



## Corners

# CAATfeed

### **Martin Stephens of CAAT Receives Prestigious Russell and Burch Award at 9<sup>th</sup> World Congress in Prague**

Dr Martin Stephens of CAAT was honored by his peers with the Russell and Burch Award, presented by The Humane Society of the United States to scientists who advance alternative methods to animal testing and promote the 3Rs (Replacement, Reduction, and Refinement). The award is named in honor of William Russell and Rex Burch, the scientists who formulated the 3Rs approach and authored the classic text, *The Principles of Humane Experimental Technique* (available in its entirety on Altweb at [http://altweb.jhsph.edu/pubs/books/humane\\_exp/het-toc](http://altweb.jhsph.edu/pubs/books/humane_exp/het-toc)). Stephens was chosen as a clear standout for the award by his peers in the field as judged by 13 past winners. The award, which comes with a trophy and a \$10,000 prize, was presented at the 9<sup>th</sup> World Congress on Alternatives and Animal Use in the Life Sciences, which was held in Prague, August 24-28.

Dr Stephens has dedicated 30 years to promoting the 3Rs, first with animal protection organizations and more recently as an academic. He was an important figure in the development of the game-changing vision for *Toxicity Testing in the 21<sup>st</sup> Century: A Vision and a Strategy* and has been a tireless advocate for its implementation. This work has been a continuation of earlier efforts to promote alternative methods through scores of

publications and presentations. He was the driving force behind the AltTox.org website for its first several years. He is a bridge-builder and a door opener, championing the 3Rs in numerous influential bodies, both national and international, including his leading role in founding the International Council on Animal Protection in OECD Programmes (ICAPO).

### **CAAT's Georgina Harris Wins Young Scientists Award**

Georgina Harris, research technologist at CAAT and PhD student at the Bloomberg School of Public Health, received a Young Scientist award at the 9<sup>th</sup> World Congress in Prague. Georgina traveled to the event on a WC9 Travel Grant and an ASCCT Travel Award. We congratulate Georgina and all of the contributors on the recognition of this outstanding scientific work titled: "A human iPSC-derived 3D model for the assessment of gene/environment interactions during neuro-development."

### **New Glossary of Reference Terms for Alternative Test Methods and their Validation**

This new glossary, first published in ALTEX and now available on Altweb, provides technical references to support work in the field of alternatives to animal testing. Giving the ever-increasing number of alternative test methods and

approaches being developed over the last decades, a combination, revision, and harmonization of earlier published collections of terms used in the validation of such methods was required. With this glossary we intend to provide guidance on issues related to the validation of new or updated testing methods consistent with current approaches. Moreover, because of new developments and technologies, a glossary needs to be a living, constantly updated document. The Internet-based version of this compilation may be found on Altweb, and will accommodate new data and terms: [http://altweb.jhsph.edu/resources/validation\\_glossary.html](http://altweb.jhsph.edu/resources/validation_glossary.html)

### **CAAT Europe Policy Coordinator Francois Busquet Coordinating an MEP-3Rs Scientist Pairing Scheme**

MEPs will be paired with 3Rs scientists from the same country and nationality, who will then accompany them during their mandate. MEPs will benefit from the scientists' knowledge and, in turn, support local 3Rs initiatives/issues and – if relevant – refer them for EU-level discussions. Sirpa Pietikainen (Finland), Vice President of the Intergroup on Animal Welfare at the European Parliament, has agreed to take the leadership for this initiative.

The network will launch in early 2015 with a reception entitled "Meet your 3R-scientist" with yearly meetings to share best practices from each country.



## CAAT Events

### Symposium on Social Housing of Laboratory Animals

– October 5-6, 2014  
 – University of Colorado Anschutz  
 Medical Campus

Sponsored by CAAT, Office of Laboratory Animal Welfare (NIH), APHIS and AWIC (USDA), and University of Colorado, Denver

This symposium, which was held on the University of Colorado Anschutz Medical campus, brought together experts in animal behavior and welfare to address common issues in trying to achieve the mandate for social housing for social species. The first day focused on nonhuman primates and the second on ruminants, rabbits, rodents and pigs. Participants were encouraged to discuss special issues they are facing at their institutions.

### Green Toxicology: Application of Predictive Toxicology

– October 23, 2014  
 – Dübendorf, Switzerland

Organized by the Swiss Federal Laboratories for Materials Science and Technology (Empa) and CAAT.

Green toxicology is the application of predictive toxicology to the production of chemicals with the specific intent of improving their design for hazard reduction. The twelve principles of green chemistry outline a strategy to reduce hazard through molecular and process design. Reducing toxicity is at the core of green chemistry and sustainability, therefore the input of toxicologists early in the chemical enterprise is essential to inform the choices of molecular designers in selecting less hazardous design strategies. Information derived from mechanistic and computational toxicology forms the nexus of toxicology and green chemistry, and this conference provided a forum for collaboration among academia and industry working in complementary fields to discover common ground in the quest for safer chemicals. This workshop was sponsored by BASF, NanoCASE, Novartis, Roche, Sigma-Aldrich, and Syngenta.

### Progress on Replacement of Animals for Cosmetic Testing and Other Issues – and a 75<sup>th</sup> Birthday Party for Alan Goldberg

– November 20, 2014  
 – Baltimore, MD

2014 is a big year of change for CAAT, most evidently with Alan Goldberg's retirement from the organization he founded in 1981. A celebration honoring Alan's 75<sup>th</sup> birthday and all his groundbreaking work with CAAT will take place in Baltimore on November 20, 2014, along with an examination of progress made on the replacement of animals for cosmetic testing and other issues. Full details 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

Systematic review and related evidence-based approaches are beginning to be translated from healthcare to toxicology. They provide transparent, objective, and consistent tools to identify, select, appraise, and extract evidence across studies. The workshop, hosted by the Evidence-Based Toxicology Collaboration, will showcase these emerging efforts and address opportunities and challenges to the expanded use of these tools within toxicology.

Agenda and registration information may be found at: [http://caat.jhsph.edu/programs/workshops/ebtc\\_nov2014.html](http://caat.jhsph.edu/programs/workshops/ebtc_nov2014.html)

## Recent Publications

Estevan, C., Fuster, E., Del Río, E. et al. (2014). Organophosphorus pesticide chlorpyrifos and its metabolites alter the expression of biomarker genes of differentiation in d3 mouse embryonic stem cells in a comparable way to other model neurodevelopmental toxicants. *Chem Res Toxicol* 27, 1487-1495. <http://dx.doi.org/10.1021/tx500051k>

Pamies, D., Bal-Price, A., Fabbri, M. et al. (2014). Silencing of PNPLA6, the neuropathy target esterase (NTE) codifying gene, alters neurodifferentiation of human embryonic carcinoma stem cells (NT2). *Neuroscience*. <http://dx.doi.org/10.1016/j.neuroscience.2014.08.031>

Pamies, D., Hartung, T. and Hogberg, H. T. (2014). Biological and medical applications of a brain-on-a-chip. *Exp Biol Med (Maywood)* 239, 1096-1107. <http://dx.doi.org/10.1177/1535370214537738>

Pamies, D., Sogorb, M. A., Fabbri, M. et al. (2014). Genomic and phenotypic alterations of the neuronal-like cells derived from human embryonic carcinoma stem cells (NT2) caused by exposure to organophosphorus compounds paraoxon and mipafox. *Int J Mol Sci* 15, 905-926. <http://dx.doi.org/10.3390/ijms15010905>

Pamies, D., Vilanova, E. and Sogorb, M. A. (2014). Functional pathways altered after silencing Pnpla6 (the codifying gene of neuropathy target esterase) in mouse embryonic stem cells under differentiation. *In Vitro Cell Dev Biol Anim* 50, 261-273. <http://dx.doi.org/10.1007/s11626-013-9691-4>

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-e98892. <http://dx.doi.org/10.1371/journal.pone.0098892>

Sogorb, M. A., Pamies, D., de Lapuente, J. et al. (2014). An integrated approach for detecting embryotoxicity and developmental toxicity of environmental contaminants using in vitro alternative methods. *Toxicol Lett* 230, 356-367. <http://dx.doi.org/10.1016/j.toxlet.2014.01.037>



## News from the President of ECOPA on behalf of its Board

ECOPA General Assembly took place during the 9<sup>th</sup> World Congress on Alternatives and Animal Use in the Life Sciences in Prague where revised statutes were discussed. Final approval will take place at the next GA in 2015. Minutes of the GA meeting 2014 are available on request from the ecopa secretary (caat-eu-policy@uni-konstanz.de).

## News from the ECOPA members

### FRANCOPA

The French platform held its second semiannual board meeting: a new working group on the teaching and learning alternatives to animal testing will be launched in October. The second newsletter on vaccines and the 3Rs strategy will be released in October. FRANCOPA will release this winter its updated status report on alternatives to animal testing in France. The French trade association of the veterinary medicines and diagnostics manufacturers joined FRANCOPA. A workshop on alternatives methods will be co-organized by FRANCOPA and OPAL (<http://www.opal-association.org>) in October 2015.

### IPAM

An exhibition on the past, present and future of 3Rs will be held in November-December 2014.

Relevant publications on the topic are: Caloni, F., Ferrari, M. and De Angelis, I. (2014). Toxicology and stem cells: new frontiers. *ALTEX* 31, 94.

Romanelli, V., Costa, P., Granata, P. et al. (2014). Science and awareness: a journey inside the 3Rs. Didactic exhibition project. *ALTEX Proc* 3, 277.

### ZonMw

We would like to inform you about a new initiative organized by ZonMw, German Federal Ministry of Education and Research (BMBF) and Projektträger Jülich. Together we are launching a new call on “Innovative Systems Toxicology for Alternatives to Animal Testing,” in short InnoSysTox. More detailed information about this joint call can be found on the ZonMw website: <http://bit.ly/1nZLxOv>. A partnering tool is available through the Project Management Jülich website: <http://bit.ly/1sAV2ck>. All applications must be submitted via the ZonMw website.

In the Netherlands this joint call is part of the ZonMw “More Knowledge with Fewer Animals” program (MKMD).

#### *Application deadline*

December 16, 2014, 15:00 h

#### *Aim of the joint call*

- Development of innovative systems biology-based 3R methods in toxicology and/or
- Application of new and existing systems biology-based 3R methods in toxicology.

#### *Sums available*

A total budget of up to M€2.9 is available for the call. ZonMw will contribute up to M€1.4 of this sum and will allocate grants to the Dutch groups. BMBF will contribute up to M€1.5 and allocate grants to the German groups.

Each joint project consortium may apply for:

- a maximum budget of k€750;
- a joint project timescale of a maximum of three years.

If you have any questions, please contact the staff of the “More Knowledge with Fewer Animals” program at MKMD@zonmw.nl.

## 3R Research Foundation Switzerland

Logistics and organization of the submission of a Swiss national research program for 3R research support to the state secretary for education and research. The proposal is currently undergoing a feasibility evaluation.

### NORECOPA

The Norwegian consensus-platform Norecopa in collaboration with the USDA’s Animal Welfare Information Center (AWIC) has launched a new database called 3R Guide (<http://www.3RGuide.info>). The database is designed to give researchers an overview of all major guidelines, databases, information centers, journals and email discussion groups that are relevant to the 3Rs when planning animal research. All entries have been indexed by type (e.g. guidelines), category (e.g. blood sampling) and 3R relevance (Replacement, Reduction, Refinement). Free-text searches can also be performed. Searches in the 3R Guide automatically retrieve hits in Norecopa’s other databases, including TextBase (literature within laboratory animal science) and NORINA (3R alternatives to animal use in education and training). Norecopa is currently seeking funds to further develop the search engine.

## German National Consensus Platform SET

### *Funding of 3R Research Projects*

The German SET Foundation focuses on financial support of research projects which promote the development of alternative methods. Currently eight projects are being supported aiming at refinement, reduction and replacement of animal experiments.



1. *Development of an animal-free batch testing method for alum-adjuvanted Rabies vaccines*  
Dr Max Bastian, Paul-Ehrlich-Institut, Langen (Germany)
  2. *A review of German biomedical and animal research applications from 2010 to identify how refinements are used in experiments involving rodents*  
Kathrin Herrmann & Prof. Dr Heidrun Fink, FU Berlin (Germany)
  3. *Assessment of active accumulation of xenobiotics into milk with help of MDCKII-bABCG2 cells: A novel in vitro model of the lactating bovine mammary gland*  
Dr Sandra Halwachs, University of Leipzig (Germany)
  4. *Development and Characterization of an In Vitro Skin Model of Atopic Dermatitis*  
Dr Sarah Hedtrich, Freie Universität Berlin (Germany)
  5. *Analysis of toxicity induced cell degeneration in an organotypic porcine retinal culture*  
Dr Stephanie Joachim, University Eye Hospital, Ruhr-University Bochum & Dr Sven Schnichels, University Eye Hospital Tübingen (Germany)
  6. *Cell culture-based in vitro method for determining the activity of the botulinum toxin*  
Prof. Dr Gerhard Püschel, University of Potsdam (Germany)
  7. *Optimized cryopreservation of primary human hepatocytes for pharmacological and toxicological research*  
Dr Gesine Pless-Petig & Prof. Dr Ursula Rauen, University of Duisburg-Essen (Germany)
  8. *Establishment of a Hybrid Preparation Technique for the Simultaneous Investigation of Histology and Broncho-Alveolar Lavage Parameters of the Rat Lung*  
Prof. Dr Martin Wiemann, IBE R&D gGmbH Institute for Lung Health, Münster (Germany)
- Detailed descriptions of these research projects can be found on our homepage <http://www.stiftung-set.de/projects/projectlist.html>.

#### *Support of ALTEX*

The SET Foundation supports the ALTEX journal on a regular basis.

#### *Support of young scientists to participate in the 9<sup>th</sup> World Congress (WC9) in Prague*

To support the participation of young scientists in the “9<sup>th</sup> World Congress on Alternatives and the Use of Animals in the Life Sciences” the Alternative Congress Trust Germany and the German SET Foundation financed in a joint venture “Young Scientists Travel Awards.” Forty-one applicants for these grants received travel support in-kind such as waiving of the congress fee and accommodation costs. In addition the five best applicants were awarded reimbursement of their travel expenses. Among the 41 recipients of the travel grants 23 were from Europe and 18 from overseas. A special session with nine oral presentations “Session X-1 – Young Scientists Travel Award Short Presentations” was added to the program and well appreciated by the audience. In addition, two of the awardees gave invited presentations in other sessions. At the end of the special session, four young scientists were awarded with a Diploma for their excellent presentations: Gamze Ates (Vrije Universiteit Brussels, BE), Ellen van den Bogaard (Radboud University, Nijmegen, NL), Georgina Harris (Johns Hopkins University, Baltimore, USA) and Tobias Hasenberg (Technische Universität Berlin, DE).



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



## News from NICEATM and ICCVAM

### NICEATM Partners with PCRM to Hold Adverse Outcome Pathway Workshop

Scientists from 21 countries, representing industry, academia, regulatory agencies, and special interest groups, attended the workshop “Adverse Outcome Pathways: From Research to Regulation.” The workshop, held September 3-5 at NIH in Bethesda, Maryland, considered how the adverse outcome pathway (AOP) concept could improve regulatory assessments of chemical toxicity. NICEATM co-sponsored the workshop with the Physicians Committee for Responsible Medicine (PCRM).

Workshop plenary presentations were followed by breakout sessions that considered AOP applications, development of new AOPs, and challenges to AOP adoption. The closing session summarized the breakout group discussions and emphasized a need to maintain the collaborations and momentum that were generated.

Presentations and links to webcasts from the workshop will be posted on the workshop webpage <http://1.usa.gov/1qmW9rj>, with a workshop report to be published early next year.

### NICEATM and ICCVAM Activities at WC9

NICEATM scientists presented nine posters and three talks at the 9<sup>th</sup> World Congress on Alternatives and Animal

Use in the Life Sciences (WC9), which took place August 24-28 in Prague, Czech Republic. Topics of NICEATM presentations included development of curated reference databases and computational and high-throughput approaches to chemical screening and prediction of toxicity. In addition to the NICEATM presentations, ICCVAM committee members from the National Institute of Environmental Health Sciences and the U.S. Food and Drug Administration co-chaired five sessions and gave six platform presentations.

NICEATM also supported a workshop convened as a satellite meeting to WC9 to consider implementation and regulatory acceptance of *in vitro* alternatives to the murine histamine sensitization test (HIST) for safety testing of acellular pertussis vaccines.

Information on NICEATM and ICCVAM activities at WC9 is available at <http://ntp.niehs.nih.gov/go/41583>.

### ICCVAM Advisory Committee Meets

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) met on September 16 at NIEHS. SACATM advises NICEATM, ICCVAM, and the NIEHS director on ICCVAM activities.

ICCVAM members and NICEATM staff presented updates on NICEATM and ICCVAM activities and interactions, U.S. federal agency 3Rs activities, and

NICEATM workshops on aquatic models, alternatives for pertussis vaccine testing, and adverse outcome pathways to the Advisory Committee.

Presentations and other materials from the September 16 meeting are available on the NTP website at <http://1.usa.gov/1w5wsRU>.

### ToxCast Data Summit Highlights Data Use

NICEATM scientists joined over 200 representatives from industry, academia, regulatory agencies, and nongovernmental organizations for the second ToxCast Data Summit on September 29-30. The EPA's Toxicity Forecaster, or ToxCast™, program uses high-throughput screening assays to test chemicals for biological activity that may lead to adverse health effects. To date, over 2000 chemicals have been evaluated in 700 assays. The data from these tests are publicly available, and the ToxCast Data Summit was the first opportunity for researchers to present the results of research projects using this data.

NICEATM gave presentations on using ToxCast data to predict potential skin sensitizers and consideration of ToxCast data in the context of the estrogen and androgen receptor pathways. NICEATM presentations from the ToxCast Data Summit are available at <http://ntp.niehs.nih.gov/go/730879>.

Workshop organizers will soon post the slides and video from the meeting on



the workshop website <https://sites.google.com/site/toxcastdatasummit/final-materials> and will be developing a report from the workshop.

### Submissions for Tox21 Data Challenge Due November 14

The National Center for Advancing Translational Sciences (NCATS) is accepting submissions through November 14 for the 2014 Tox21 Data Challenge. The goal of the Challenge is to “crowd-source” analysis of Tox21 data by independent researchers to reveal how well they can predict compound interference

in biochemical pathways using only chemical structure data. The computational models produced from the Challenge could become decision-making tools for government agencies in determining which environmental chemicals and drugs are of the greatest potential concern to human health.

Information about the Challenge and a link to data downloads are available at <https://tripod.nih.gov/tox21/challenge/>. Challenge winners will be recognized on the NCATS website and via social media, and have an opportunity to submit a paper for publication in a special thematic issue of “Frontiers in Environmental Science.”

### NICEATM Publications

NICEATM scientists contributed to an evaluation of the ability of two high-throughput assays used in the Tox21 program to identify substances that interact with the estrogen receptor.

Huang et al. (2014). Profiling of the Tox21 10K compound library for agonists and antagonists of the estrogen receptor alpha signaling pathway. *Nature Scientific Reports* 4, 5664. <http://dx.doi.org/10.1038/srep05664>



## IIVS News & Views

### China FDA Signs MOU with US Laboratory to Promote Non-Animal Testing Methods in China

The Institute for In Vitro Sciences (IIVS) signed a Memorandum of Understanding with the National Institute for Food and Drug Control (NIFDC)<sup>1</sup>, a subordinate agency of the China Food and Drug Administration (CFDA) in June of this year. The two organizations will work collaboratively on a number of projects focused on cosmetic safety testing designed to promote *in vitro* (non-animal) techniques in China.

In November 2013, IIVS held a week long training workshop at the NIFDC laboratory in Beijing to teach *in vitro* techniques for cosmetic safety testing to Chinese regulators including those from provincial agencies. Under the new memorandum, the NIFDC will coordinate with IIVS to hold an annual training workshop titled “Techniques for In Vitro Assays for Cosmetics Testing”. Additionally the NIFDC agrees to participate in validation projects of new models or techniques developed within China.

The MOU was signed by Dr Wang Youchun, Deputy Director-General of NIFDC and Erin Hill, Co-founder and

Vice President of IIVS. “The NIFDC has a great responsibility to introduce *in vitro* techniques to a large number of regulators within China” states Ms Hill. “IIVS is honored to assist them in achieving this goal. Additionally our expertise in validation projects will be helpful as new *in vitro* methods are developed within China.”

Since 2012, the Institute for Food and Cosmetic Control (IFCC) within the NIFDC has been responsible for the testing of cosmetics for pre-market approval, post market surveillance, and investigations in response to consumer complaints. A new *in vitro* laboratory is currently un-

<sup>1</sup> NIFDC areas of responsibility cover quality control of pharmaceutical products, biological products, medical devices, food, healthy food, cosmetics, reference standards, laboratory animals, and drug safety evaluation. The Institute for Food and Cosmetics Control (IFCC), an internal institute of NIFDC, is responsible for safety evaluations of food, healthy food and cosmetics.



der construction which will be used for, among other things, hands-on training. Dr Wang Gangli, Director of the IFCC commented, "With our expanded laboratory space and support from IIVS experts, we will be able to organize workshops to train many more scientists within China on the use of *in vitro* methods for testing of cosmetics."

Following the signing ceremony IIVS visited the laboratories of the NIFDC center responsible for the testing of drugs, the National Center for Safety Evaluation of Drugs (NCSED). The center Director, Dr Wang Jufeng, and staff discussed potential collaborations and uses of *in vitro* methods in the evaluation of drugs within China.

### Dr Holger Behrsing Joins IIVS

IIVS is pleased to announce the addition of Dr Holger Behrsing as Principal Scientist. Dr Behrsing will largely be responsible for developing and optimizing *in vitro* methods for the new inhalation toxicology program at IIVS. IIVS is expanding its expertise to meet the growing needs of the chemical, consumer and household products industries, for industrial hygiene support, and anticipated tobacco product regulatory requirements for inhalation and respiratory toxicology. In addition to developing laboratory programs, Dr Behrsing will liaise with stakeholder groups to coordinate technical workshops and symposia to explore the use of *in vitro* methods for inhalation toxicology purposes.

Dr Behrsing completed his doctoral degree in pharmacology and toxicology at the University of California, Davis and subsequently accepted a post-doctoral appointment at SRI International where he developed *ex vivo* liver and lung slice technologies. Dr Behrsing joined SAIC-Frederick (now Leidos Biomedical) to lead the Predictive Toxicology Laboratory where he facilitated transfer of the slice technology to the NCI and developed a range of *in vitro* models to test chemotherapeutic agents. Dr Behrsing has expertise establishing and working with a variety of cell lines, primary cells

and tissues including those of human origin. IIVS looks forward to Dr Behrsing's contributions in applying novel endpoint methods to complement our considerable cell and tissue culture platforms.

### International Training Program Presented in Vietnam

While many countries have implemented *in vitro* methods to determine the safety of cosmetic and personal care products, some still rely heavily on animal testing. Recently some of these countries have shown an interest in moving toward more predictive and relevant *in vitro* test methods. There is a particular interest in adopting those methods that have been evaluated and deemed acceptable by the Organization for Economic Cooperation and Development (OECD). Often, laboratories and government regulators require training in the science behind *in vitro* methods, and help in understanding how to interpret the data from these new systems. Recognizing this need, IIVS works with a variety of collaborators to help fund its training programs aimed at assisting these countries in the adoption of *in vitro* methods.

Recently, IIVS scientists traveled to Ho Chi Minh City, Vietnam to visit the Ministry of Health (MOH). IIVS Study Directors, Allison Hilberer and Nathan Wilt, met with the directors and employees of MOH for introductory lectures and hands-on training on the Bovine Corneal Opacity and Permeability (BCOP) assay, an *in vitro* method to assess eye irritation. The laboratory and lecture work also included discussion of specific case studies that will provide practical knowledge for the MOH group to use during their post-market testing of a number of different product types.

This was the first experience that many of the participants had with the BCOP assay. The group, largely from the pharmacology department, showed good laboratory techniques and were eager to begin implementing the method on their own. The trip was funded by Cruelty Free International (CFI). Attending on behalf of CFI was Nick Palmer, who also gave a

presentation on the use of non-animal testing. A critical piece of equipment for the assay was generously donated by BASF. IIVS will continue to provide support to the Vietnam MOH group as they begin to utilize the BCOP assay in their laboratory.

### IIVS Provides Training during the Second Annual Guangzhou Alternatives Conference

The 5 day conference, organized by Dr Shujun Cheng of the Guangdong Inspection and Quarantine Bureau (GCIQ), provided lectures on *in vitro* techniques to over 90 participants from industry and the Chinese regulatory community. An essential component of the meeting was to provide hands-on training in a variety of methods for roughly 20 attendees. IIVS scientists, funded by a grant from the Humane Society International, The Humane Society of the United States and the Human Toxicology Project Consortium, provided training on the BCOP and 3T3 Phototoxicity Assay. Scientists from the CIQ provided training on the CAMVA assay and L'Oréal introduced skin irritation testing with their 3D tissue construct.

Following the training in China, IIVS Senior Scientist, Dr Quanshun Zhang, traveled with Humane Society International (HSI) representatives to address law makers in Japan and Taiwan. The goal of these meetings was to give policy makers a global perspective on the legislative, scientific and corporate efforts regarding the use and acceptance of non-animal testing methods.

### The Reconstructed Skin Micronucleus Assay

Why do we need a new genotox assay? A number of non-animal test methods exist for genotoxicity testing and, in fact, several were among the first *in vitro* assays standardized for regulatory approval. These methods, e.g., the Ames assay, mouse lymphoma assay, and *in vitro* micronucleus test, are well



characterized and understood, have been included in many publications, and are used by regulatory agencies throughout the world. They are often the first step in the genotoxicity testing of cosmetic ingredients. However, these tests are very sensitive and are known to often provide “false,” or misleading, positive results. Since this high false positive rate is well known, if initial tests show the material is potentially genotoxic, many companies perform further investigations using animal-based genotoxicity assays, which are less likely to produce false positive results.

However, the EU’s 7<sup>th</sup> Amendment to the Cosmetics Directive, effective as of March 2009, prohibits the use of *in vivo* genotoxicity assays to assess the safety of cosmetic ingredients. Thus many ingredients that are safe might be unavailable for use in cosmetics because they were deemed “positive” in the first tier *in vitro* battery and cannot be retested in the less sensitive animal assay. Developing a new *in vitro* genotoxicity assay that is more predictive of human response and is relevant to cosmetics use thus became extremely important.

This line of investigation led to the development of the Reconstructed Skin Micronucleus Assay (RSMN) by IIVS and the Procter & Gamble Company.

In the RSMN, the test material is dosed onto the topical surface of a three dimensional human skin tissue construct (EpiDerm™, MatTek Corporation, Ashland, MA), similar to the way a human

cosmetic user might be exposed through skin application. After treatment and a suitable time for cell recovery, cells are collected by trypsinization of the tissues. By exposing the tissue to cytochalasin B (a cytokinesis blocker) along with the test material, actively dividing basal cells within the EpiDerm tissue can be identified as binucleated cells, i.e. proof that they have undergone a nuclear division. These binucleated cells are evaluated microscopically to identify micronuclei that result from DNA damage. This protocol is similar to a previously used rodent skin micronucleus method (Nishikawa et al., 2005, *Mutat Res* 588, 58-63).

IIVS is participating – along with partners which have included the Procter & Gamble Company, BioReliance, and L’Oréal – in a prevalidation of the assay sponsored by Cosmetics Europe. Previously the assay had been shown to have good intra- and inter-laboratory reproducibility. Current efforts focus on evaluation of a broad range of chemicals, e.g., expanding the database to include substances which require metabolic activation. Twenty eight chemicals have been tested thus far and data review is currently underway.

Because the assay has shown initial promise, IIVS has worked with scientists from the FDA Center for Food Safety and Applied Nutrition (FDA CFSAN) to provide them with technical training. Scientists from the FDA CFSAN visited IIVS for several days to

receive hands-on training on our protocol for the method. The FDA scientists have since been able to set up their own laboratory (based on our specifications), and worked our protocol into an internal SOP for the method. Their early work has also been promising indicating that they were able to successfully transfer the method into their laboratory.

## Recent Publications

### *The Role of the Study Director in Non-clinical Studies*

IIVS’ Gertrude-Emilia Costin and Hans Raabe recently co-authored the chapter “In Vitro Toxicology Models” in the book *The Role of the Study Director in Nonclinical Studies: Pharmaceuticals, Chemicals, Medical Devices, and Pesticides* edited by William J. Brock, Barbara Mounho and Lijie Fu and published by Wiley. More information about the book can be found on the Wiley publishing website.

### *Handbook of Toxicology*

Hans Raabe served as co-author with industry consultant Dr John Harbell for a chapter in the third edition of the *Handbook of Toxicology* titled “In Vitro Methods for the Prediction of Ocular and Dermal Toxicity” edited by Michael J. Derelanko and Carol S. Auletta and published by CRC Press. More information about this book can be found on the CRC website.





# IUF

LEIBNIZ RESEARCH  
INSTITUTE FOR  
ENVIRONMENTAL  
MEDICINE

## Relevance of alternative methods for research in environmental medicine

The IUF – Leibniz Research Institute for Environmental Medicine, founded in 2001, is since 2014 a member of the Society ALTEX Edition. The IUF analyzes how environmental factors damage human health. In this regard it focuses on ambient relevant particles / particulate matter including nanoparticles, non-ionizing radiation as well as chemicals. The IUF investigates how these environmental noxae cause premature aging of the brain, the lung, the cardiovascular system and the skin, and whether and how they are immunotoxic or cause (developmental) neurotoxicity. The research at the IUF, which is conducted by 14 groups made up of over 120 researchers and made possible by a ca. 9 M€ annual research budget serves to improve risk assessment and the development of novel molecular preventive measures. This research mission implies a holistic assessment of the environmental burden that individuals are exposed to, and also of the reaction and modulation of the human system.

As both improved risk assessment and the development of novel molecular preventive measures aim to protect humans, the IUF is particularly concerned about the development of experimental model

systems which are of clear-cut relevance to human physiology. In this regard, it should be noted that with the emergence of transgenic animals the hope was awakened to have found a robust tool for understanding human physiology. Thus, transgenic animals have become the predominant tools in many research areas of environmental medicine. While they are to some extent indeed helpful for mechanistic understanding of toxicity pathways of environmental noxae, it has been more and more realized that species differences in pharmaco-/toxicokinetics and -dynamics between humans and animals prohibit meaningful extrapolation in cause – effect relationships between species. In fact, it is barely possible to predict the responses of humans towards pharmaceuticals and chemicals by extrapolation from studies with rodents. As an example, at least 20% of pharmaceuticals have to be taken out of the market as they induce severe side effects in human patients without any indications of these adverse reactions in test animals.

The IUF therefore focuses on the development and use of novel alternative methods, e.g., 3D human cell cultures, as we are convinced that the field can best benefit from human-relevant systems, which are functional, properly reflect human physiology and can mirror human diversity. Such organoid cultures are also genetically modified for human-relevant

pathway analyses. In addition to the cell-based *in vitro* methods, the IUF utilizes *C. elegans* as an *in vivo* model organism confirming pathway-related phenotypes caused by environmental exposures. To pave the way for the future, the IUF currently also works with induced pluripotent stem cells with the intention to study adverse effects of environmental exposure on disease models as well as genetic susceptibility. The combination of these models with omics techniques, medium throughput and high content imaging approaches opens the door to a human-relevant and holistic evaluation of risks to human well-being and the development of intervention strategies conserving human health.

The human 3D neurosphere model, which involves primary human neuronal progenitor cells and has been developed at the IUF, is an outstanding example for the efforts and success of the IUF regarding the development of human-relevant methodologies. This *in vitro* system can be employed to examine neurodevelopmental as well as neurotoxicity of compounds, and is also useful for investigating environmentally-driven brain aging.

In aggregate, our strong and growing interest in using non-animal, human-relevant methodologies was the reason the IUF has chosen ALTEX as its official organ.