketed for babies and children may make non-stinging claims. Unfortunately, many sunscreens, particularly those formulated for children, are viscous and aqueous insoluble. Using the standard NociOcular protocol, the samples do not form either a solution or a homogeneous suspension in the prescribed buffers. The inability to obtain either a solution or a homogenous suspension results in dosing challenges, and subsequent results are often variable or inconclusive. In order to overcome this technical challenge, a solvent composed of detergent along with traditional assay buffer was prepared. The use of this new solvent resulted in a more homogenous mixture that allows dosing. When diluted in the assay buffer without detergent, the baby sunscreen showed results similar to those of the non-stinging negative control. However, when prepared in the detergent, it was possible to transfer a more homogenous sample of the product, and the cells responded in a manner that correlates to consumer reviews of the product. Also worthy of note, when the amount of detergent used was increased, the suspension became more homogenous and resulted in a greater number of TRPV1 channels being activated. The detergent used for the dilutions, when tested alone, did not predict an eye stinging response would occur and did not change the assessment of an adult shampoo previously evaluated without the detergent. It is hypothesized that by increasing the solubility through the higher concentration of detergent, the cells were exposed to a dose that was more representative of what occurs in actual use.

As IIVS continues its research on the NociOcular assay, the validity of alternate solvents will be further explored. Increasing the detergent allowed for an increased response; therefore, future research will help to determine which alternate solvents best represent the results observed in clinical studies and consumer surveys for these types of products. Additionally, the testing of additional products, focusing on ophthalmic solutions used in the eye or other products used in close proximity to the eye, will be conducted. IIVS would also like to commence the testing of potential sting-inducing ingredients of products. If you would like more information about the NociOcular assay and IIVS' efforts, please contact IIVS Study Director Kimberly Norman at knorman@iivs.org.