News from the American Society for Cellular and Computational Toxicology

This summer and fall has already been a busy time for the ASCCT. In June, Dr Nicole C. Kleinstreuer, Postdoctoral Fellow at EPA's National Center for Computational Toxicology, presented her work on the Virtual Embryo Project, which focuses on elucidating developmental toxicity pathways to inform new predictive tools. In August, Dr Russell S. Thomas, Director of the Institute for Chemical Safety Sciences and the Center for Genomic Biology and Bioinformatics at the Hamner Institutes for Health Sciences led an interesting discussion of his work on the use of high-throughput *in vitro* screens for hazard and risk assessment.

The first annual meeting of ASCCT was held September 21 at the Lister Hill Auditorium, National Library of Medicine, National Institutes of Health in Bethesda, MD. This location helps to accomplish the ASCCT goal of including regulators in its activities. Participants from government, industry, testing laboratories, and animal protection NGOs gathered to view plenary and member lectures, a poster session, and a cocktail reception. Presentations included both overviews of the state of the art of the regulatory applicability of new approaches to toxicology and detailed presentations of new methods. The intimate setting provided opportunity for frequent and spirited discussion amongst the attendees.

Attendees were welcomed by ASCCT president Rodger Curren of the Institute for In Vitro Sciences, then treated to a plenary lecture by Melvin Andersen of the Hamner Institutes for Health Sciences. Dr Andersen provided an overview of the evolution of *in vitro* and *in silico* approaches, in the context of the National Academies' *Toxicity Testing in the 21st Century: A vision and strategy* report, released five years ago. He also discussed some recent case studies underway at the Hamner that are intended to put the NAS vision into action. Susanne Fitzpatrick of the Food and Drug Administration next presented an overview of coordinated efforts between the FDA, the National Institutes of Health, and the Defense Advanced Research Projects Agency (DARPA) to provide funding for human cell-derived 3D tissues and organs on chips, using microfluidic technology. The projects that received funding will work in a coordinated and collaborative manner, working with the FDA to ensure the optimized assays are appropriate for regulatory use. Catherine Willett of the Humane Society of the United States provided an overview of the concept of Adverse Outcome Pathways (AOPs) and gave examples that are being developed at the Organization of Economic Coordination and Development, by the US EPA and others. Adverse Outcome Pathways are "conceptual construct(s) that portrays existing knowledge concerning the linkage between a direct molecular initiating event (e.g., a molecular interaction between a xenobiotic and a specific biomolecule) and an adverse outcome at a biological level of organization relevant to risk assessment" (Ankley et al., 2010, *Environ Tox Chem, 29*, 730-741). AOPs can be written in manuscript form, or portrayed graphically in tools like Effectopedia.

Dr Willett's talk was one of three selected from submitted abstracts. Two other presentations were given from submitted abstracts, by Can Jin of ACEA Biosciences and Rohan Kulkarni of Bioreliance by SAFC. Dr Jin presented her research applying label-free impedance-based Real Time Cell Analysis technology to monitor native endocrine signaling pathways in human cells, and Dr Kulkarni presented his work quantifying a high-throughput micronucleus assay in CHO cells by flow cytometry.

A poster session drew colleagues from all over the US and as far away as Japan. Presenters covered research and devel-

opment of cellular and computational methods for skin and eye irritation, sensitization, genotoxicity, cell transformation, and neurotoxicity. A few attendees presented policy approaches to reduction of animal use in testing.

ASCCT members also held their first in-person all-member business meeting, with an election of three new members of the Board of Directors and Officers. Another business meeting by web conference and an online election will be held for members who could not travel, and the results of the Board election will be published online in November.

Following the meeting, founding member the Alternatives Research & Development Foundation (ARDF) sponsored a lovely reception. Sue Leary, president of ARDF, presented the William and Eleanor Cave Award to Melvin Andersen. Dr Andersen received the award, which carries a $ 5000 prize, for his personal evolution from animal toxicologist to visionary leader of the toxicity testing for the 21st century paradigm shift.

Finally we are pleased to announce that membership in the Society passed 100 this summer. The Society also gained two additional organizational sponsors: People for the Ethical Treatment of Animals and Research Institute for Fragrance Materials. Visit http://www.ascctox.org to find out how you can become part of this dynamic and growing community of scientists and policy makers dedicated to advancing cellular and computational methods for toxicity testing.

CAAT*feed*

Joint ALEXANDRA Association/ BASF/Beiersdorf/L'Oréal CAAT Information Day on Organotypic 3D Cell Culture Models and Engineered Tissues: October 25, 2012

The first 3D cultures have been established to better mimic *in vivo* with respect to functional and physiological parameters than classical 2D *in vitro* models. Now, engineered tissues and 3D-cell cultures modeling the liver or aspects of the nervous system, the skin, the respiratory system, the intestine, the heart, and other organs are employed more and more in research. However, the use in toxicological research is still relatively limited, and only few of these systems have found regulatory acceptance.

3D models pose technical challenges in addition to those of 2D cell cultures. They are based on a more complex experimental set-up, which necessitates the use of modified or different endpoints compared to 2D cultures. This information day will focus on the applicability of 3D systems to model human physiology and pathophysiology with particular focus on toxicological risk assessment. The systems presented will include hepatic, neuronal, lung, and skin models. Besides an in-depth discussion of available systems, the way forward and hurdles to regulatory acceptance will be highlighted.

Speakers will include representatives from:

- Academia from Europe and USA
- Multinational chemical and cosmetic companies
- Animal welfare organizations

Presentations will be followed by a discussion to address questions of the audience. The complete program will be available for viewing on the CAAT-Europe and CAAT websites: http://caat.jhsph.edu/

The Information Day will be preceded by a workshop on the topic (October 22-24, by invitation only) on the same topic and the CAAT-Europe board meeting on October 24th.

1st European Meeting of the CAAT Refinement Working Group, Konstanz, October 26, 2012

CAAT-Europe announces the inaugural meeting of the European Refinement Initiative on Friday, October 26, 2012 at the Steigenberger Inselhotel in Konstanz, Germany from 10:00-18:00. Representatives from pharmaceutical industries, contract research organizations, and animal breeders are invited to share non-competitive information and discuss ways to refine procedures involving animals. The topics could include housing, enrichment, anesthesia/analgesia, humane endpoints, procedures, biomarkers, imaging, behavior assessment, and

personnel training. For more information or registration for future meetings, please contact Nina Hasiwa at: caat-eu@uni-konstanz.de

CAAT/ESTIV/IVTIP Pre-congress Meeting to the Annual ESTIV Conference 2012 on "The Economics of Alternative Methods," Lisbon, Portugal, October 16, 2012 (13:30-16:30)

Organized jointly between ESTIV, CAAT, and IVTIP and chaired by Thomas Hartung (CAAT Director) and Greet Schoeters (ESTIV President) the meeting will feature:

- *The most important -omics is economics* (Thomas Hartung, Johns Hopkins, USA);
- *Economics of animal testing within REACH* (Costanza Rovida, CAAT-Europe, Italy);
- *Reducing economical constraints to enhance the industrial applicability of in vitro testing* (Erwin Roggen, Novozymes, Denmark);
- *Towards cost reduction of 3D human tissue models: possible ways forward based on an Open Source concept* (Bart De Wever, The ALEXANDRA Association, Monaco);
- *Reducing the costs and the number of animals by the use of in vitro test methods supporting grouping, waiving, and read-across* (Albert Poth, Harlan Cytotest Cell Research, Germany);
- *The economics of alternative methods relative to in vivo testing: pros and cons* (Bennard van Ravenzwaay, BASF, Germany);
- *In vitro efficacy testing of anti-cancer drugs in canine primary cell culture models: a cost-effective approach to increase drug target selection for cancer treatment in dogs and humans* (Robert Barthel, Oncobiotek, France);
- *Impact of toxicity testing on the costs versus benefit balance and possible ways forward* – a roundtable discussion

For information: http://www.estiv2012.com/precongressworkshop.htm

CAAT/Istitute Superiore di Sanità/Italian Platform for Alternative Methods (IPAM) Joint International Symposium on "Alternative *In Vitro* Methods to Characterize the Role of Endocrine Active Substances (EASs) in Hormone-targeted Tissues," Rome, Italy, December 17, 2012

The CAAT-IPAM-ISS symposium will focus on the use of human cell cultures in the field of endocrine disruption with the main goal of promoting the assessment and validation of alternative methods to animal experimentation in agreement with the requirements of Directive 2010/63/EU and REACH policy, and to support different fields of investigation such as toxicology, environmental sciences, and biomedical research.

The CAAT-IPAM-ISS symposium will be open to scientists from academia, regulatory bodies, and stakeholders; participation of young scientists and PhD and undergraduate students will be favored by waiving the registration fee.

Registration deadline: November 12, 2012

For information:
http://www.nature.com/natureevents/science/events/17365-Alternative_in_vitro_methods_to_characterize_the_role_of_Endocrine_Active_Substances_EASs_in_hormone_targeted_tissues
or:
http://www.iss.it/binary/ampp/cong/CAAT_IPAM_ISS_symposium_First_Announcement_1Ago12_v4.pdf
Contact:
Dr Laura Narciso, Department of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy, Tel. 064990-2512, Fax 064990-3014, e-mail: laura.narciso@iss.it

CAAT to receive $ 2 million sub-grant to Wake Forest University from the US Defense Threat Reduction Agency

In context of recently intensified US funding for "human-on-a-chip" approaches, CAAT will receive funding in a consortium led by Wake Forest University. The project aims to develop a body-on-a-chip platform (INtegrated Organoid Testing System, INGOTS), a modular, scalable, microfluidic system with multi-tissue system integration (mimicking human organ function) to be achieved through the creation of re-sealable microfluidic devices capable of cell culture and analysis. The predicted budget for CAAT is $ 2.1 million (direct plus indirect). A key role for CAAT will be to provide advice to the consortium on quality assurance and validation of the systems to be developed. Furthermore, metabolomics measurements and mitigation strategies for identifying alternative cell systems will be supported.

The work is closely related to the recent article: Hartung T. and Zurlo, J. (2012). Alternative approaches for medical countermeasures to biological and chemical terrorism and warfare. *ALTEX 29*, 251-260 (available on the ALTEX and Altweb websites).

New supporters and Advisory Board members

CAAT welcomes two new supporters and members of the advisory board: David K. Wilcox, President of the Research Institute for Fragrance Materials (RIFM) and Pierre Sivac, President of the International Fragrance Association (IFRA).

Job opening: Post-Doctoral researcher (Human Toxome Project)

CAAT is involved in several scientific projects implementing the vision of *Toxicity Testing in the 21st Century* as formulated by the 2007 National Research Council report. The center is leading the NIEHS-funded Human Toxome Project, which is a collaborative effort between CAAT, Georgetown University, The Hamner Institute, Agilent, and the US Environmental Protection Agency (EPA) to map pathways of toxicity. CAAT is seeking a postdoctoral researcher to analyze and integrate multiple data streams

including high-throughput screening data from the EPA ToxCast project.

Experience: The candidate will develop/use computational tools to identify and evaluate putative predictors of toxic effects due to endocrine disruption. Required skills include empirical analysis of using HTS, metabolomics, and proteomics data using machine learning methods and pathway analysis using in-house and publicly available tools. Demonstrated experience with computer programming using open-source tools (R and/or Python) is a must.

Posted Qualifications: PhD in computational biology/toxicology, bioinformatics, physics, computer science, mathematics, or related field. Have some knowledge of pathway analysis approaches and experience in applying machine learning techniques to noisy data sets. Highly motivated and a self-starter, with the ability to work efficiently with minimal supervision. Additional education may substitute for required experience and additional related experience may substitute for required education, to the extent permitted by the JHU equivalency formula.

Location: The position is located in Research Triangle Park, North Carolina, at the National Center for Computational Toxicology of the US Environmental Protection Agency (EPA) and partly at the Johns Hopkins Center for Alternatives to Animal Testing (CAAT), in Baltimore, Maryland, USA.

Contact: Andre Kleensang:
akleensa@jhsph.edu

CAAT met with European Commission's Chief Scientific Adviser Anne Glover in June 2012

Anne Glover is a Scottish biologist who was appointed as the European Commission's first-ever Chief Scientific Adviser in December, 2011. She met with Thomas Hartung and Francois Busquet for one hour to discuss the Human Toxome project and related matters.

Thomas Hartung now Editorial Board member of PeerJ Open Access journal

CAAT Director Thomas Hartung is now an editorial board member of PeerJ, a recently launched Open Access journal. PeerJ is an inclusive, broad-based journal publishing across all of the biological and medical sciences, and is aimed at dramatically lowering the financial barriers for authors to publish their work in a high-quality, open access venue. Instead of charging authors a fee to publish each article (as is common with most open access publishers), PeerJ operates with a lifetime membership model, allowing authors to publish throughout their careers. Only articles that are soundly conducted and correctly reported will be published after being subject to rigorous peer review.

Paul Locke appointed to Council of the National Academy of Sciences Institute for Laboratory Animal Research (ILAR)

Paul Locke, Director of CAAT's Policy Program, was recently appointed to the Council of the National Academy of Sciences Institute for Laboratory Animal Research (ILAR). ILAR evaluates and reports on the scientific, technological, and ethical use of animals and related biological resources, and on non-animal alternatives in non-food settings, such as research, testing, education, and production of pharmaceuticals.

Martin Stephens appointed to Board of Chimp Haven

Senior Research Associate Martin Stephens has been appointed to the board of directors of Chimp Haven, a nonprofit organization that operates a sanctuary near Shreveport, Louisiana for chimpanzees formerly used in research and entertainment. The organization was founded in 1995 to establish a setting in which chimpanzees could live in large social groups in spacious, outdoor habitats.

Following passage of the 2000 CHIMP Act, which called for the establishment of a national sanctuary system for chimpanzees no longer needed in biomedical research, the government selected Chimp Haven to run the mandated national chimpanzee system. The National Institutes of Health, which has funded much of the biomedical research on chimpanzees over the years, provided partial support for the building of Chimp Haven sanctuary in 2002.

Stephens, who joined the CAAT staff last year, had been an advocate for chimpanzees in laboratories in his earlier work with The Humane Society of the United States. According to Stephens, "The least we owe these individuals for decades of confinement and experimentation is a comfortable retirement in a naturalistic setting."

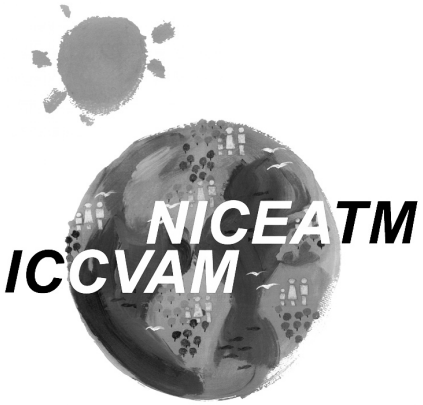
Recent publications

Balmer, N. V., Weng, M. K., Zimmer, B., et al. (2012). Epigenetic changes and disturbed neural development in a human embryonic stem cell-based model relating to the fetal valproate syndrome. *Hum Mol Genet 21*, 4104-4114. Epub 2012 Jun 20.

Weng, M. K., Zimmer, B., Pöltl, D., et al. (2012). Extensive transcriptional regulation of chromatin modifiers during human neurodevelopment. *PLoS One 7*, e36708. Epub 2012 May 9.

Zimmer, B., Lee, G., Balmer, N. V., et al. (2012). Evaluation of developmental toxicants and signaling pathways in a functional test based on the migration of human neural crest cells. *Environ Health Perspect 120*, 1116-1122. Epub 2012 May 9. PMID: 22571897

Leist, M., Hasiwa, N., Daneshian, M., and Hartung, T. (2012). Validation and quality control of replacement alternatives – current status and future challenges. *Toxicol. Res 1*, 8-22. DOI:10.1039/C2TX20011B (Review Article)



News from NICEATM and ICCVAM

U.S. Agencies accept ICCVAM-recommended *In Vitro* Estrogen Receptor Testing Methods

U.S. Federal agencies have agreed with recommendations made by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) on test methods that use human cells to screen substances for their potential to interact with the estrogen receptor. ICCVAM evaluated the scientific validity of the BG1Luc estrogen receptor (ER) transactivation (TA) agonist and antagonist assays and recommended how they could be used to identify substances that induce or inhibit human ER activity *in vitro*. The ICCVAM recommendations were based on data from an international validation study coordinated by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).

The U.S. Environmental Protection Agency (EPA) responded to the ICCVAM recommendations by noting that they regard the BG1Luc ER TA test method as an alternative to the ER TA test method currently used in their Endocrine Disruptor Screening Program (EDSP). Several Federal agencies indicated that they would communicate the ICCVAM recommendations to stakeholders and encourage their appropriate use. The BG1Luc ER TA test methods are examples of those advocated by the National Research Council's 2007 report on Toxicity Testing in the 21st Century, which emphasizes methods that can detect perturbations in toxicity pathways.

Endocrine-active substances cause adverse health effects by interfering with the biochemical pathways that constitute normal hormone function. Evidence suggests that exposure to endocrine-active substances may cause reproductive and developmental problems in humans and wildlife. Such exposure may also increase cancer incidence in humans.

Through the EDSP, the EPA screens pesticides and environmental contaminants for their potential to affect the endocrine systems of humans and wildlife. At the request of EPA, NICEATM and ICCVAM reviewed the validation status of *in vitro* test methods that might be suitable for inclusion in the EDSP and developed guidance for future validation studies.

NICEATM then conducted validation studies of *in vitro* test methods that could identify potential endocrine-active substances without using animals. The BG1Luc ER TA agonist and antagonist assays, also known as the LUMI-CELL® ER test methods, were the subjects of one of these validation studies. NICEATM coordinated an international validation study of the BG1Luc ER TA agonist and antagonist assays at laboratories in Europe, the United States, and Japan.

Based on the results of the NICEATM-sponsored validation study, ICCVAM recommended that the BG1Luc ER TA test method could be used as a screening test to identify substances with *in vitro* ER agonist activity or ER antagonist activity. The EPA agreed with the ICCVAM conclusion that the accuracy of this assay is at least equivalent to that of the method currently used in the EDSP Tier 1 screening battery and indicated that they regard the BG1Luc ER TA test method as an alternative to the current method.

NICEATM also nominated the BG1Luc ER TA test methods for evaluation in the U.S. government's Tox21 program. The assays have now been adapted to a high throughput format using 1536-well plates by the National Center for Advancing Translational Sciences and have been used to screen all compounds in the Tox21 10K chemical library consisting of over 9,000 chemicals, pharmaceuticals, pesticide ingredients, and other substances.

More information about the ICCVAM evaluation of the BG1 test method is available on the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/methods/endocrine/end_eval.htm

ICCVAM seeks comments and data on nominated methods

ICCVAM recently requested public comments, nominations of experts, and data submissions on nominated test methods for three safety testing applications.

In Vitro Test Method to Assess Allergic Contact Dermatitis Hazard Potential

The electrophilic allergen screening assay (EASA) is an *in vitro* test method that may be useful for identification of substances with the potential to produce allergic

contact dermatitis (ACD). NICEATM recently requested public comments on the EASA, which was nominated for validation studies to evaluate its usefulness and limitations as a screening assay to identify potential sensitizers. NICEATM also requested data generated using *in vivo* and *in vitro* test methods for assessing ACD hazard potential. Data will be used to develop integrated testing and decision strategies that will also consider incorporation of the EASA following adequate validation studies.

Sensitizers are substances with the potential to cause ACD, and skin sensitization is the process by which a sensitizer induces the development of ACD. The initial molecular event in the pathway leading to skin sensitization involves binding of the potential sensitizer to proteins in the skin. The EASA identifies a potential sensitizer by measuring binding of a test substance to chemical probes that contain structures commonly found in skin proteins.

The nomination of the EASA for validation studies was considered at the recent meeting of the ICCVAM advisory committee (see article below); the advisory committee agreed with ICCVAM's draft high priority for the proposed studies.

For more information about the NICEATM-ICCVAM evaluation of the EASA, please visit the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/methods/immunotox/EASA.htm

Up-and-Down Procedure for Acute Dermal Systemic Toxicity Testing

NICEATM and ICCVAM are planning to convene an independent scientific peer review panel to assess the validation status of an up-and-down procedure (UDP) for acute dermal systemic toxicity testing. NICEATM requested nominations of scientific experts to be considered for the panel and data for substances tested in *in vivo* acute dermal and oral systemic toxicity tests.

Poisoning by dermal exposure (absorption through the skin), while not as common as poisoning by ingestion, accounted for over 172,000 poisonings in the U.S. in 2010. Alternative test methods for acute dermal systemic toxicity testing are an ICCVAM priority because such testing is required by multiple U.S. regulatory agencies, can involve large numbers of animals, and can result in significant pain and distress to test animals. NICEATM is developing a UDP procedure for acute dermal systemic toxicity testing, which is one of the four most commonly conducted product safety tests worldwide. If accepted, this procedure could reduce the number of animals required for this testing compared to current guidelines.

For more information about the NICEATM-ICCVAM evaluation of the dermal UDP, please visit the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/methods/acutetox/udp-dermal.htm

In Vitro Test Methods to Assess Eye Injury Hazard Potential

NICEATM and ICCVAM are planning to convene an independent scientific peer review panel to assess the validation status of *in vitro* tests and integrated non-animal testing and decision strategies proposed for identifying eye injury hazard potential of chemicals and products. NICEATM has requested nominations of scientific experts who can be considered for the panel. NICEATM has also requested data from substances tested in *in vitro* tests for identifying eye injury hazard potential. Of particular interest are data generated in the short time exposure (STE) and isolated rabbit eye (IRE) tests and data from approaches using two or more *in vitro* tests. However, NICEATM also requests data from other *in vitro* tests, such as BCOP and HET-CAM, as well as corresponding *in vivo* data from any ethical human or animal studies or accidental human exposures.

Past ICCVAM evaluations of *in vitro* eye safety test methods have supported national and international acceptance of several methods that can be used to obtain eye hazard classification data on chemicals and products without using animals. NICEATM and ICCVAM are currently evaluating additional *in vitro* test methods for their potential usefulness for this purpose. The IRE test is an organotypic test method that evaluates the eye injury potential of a test substance by measuring corneal opacity, corneal swelling, epithelial integrity, and fluorescein staining. The STE test measures the viability of cultured cells from an established rabbit corneal epithelial cell line following test substance exposure.

For more information about the ongoing NICEATM and ICCVAM evaluations of eye safety test methods, please visit the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/methods/ocutox/STE-IRE.htm

NICEATM-ICCVAM and International Partners Convene Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing

Over 80 international scientific experts from the United States, Europe, and Asia attended the "International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing: State of the Science and the Way Forward" on September 19-21. Scientists representing industry, government, and academia met to review available methods and approaches for *Leptospira* vaccine potency testing as well as recent advances in science and technology. Participants also developed a strategy to achieve global acceptance and implementation of scientifically valid alternative methods.

NICEATM, ICCVAM, and partner organizations in the International Cooperation on Alternative Test Methods (ICATM) organized the workshop, which was hosted by the U.S. Department of Agriculture (USDA) Center for Veterinary Biologics at the National Centers for Animal Health in Ames, Iowa. Cosponsors of the workshop included the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), the International Alliance for Biological Standardization, and the Animal Health Institute.

Leptospirosis is a bacterial zoonotic disease caused by spirochetes of the genus *Leptospira*. An estimated 500,000 human cases of leptospirosis occur worldwide each year with a fatality rate of up to 25% in some regions. Designated as a Neglected Tropical Disease

by the U.S. National Institutes of Health and a Neglected Zoonotic Disease by the World Health Organization, leptospirosis is a global public health priority.

In the United States and other countries, *Leptospira* vaccines are used in cattle, swine, and dogs to protect them from disease and to reduce the risk of animal-to-human transmission. Manufacturers test the potency of vaccine lots prior to their release to ensure their effectiveness. However, methods currently used to test the potency of Leptospira vaccines use large numbers of laboratory animals that experience significant pain and distress, accounting for over one third of the animals reported to the USDA in this category.

An international workshop organized in 2010 by NICEATM, ICCVAM, and their international partners identified *Leptospira* vaccines as a high priority for future research, development, and validation of alternative test methods. The September 2012 workshop was convened to consider methods and approaches for *Leptospira* vaccine testing that could provide improved accuracy, efficiency, and worker safety and are more humane and use fewer or no animals.

A summary of the conclusions and recommendations from the workshop is available on the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/meetings/LeptoVaccWksp-2012/LeptoVaccWksp.htm

A report from the workshop, which will include manuscripts contributed by many of the speakers, will be published in 2013 as a special issue of *Biologicals*.

ICCVAM Advisory Committee meets

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) met on September 5 and 6. SACATM is composed of representatives of regulated industries and other ICCVAM stakeholders. SACATM advises the Director of the National Institute of Environmental Health Sciences (NIEHS), ICCVAM, and NICEATM about ICCVAM activities and Federally mandated ICCVAM functions.

At the September meeting, SACATM was provided with an overview of NICEATM and ICCVAM activities and progress over the past year. The program included a discussion of the October 2011 NICEATM-ICCVAM workshop on alternative methods for human and veterinary rabies vaccine testing and two upcoming workshops on alternatives for *Leptospira* and pertussis vaccine testing. SACATM gave a high priority to an ICCVAM nomination of an electrophilic allergen screening assay, an *in vitro* test method for identification of potential skin sensitizers (see article above). SACATM also discussed the draft NICEATM-ICCVAM Five-Year Plan for 2013-2017.

Representatives from NIEHS, EPA, and the National Institutes of Health updated SACATM on research and development activities relevant to the future development of alternative test methods, including new programs to create integrated microphysiological systems and stem-cell derived three dimensional micro-organs. Updates on current activities were also presented by representatives of ICATM member organizations, including the Korean Center for the Validation of Alternative Methods, Health Canada, the Japanese Center for the Validation of Alternative Methods, and EURL ECVAM.

Materials from the September SACATM meeting, including the agenda, background materials, public comments submitted, and all presentations, are available on the NTP website at: http://ntp.niehs.nih.gov/go/8202

Minutes from the meeting will be available on this page later this year.

NICEATM-ICCVAM requests nominations and submissions of test methods with potential regulatory applications

NICEATM and ICCVAM welcome nominations and submissions from the public for new or revised alternative safety testing methods and integrated testing and decision strategies with the potential to improve the accuracy of safety assessments and the potential to reduce, refine, or replace the use of animals. Test methods and testing that incorporate advances in science and technology are especially encouraged.

- *Nominations* can be submitted for proposed test method validation studies, specific test method or validation issues, or requests for test method evaluations. Such nominations are typically addressed with international validation studies, workshops, conferences, or test method independent scientific peer review meetings.
- When validation studies for a test method or testing strategy have been completed that adequately characterize its usefulness and limitations for a specific proposed regulatory requirement or application, a *submission* can be sent to ICCVAM for review and technical evaluation of the test method. ICCVAM then develops a test method evaluation report and formal recommendations that are forwarded to U.S. Federal agencies for acceptance consideration.

Organizations or individuals that wish to propose nominations or submissions of promising test methods or integrated testing strategies are encouraged to contact NICEATM for information and guidance on preparing proposals. Submission and nomination guidelines are also available on the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/SuppDocs/submission.htm

On behalf of the Tox21 Consortium and its Assays and Pathways Working Group, NICEATM also invites the nomination of pathway-based *in vitro* assays that can be considered for incorporation into the Tox21 high-throughput screening initiative. For more information about NICEATM support of Tox21, please visit the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/Tox21/Tox21.htm

About NICEATM and ICCVAM

ICCVAM is composed of representatives from 15 U.S. Federal regulatory and research agencies that require, use, or generate toxicological and safety testing information. ICCVAM evaluates the usefulness and limitations of new, revised, and alternative safety testing

methods and integrated testing strategies with regulatory applicability, with an emphasis on those that may further reduce, refine, or replace the use of animals in testing. ICCVAM then provides recommendations on the scientific validity of evaluated methods and strategies to U.S. Federal agencies.

U.S. Federal law established ICCVAM as a permanent interagency committee under NICEATM. NICEATM, which is located within NIEHS, administers ICCVAM and provides scientific and operational support for ICCVAM activities. Consistent with the NTP mission, NICEATM also conducts and coordinates international validation studies on high-priority improved safety testing methods and strategies.

NICEATM and ICCVAM promote the scientific validation and regulatory acceptance of innovative safety testing methods that more accurately assess the health hazards of chemicals and products while reducing, refining (enhancing animal well-being and lessening or avoiding pain and distress), and replacing animal use. NICEATM and ICCVAM collaborate to evaluate new and improved test methods and strategies applicable to the needs of U.S. Federal agencies and work to achieve national and international harmonization of safety testing methods.

Questions about NICEATM and ICCVAM activities are welcomed and can be directed to Dr William S. Stokes, Director, NICEATM, at niceatm@niehs.nih.gov; phone +1 919 541 2384; fax +1 919 541 0947. Copies of documents mentioned in this update can also be obtained by contacting NICEATM.

Information on the availability of NICEATM and ICCVAM draft documents, requests for nominations of experts to participate at workshops and on peer review panels, and specific information about NICEATM-ICCVAM meetings are communicated via the ICCVAM-all e-mail list and in notices posted in the U.S. *Federal Register*.

Subscribers to the ICCVAM-all e-mail list are notified directly of NICEATM-ICCVAM activities. Subscribers receive e-mail notification of NICEATM-ICCVAM *Federal Register* notices, availability of NICEATM-ICCVAM reports, notices of upcoming meetings, requests for public comments or data, and other events of interest to our stakeholders. If you would like to subscribe to the ICCVAM-all list, or for more information, please visit the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/contact/ni_list.htm

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IIVS News & Views

Dermal Absorption Workshop

In May of this year IIVS and the Physicians Committee for Responsible Medicine (PCRM) hosted a cadre of international scientific and North American regulatory experts in dermal absorption, for a workshop intended to pave the way for the adoption of an *in vitro* method for pesticide risk assessment. Despite the adoption of OECD Test Guideline 428 on *in vitro* dermal absorption techniques in 2004, pesticide regulators in North America often do not accept data generated using human skin *in vitro*, a technology commonly used to evaluate dermal absorption of ingredients in personal care products. Regulators cite the inconsistency of experimental protocols historically submitted for regulatory review as a major reason behind the lack of acceptance of *in vitro* data. There are several key protocol elements that can vary within the accepted guidance, such as skin thickness, species, and preparation procedures, receptor fluid composition, and tape stripping or other post-exposure procedures.

Steering group members from IIVS, PCRM, California EPA, and Canada PMRA planned the workshop to ensure that discussion of these and other key variables would be supported by data collected in top laboratories and agrochemical companies. The workshop participants then discussed each varia-

ble and made several recommendations for "best practices" for the conduct of *in vitro* dermal absorption studies. The workshop also determined reporting guidelines to ensure companies provide enough detail for regulators to interpret submitted *in vitro* studies. While the recommendations are not binding, participants agree that studies conducted according to them will improve acceptability and comparability of data across studies and laboratories, soon paving the way for broad acceptance of the *in vitro* method. Finally participants encouraged the conduct of a meta-analysis of existing *in vitro* and *in vivo* dermal absorption data with agrochemicals. A workshop report detailing the recommendations and reporting guidelines is in progress.

Workshop for Russian Delegates

Furthering its goal to technically assist in the adoption of non-animal methods in countries where animal testing has historically been required, IIVS hosted a four-day workshop for eleven attendees from Russia. Through a combination of lectures and hands-on laboratory training, the delegates gained firsthand knowledge of how non-animal test methods can be used to assess the safety of cosmetic and personal care products.

"Scientists in Russia are interested in learning more about non-animal test methods. This course gave an extremely useful technical overview and also highlighted how the data from these methods can be incorporated into a product safety testing program." said Tatiana Puchkova, Chairman of the Board of Perfumery and Cosmetic Association of Russia which organized this training for Russian authorities and leading toxicologists. "This is the beginning of an extensive collaboration with IIVS to establish non-animal safety testing in Russia. This effort is extremely important for further harmonization and plans to enter the OECD."

Although several non-animal tests have been formally validated and accepted by the OECD, the corresponding Test Guidelines often lack sufficient detail for users to execute the new methods or interpret resulting data. The workshop is one example of many educational and training activities in IIVS' International Outreach Program focusing on bridging information gaps to make non-animal test methods more readily adoptable by scientists around the world. Generous support from The Alternatives Research & Development Foundation, The Colgate Palmolive Company, and Humane Society International enabled IIVS to provide the workshop for the Russian attendees. Additional IIVS training programs are being planned for Russia as well as the US, China, and Brazil.

Next IIVS Webinar: November 12, 2012 "An *In Vitro* Ocular Hazard Testing Strategy for EPA Registered Antimicrobial Cleaning Products: A Collaborative Success Story" presented by Dr Rodger Curren, President IIVS

The majority of cleaning products in the US do not undergo a pre-market registration process. However, once an "anti-microbial" claim is made for the product, it is regulated by the US Environmental Protection Agency's Office of Pesticide Programs (US EPA OPP) and animal testing for hazard identification is required before the product can be sold. Investigation of whether an *in vitro* approach for evaluating the safety of anti-microbial cleaning products (AMCP) could replace some of the required animal tests was suggested by OPP's Pesticide Program Dialog Committee. Both the EPA and industry recognized that many essentially identical products had been marketed safely for years – without the anti-microbial claims and without animal testing. All parties believed that eye irritation should be the first animal test replaced. They agreed that a predictive, conservative *in vitro* testing strategy to label for eye irritation hazard had the potential to satisfy industry's need for non-animal testing while providing necessary data for regulatory approval. Key approaches which ultimately led to the success of the program were:

– Agreement by all parties on the purpose and goal of the study
– Addressing known toxicity mechanisms whenever possible
– Providing transparency in data collection and analysis

The webinar is complimentary but registration is required. Please visit http://www.iivs.org to register.

Registration for IIVS Training Workshop now open

"Practical Methods for In Vitro Toxicology" will be held 14-17 January, 2013 at the IIVS laboratory facility in Gaithersburg, Maryland USA. The 3½ day course will include lectures given by experts in the field of *in vitro* toxicology as well as hands-on laboratory experience. For registration details or to view a sample agenda please visit http://www.iivs.org.

News from ecopa

This year's ecopa annual meeting will be hosted by ecopa and the 3R Research Foundation Switzerland, the platform for 3R alternatives in Switzerland, at the Technopark in Zurich on November 20, 2012. It is part of the Joint 25th Jubilee Meeting of the 3R Research Foundation Switzerland held in conjunction with the Swiss Laboratory Animal Science Association (SGV), Swisstox, and ecopa on November 19 and 20. On both days two parallel sessions will be held with over 40 speakers and lecturers from Europe, including Switzerland, and the US.

The session "The 3Rs as a European Consensus" on November 20 at 15:40 will kick off with "ecopa: Who? What? Where? Challenges." by L. Knudsen, University of Copenhagen, Denmark, and will continue with reports of the national consensus platforms on their progress in 2012. The ecopa General Assembly will run from 17:30 to 19:00.

Further information: Official announcement of the joint meeting: http://bit.ly/QK0lTQ

Program for the "Joint 25th Anniversary Meeting SGV – 3R Research Foundation- Swisstox- ecopa": http://bit.ly/QwSx6g

Parallel sessions of SGV 2012 on refinement: http://bit.ly/QUEfOG

The Austrian platform zet, Centre for Alternative and Complementary Methods to Animal Testing, in Linz, is committed to its motto "animal welfare through science," promoting the rethinking process in life sciences and research regarding the use of *in vivo* models.

In 2007 zet founded the zet-Life Science Laboratory (zet-LSL) now integrated into the BioMed-zet Life Science GmbH. In this new state-of-the-art facility zet is realizing its scientific goals of "developing alternative and complementary methods to animal testing using modern cell and molecular biology techniques."

One of zet's recent success stories focuses on the bioavailability of orally administered drugs using *in vitro* models. For this purpose the effects of acetaminophen (APAP) aka Paracetamol were studied, as its net intestinal absorption and that of co-administered drugs are thought to be affected by different mechanisms. Caco-2 cells were used as a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells. The Caco-2 *in vitro* model is widely used across the pharmaceutical industry to predict the absorption of orally administered drugs. The zet studies aim to examine whether APAP has an influence on the permeability of small molecules in the Caco-2 barrier model, on the bioavailability of substances, on regulation of para-cellular transport mechanisms, and on cell-contact structures, e.g., tight junctions.

For this an intestinal barrier model using Caco-2 cells was established by culturing these cells for 21 days, followed by APAP-administration for 24 h after differentiation. By adopting scanning electron microscopy technology, high content imaging systems, immunostaining approaches, and impedance measuring systems the effect of APAP on the cell membrane topography and microvilli, on membrane permeability, on epithelial coherence, and on tight junction proteins could be investigated.

It could be shown that APAP has the capacity to impair the bioavailability of co-administered substances through different mechanisms: reduced number of microvilli on the surface of Caco-2 cells, decreasing the para-cellular transport activity and increasing the trans-cellular transport activity across the intestinal barrier model. Moreover, these studies could reveal that APAP may reduce the bioavailability of administered substances through remodelling the tight junction ZO-1 and its tyrosine phosphorylation due to decreasing para-cellular transport activity of the small molecules in the net intestinal absorption.

These studies clearly describe the potential of the *in vitro* barrier model with Caco-2 cells as a tool for research and for safety sciences. Such models could be recruited for making a human-relevant preselection of test substances and drugs, reducing and replacing animals in this regard.

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