News from the American Society for Cellular and Computational Toxicology

The American Society for Cellular and Computational Toxicology has had a very busy fall and looks forward to growing in 2012! We invite those interested in individual, student, or organizational memberships to visit www.ascctox.org for more information. Thank you to our founding sponsors: Alternatives Research and Development Foundation, Avon, Bioreliance, The Center for Alternatives to Animal Testing at Johns Hopkins University, The Clorox Company, The Colgate-Palmolive Company, The Hamner Institute for Health Sciences, The Institute for In Vitro Sciences, SC Johnson, and the Physicians Committee for Responsible Medicine.

In August of 2011 the ASCCT launched its popular member webinar program with a presentation by Dr Patricia Schmieder of the EPA's National Health and Environmental Effects Research Laboratory. Dr Schmieder described MetaPath, a Metabolism Pathway Database and computational tool developed by the EPA. Drs Gilman Veith and Hristo Aladjov of the International QSAR Foundation followed in October with a presentation of Effectopedia, an online encyclopedia of adverse effects pathways. Finally, the first webinar of 2012 was given by Dr Martin Stephens of The Center for Alternatives to Animal Testing at Johns Hopkins University, who described the efforts of the Evidence-Based Toxicology Collaboration to help address the challenge of critically assessing the new toxicology testing methods as well as the data generated by these and traditional methods. Every couple of months, ASCCT members are treated to a topical presentation and Q & A session with top experts in their fields and can access recordings of the webinars afterwards.

The ASCCT exhibited at the World Congress for Alternatives and Animals in the Life Sciences in Montreal, and also co-hosted a session with the European Society for Toxicology in Vitro (ESTIV) titled *Comparing the challenges of implementing new non-animal methods in the US and Europe*. Speakers Bas Blaauboer, Chantra Eskes, Horst Spielmann, Marilyn Aardema, and Kristie Sullivan treated the standing-room-only crowd to perspectives from academia and industry from both sides of the Atlantic. Look for a summary of the session in the proceedings.

The ASCCT exhibited at the Society of Toxicology meeting in San Francisco in March and is now planning its first annual meeting for the Washington, DC area in the Fall of 2012. We invite you to join and welcome your contributions.

CAAT*feed*

CAAT European Policy Program

The CAAT European Policy Program will be supervised by Dr. Paul Locke, who has directed CAAT's US Policy Program since 2004. The European Policy Program will help cement CAAT's role as a transatlantic bridge for the 3Rs and alternatives and as a global scientific voice for bringing the 3Rs and humane science into law, regulations, and guidance. Francois Busquet will be the CAAT representative in Brussels. Dr. Busquet, an expert in zebrafish embryo and its applicability to toxicity and safety testing and research, worked for ECVAM, European Commission until January 2012.

CAAT has been active in transatlantic policy issues for some time. In June 2010, it sponsored a symposium in Washington, DC entitled "International harmonization in toxicity testing: An EU perspective on the way forward." CAAT is also an active member of the American Consortium on European Studies (ACES) and an EU Center for Excellence supported by DG RELEX of the European Commission, and it organizes many information days in cooperation with CAAT-Europe in Konstanz. One of the goals of the European Policy Program is to serve as a voice of science to political decision makers in the EU and to act as a conduit so that cutting edge humane science is available to make policy on both sides of the Atlantic.

The objective of the policy program is to enhance and extend CAAT's visibility at the EU legislator level and to provide information, primarily to the EU Parliament but also to other EU and national institutions as appropriate. In order to accomplish this objective, the following activities will be undertaken:

– Dissemination and support of CAAT's innovation and scientific activities in Europe by arranging face-to-face meetings with Members of the European Parliament (MEPs) and CAAT representatives
– Capturing MEPs' attention regarding CAAT's rational, science-driven approach to chemical safety testing and animal welfare issues
– Attending specific European Parliamentary (EP) related events, i.e., EP Committees on Environment, Public Health and Food Safety (ENVI), Industry, Research and Energy (ITRE), and Internal Market and Consumer Protection (IMCO) as well as European Parliament's Intergroup on the Welfare and Conservation of Animals
– Close follow-up on the MEPs' work at the EP Committee level and at Plenary Sessions at Parliament in Brussels and Strasbourg.
– Communicating CAAT's position to MEPs on draft EU legislation for amendments when proposed by the European Commission or MEPs
– Informing CAAT firsthand about news from the EP and EC and about future EU policy programs (e.g. Horizon 2020), available EU funding, or potential EU collaborations with CAAT
– Development of IT tools to keep policy makers and CAAT informed of their respective agendas
– Identifying partnerships with lobbying organizations, especially CAAT sponsors and partners, to create synergies
– Providing scientific and policy perspectives of the EU to the US, and vice versa

Contact: Francois Busquet (caat-eu-policy@uni-konstanz.de)

Open Workshop on the "Human Toxome Project"

May 15, 2012, European Parliament, Brussels

This workshop, presented and organized by Vittorio Prodi, Member of the European Parliament and ENVI and ITRE committees, will be held at the European Parliament in Brussels from 12:00 to 14:00 in Room PHS 1A002. The program will include speakers from the Office of the European Commissioner for Research, Innovation and Science, Office of the European Commissioner for Health and Consumer Policy, Thomas Hartung for CAAT, and representatives of CEFIC, HSI, and EFPIA. Presentations will be followed by questions from the audience. The program is available at: http://cl.ly/1u3y0o3D0x1K050D0S3d

Congressional Briefing: EU and US animal welfare law in research and safety assessment: Similarities, differences and harmonization

April 17, 2012, 5:00 PM, Capitol Hill, Washington, DC, Cannon House Office Building Room 121

Speakers include the Honorable James Moran, Co-chair of the Congressional Animal Protection Caucus of the US House of Representatives, Dr. Thomas Hartung (CAAT), and Dr. Kathryn Bayne, Global Director, Association for Assessment and Accreditation of Laboratory Animal Care International.

Animals are used worldwide to support product development and research. Recently, the EU has enacted several Direc-

tives that call for enhanced protection of animals used in laboratories or, in the case of cosmetics testing, their replacement with non-animal test methods. The goal of this briefing will be to increase awareness of the EU's Directives on animal welfare and to discuss international approaches to the refinement, reduction, and replacement of animal testing. The briefing will further compare and contrast animal welfare implementation activities, point out harmonization challenges, and identify key areas where EU and US animal welfare law conflict with, or inform, each other. This briefing is sponsored by the American Consortium on EU Studies and CAAT. The American Consortium on EU Studies (ACES) is a partnership of five universities in the Washington, DC area, including CAAT at Johns Hopkins University. The partnership was established to improve academic and public understanding of the European Union and U.S.-EU relations. It seeks to strengthen education and research opportunities and to create new synergies among scholars, students, policymakers, the private sector, representatives of governmental and non-governmental organizations, and the media. ACES is recognized by the European Commission as the EU Center of Excellence in Washington, D.C. For more information see: http://transatlantic.sais-jhu.edu/ACES/index.htm

Thomas Hartung and the Human Toxome Project in *Science* Magazine: Animal-Free Toxicology: Sometimes in Vitro is Better

by Jeffrey. M. Perkel
Science, March 2, 2012, p. 1122-1125
"Animal-based testing is expensive and time-consuming, morally and ethically troubling, and most significantly, often a poor predictor of human toxicity. Driven both by legislative mandate and scientific need, a new suite of in vitro and cell culture-based animal-free methods [is] gaining a foothold in toxicology labs."

For the complete article see: http://altweb.jhsph.edu/news/2012/lifesciencetech.html

CAAT activities at SOT, San Francisco, CA, March 11-15, 2012

Open Forum on 21st Century Toxicology and Evidence-based Toxicology
CAAT, the Evidence-based Toxicology Collaboration (EBTC), and the Human Toxicology Project Consortium hosted an open forum on 21st century toxicology and evidence-based toxicology as a satellite meeting to the Society of Toxicology annual conference on March 11 in San Francisco. The forum offered participants an opportunity to provide informal updates on work they are doing to advance the new toxicology.

CAAT and the HTP Consortium hosted similar meetings on 21st century toxicology last year at both the SOT conference and the World Congress on Alternatives and Animal Use in the Life Sciences. This year we added evidence-based toxicology to the mix, given the work of the newly formed Evidence-based Toxicology Collaboration (EBTC) (see www.ebtox.com), for which CAAT serves as secretariat. The HTP Consortium comprises several companies and organizations, including CAAT, seeking to accelerate implementation of the NRC's 2007 report on "Toxicity Testing in the 21st Century."

Consensus Workshop: Quality Standards for Publications Dealing with In Vitro Test Systems
The consensus workshop, held March 13, was organized by CAAT-Europe as a SOT satellite meeting. The quality of *in vitro* data presented in scientific publications, particularly in the field of toxicology, is of great importance—regarding reproducibility, for example, and as the basis for evidence-based toxicology (EBT). The goal of the workshop was to engage experts in the process of assembling a comprehensive set of guidelines for authors, referees, editors, and regulators. The experts worked out a set of recommendations covering different aspects of the topic prior to the meeting. A draft document was generated and discussed during the workshop. The public feedback was very positive. Work on the subject is ongoing, and the consensus report with all contributors will be published soon.

Presentations at the SOT Annual Meeting
Thomas Hartung gave a 45-min Continuing Education Sunrise Course on "Lessons learned from Alternative Methods for Moving to Toxicology Testing for the 21st Century."

Helena Hogberg, CAAT Research Associate, gave a 20-min presentation at SOT on "The developmental neurotoxicity of lead in 3D rat primary neuronal cell cultures using transcriptomics and metabolomics approaches" (Abstract number 1703).

Thomas Hartung gave a 30-min lecture on "Mapping the Human Toxome in the Agilent workshop Knowledge-driven multi-omics integration for pathways of toxicity (PoT) research: policy and technology drivers."

Martin Stephens and Thomas Hartung participated in an 80-min roundtable session on "Scientific, Regulatory, and Public Perspectives in the Credibility and Use of Alternative Test methods in the Legislative Framework" with Linda Birnbaum (NIEHS), Steve Bradbury (EPA), and Mel Anderson (The Hamner Institutes for Health Sciences).

Other Presentations

Helena Hogberg also gave a 20-min talk at the International Conference and Exhibition on Metabolomics & Systems Biology in San Francisco, February 20-23.

Metabolomics in Toxicology and Pre-clinical Research, State-of-the-art and Potential Applications – a joint CAAT-Europe and BASF Symposium and Expert Workshop

February 13-15, 2012, Berlin, Germany
This symposium, attended by 130 registered participants, brought together opin-

ion leaders in toxicometabolomics from all over the world. As part of the transatlantic think tank for toxicology (t^4), it was sponsored by the Doerenkamp-Zbinden Foundation. State-of-the-art use of the technology, both *in vivo* and *in vitro*, was presented. The following one-and-a-half-day workshop prepared a consensus report, to be published in *ALTEX*, regarding opportunities for using the technology *in vitro*, *in vivo*, and for regulatory purposes.

Joint Convention: Scientific Roadmap for the Future of Animal-free Systemic Toxicity Testing. CEFIC, DZF, ECOPA, EUSAAT, CAAT-US, IIVS, IVTIP, ESTIV, CAAT-Europe, ToxCast, HSI, and Cosmetics Europe

March 20-21, 2012, Brussels, Belgium
The European REACH regulation, together with testing bans for cosmetic ingredients in Europe and a possible US TSCA reauthorization, point to the desire for a transition to an animal-free strategy for systemic toxicity testing. Other areas and novel products could similarly benefit from humane predictive approaches. A recent stocktaking (Adler et al. 2011, *Arch Toxicol 85*, 367-485) and its expert review (Hartung et al. 2011, *ALTEX 28*, 183-209) identified gaps in the available science. An expert workshop, presented and discussed at a multi-stakeholder forum, was held to promote the development of a roadmap to close these gaps (Basketter et al. 2012, *ALTEX 29*, 3-89). The event was hosted by CAAT and CAAT-Europe, ecopa, EUSAAT, Doerenkamp-Zbinden-Foundation, ESTIV, IVITIP, ESTIV, IIVS, Humane Society International, and ToxCast (US EPA), together with CEFIC and Cosmetics Europe. This event also benefited from the advisory comments of Eurogroup for Animals and ecopa, as well as from the contributions of the SEURAT-1 consortium and the European Chemicals Agency (ECHA).The public expert responses were constructive, and the five hours of discussion on the recommendations of the presented report were lively. The forum broadly endorsed the recommendations made. A summary of this event will be published soon in *ALTEX*.

Workshop on Alternative Methods for the Association of Pharmaceutical Industries (AFI)

April 12, 2012, Milan, Italy
AFI is the Italian Association of Pharmaceutical Industries. It was founded in 1960 as a discussion forum among scientists whose activity is somehow related to the world of pharmaceutical industries as a whole, including research & development, manufacturing, regulatory affairs, and marketing. Currently, this association includes 42 working groups covering seven areas. CAAT Europe was invited by AFI to help organize one of the first workshops on alternative methods for the toxicological characterization of chemicals. According to the classical scheme, this initial workshop explored various aspects of the topic, presenting the different approaches of *in vitro* strategies and *in silico* modeling. Additional presentations focused on the needs of the pharmaceutical industries, with discussions of specific regulatory requirements and the differences between alternative methods applied during the development versus the approval phase of a new drug, as well as alternative methods for testing biomedical devices.

ASCCT Member Webinar: Evidence-Based Toxicology

The webinar on Evidence-Based Toxicology, presented by Dr. Martin Stephens of CAAT, is available until February 2013. To access the webinar, please contact Kristie Sullivan at ksullivan@pcrm.org.

CAAT Research Grants awarded

For more than 30 years now, CAAT's research grants program has supported research on the development of *in vitro* and other alternative techniques. The following projects have received CAAT funding for the 2012-2013 grant period:

New Grants
- Human embryoid bodies as 3D *in vitro* model for developmental toxicity prediction
  Thierry Dorval, Institut Pasteur, Korea
- Novel axial elongation morphogenesis systems using embryonic stem cells to investigate teratogenic factors
  Yusuke Marikawa, University of Hawaii, HI, USA

Renewals
- Development of zebrafish-based assays for the assessment of developmental neurotoxicity of chemicals at low doses
  Nishimura Yuhei, Mie University, Japan
- Human culture model as replacement to the animal assays for assessing the potential of cosmetic ingredients to cause non-immunological contact urticaria (NICU)
  Francesca Levi-Schaffer, Hebrew University of Jerusalem, Israel
- Brain aggregate cell cultures as an *in vitro* model for developmental neurotoxicity testing
  Marie-Gabrielle Zurich, University of Lausanne, Switzerland

Upcoming Meetings

CAAT Pharmaceutical Information Day: Scientific and Animal Welfare Innovations
May 7, 2012
Baltimore, MD, USA

Speakers will include representatives from pharmaceutical companies and the National Cancer Institute. The focus of the meeting will be on new technologies used in developing therapeutics and their impact on the Three Rs.
- Thomas Hartung – Introduction
- Wayne Buck (Abbott) – Opportunities and Challenges in Practical Implementation of 3Rs in Pharmaceutical Toxicology

– Marilyn Brown (Charles River Laboratories) – Examples of the 3Rs in practice at Charles River
– Brian Berridge (GlaxoSmithKline) – Optimizing Animal Modeling Science as a Strategy for Advancing the 3Rs
– Manisha Sonee (J&J) – Integrated Approaches of Predictive Toxicology in Pharmaceutical Development
– Myrtle Davis (NIH/NCI) – Mechanistic Toxicology: A Molecular Basis for Translation and In Vitro Screening Opportunities
– Okey Ukairo (Hepregen) – TBA
– Oliver Flint (BristolMyers Squibb) – Stem Cells in Drug Safety Assessment
– Ray Kemper (Roche) – Use of Stem Cell Derived Tissues to Improve Cardiovascular Safety Assessment
– William Pennie (Pfizer) – The Use of Multiparametric in silico/in vitro Safety Tools to Streamline Drug Design
– Douglas Keller (Sanofi) – In silico methods for toxicity prediction
– Joanne Zurlo – Closing remarks

Information for registration and the meeting agenda will be forthcoming on the CAAT website and CAATwalk.
Contact: mprincip@jhsph.edu

CAAT Information Day on New Approaches to Assessing Countermeasures to Bioterrorism Agents
May 22, 2012
Baltimore, MD, USA

Late in 2011, the National Research Council published a report on Animal Models for Assessing Countermeasures to Bioterrorism Agents. The report summarized the status of current animal models to assess the efficacy of medical countermeasures, pointing out the imperfection of the existing models and the need for human clinical data to help inform the process. In addition, the committee that authored the report recommended that the Department of Defense examine scientific gaps in information needed to utilize alternative methods, identify areas in which current *in vitro* and *in silico* methods could be utilized as adjunct methods to animal models to help improve their usefulness and develop criteria for technologies to ultimately replace animal use for these purposes.

The committee also recommended changes in the standard practice of using animal models to more accurately represent a clinical course of treatment in humans. This may be done by developing a matrix to compartmentalize data from components of specific animal studies that best reflect the human situation. CAAT is organizing an information day to examine the state-of-the-art in new and developing technologies to replace animal use for assessing countermeasures to bioterrorism agents, as well as those that will modify current animal protocols to increase their efficiency and improve animal welfare.

Draft Program:
– Introduction and purpose of meeting – Thomas Hartung, CAAT
– Statement of problem and summary of NRC report and committee recommendations – George Korch, Johns Hopkins Bloomberg School of Public Health & co-chair of NRC committee
– Limitations of current animal models and recommendations for improving data gathering – Steven Niemi, Massachusetts General Hospital and co-chair of NRC committee
– *In vitro* biomarkers for *in vivo* studies – Donald Drake, VaxDesign (Sanofi-Pasteur)
– Use of human cells to detect exposure to bioterrorism agents – Marti Jett, Walter Reed Army Institute of Research
– Agency efforts to develop more predictive models – FDA, DARPA, DTRA, NIAID

More information on this one-day meeting will follow on the CAAT website.
Contact: mprincip@jhsph.edu

CAAT t^4 Workshop on Application of Toxicity Testing in the 21st Century beyond Environmental Chemicals
June 4-6, 2012

This international workshop will include representatives from the pharmaceutical, food and other industries as well as regulatory agencies to discuss how to adapt the vision of the 2007 NRC report to drug development and safety assessment. A report from this workshop will be published in *ALTEX*. *By invitation only*.

Recent Publications by CAAT/CAAT-Europe Faculty

Hengstler, G., Marchan, R., and Leist, M. (2012). Highlight report: Towards the replacement of in vivo repeated dose systemic toxicity testing. *Arch Toxicol 86*, 13-15.

Kadereit, S., Zimmer, B., van Thriel, C., et al. (2012). Compound selection for in vitro models of developmental neurotoxicity and prenatal neurotoxicity. *Frontiers in Bioscience*, in press.

van Thriel, C., Westerink, R., Beste, C., et al. (2011). Translating neurobehavioural endpoints of developmental neurotoxicity tests into in vitro assays and readouts. *NeuroToxicology*, in press.

Stephens, M. L., Barrow, C., Andersen, M.E., et al. (2012). Accelerating the development of 21st century toxicology: outcome of a human toxicology project consortium workshop. *Toxicol Sci. 125*, 327-34.

ecopa

News from ecopa

DETECTIVE
Detection of Endpoints and Biomarkers for Repeated Dose Toxicity Using In Vitro Systems

DETECTIVE (Detection of Endpoints and Biomarkers for Repeated Dose Toxicity Using In Vitro Systems) is an FP7 project part of the SEURAT-1 cluster, a European research initiative co-funded by the European Commission and Cosmetics Europe (former COLIPA) aiming at "Safety Evaluation Ultimately Replacing Animal testing". The specific goal of DETECTIVE is the identification of key biomarkers for repeated dose toxicity assessment in heart, liver and kidney.

The second DETECTIVE General Assembly took place on February 6-7, 2012 in Lisbon, Portugal. This meeting was followed by the second Annual Cluster Meeting of SEURAT-1 on February, 8-9 at the same location, organized by the COACH project, the SEURAT-1 coordination action. The SEURAT-1 cluster meeting was successful in its bridging activities between DETECTIVE and the five other projects of the cluster. Presentations were given with respect to the achievements of the first year and the priorities set for the second year.

Working sessions were dedicated to specific topics (such as the characterization and standardization of stem cells and questions related to biokinetics, mode of action and safety assessment) in which the different partners were discussing the cross-cluster integration of specific projects' results. Young scientists were invited to present, through a poster, their results already obtained during the first year of the SEURAT-1 cluster projects.

Anja Wilmes from the DETECTIVE partner Medizinische Universität Innsbruck presented the "Application of the xCELLigence system for monitoring vectorial transport and toxicity of renal epithelial cells" (prepared in collaboration with ROCHE), and received one of the three awards sponsored by Cosmetics Europe. Furthermore, the DETECTIVE project brochure was distributed during the SEURAT-1 meeting and is available for download together with the poster on the DETECTIVE project website at www.detect-iv-e.eu.

DEUTSCHER TIERSCHUTZBUND
AKADEMIE FÜR TIERSCHUTZ

Animal Welfare in Our Hearts and Minds - The Animal Welfare Academy (Akademie für Tierschutz) of the German Animal Welfare Federation (Deutscher Tierschutzbund eV.)

The Animal Welfare Academy is the scientific affiliation to Germany's biggest animal welfare organization, the German Animal Welfare Federation (Deutscher Tierschutzbund), a registered charity funded exclusively by donations and membership fees and representing the umbrella for 16 regional („Länder-") associations, more than 700 local associations and 500 animal shelters in which more than 800,000 members are organized. In the Academy, scientists from the areas of biology, veterinary science and law look into the animal welfare problems of our society from a scientific point of view. Thereby they provide the basis for targeted lobbying and other activities for the whole German Animal Welfare Federation, but also its member organizations and co-operating organizations (e.g., Eurogroup for Animals).

To generate and apply knowledge for the advancement of animal welfare in the various problem areas the Academy is structured into departments, i.e. farm animals dept., companion animals dept., le-

gal dept., wildlife dept., and research animals dept. Additionally the Academy runs a cell culture laboratory (see below).

The Academy's experts represent animal welfare in various environments, be it scientific panels, committee working groups, political (governmental, regulatory) fora, ethical committees, at congresses, fairs, etc. There, they give the interests of animals a voice which otherwise mostly would be neglected.

Another pillar of the Academy's work is the education of members of local animal protection societies to provide them with the knowledge to carry out practical animal welfare work according to sound and up-to-date knowledge. To that end the Academy offers seminars on a wide range of practical and theoretical animal welfare issues ranging from ethological aspects to rhetorical/psychological elements of communication.

The department of animal experimentation and alternatives

The main target of the dept. of animal experimentation and alternatives is the legal framework, i.e. animal welfare legislation and enforcement. For instance, in the past the Academy's experts have extensively provided input concerning the revision of Directive 2010/63/EU or the Council of Europe Convention ETS 123 on a European level. But also legislation demanding animal experiments is in the focus of the Academy's work such as European regulations (e.g. REACH, plant protection products, and – see below - biocides), OECD guidelines, etc.

The general and long-term aim of these activities is the abolition of animal experimentation. With regard to the 3Rs the focus of work is clearly on replacement, but includes contributions to reduction and refinement.

To promote the development and application of alternative methods the German Animal Welfare Federation and its Academy participate in, but also sponsor and organize scientific conferences. Moreover, they provide scientific publications and serve as members of editorial boards of journals, to which there is also financial support such as in the case of ALTEX.

National and international organizations or activities in which experts of the Animal Welfare Academy participated or still participates include: the ZEBET commission, the board of the German platform for alternatives SET, ECVAM's scientific advisory committee ESAC, the board of ecopa, EUSAAT, and others.

The cell culture laboratory

With its cell culture laboratory the Animal Welfare Academy aims at actively contributing to the practical development and evaluation of alternative methods, and to gain insight into the details of the processes of funding of alternative research. Both help to better understand hurdles and pitfalls in the practice of development and validation of alternative methods and to gain credibility in discussions about alternatives. Projects in the past include research on alternatives for the fish test in the German Waste Water Act, for the replacement of the *in vivo* micronucleus test, and for the Draize *in vivo* eye irritation test. The latter consisted in the development of a human corneal model for the prediction of ocular irritation and represents the latest research activity, which now is followed up by other laboratories in the next stage of the project where the Academy serves as an advisor. With regard to practical lab work, our cell culture laboratory at present cooperates with the company cellasys (Munich, Germany; http://www.cellasys.com) which is specialized in system solutions for online analysis of living cells.

Examples of projects on animal experimentation and alternatives

Some examples of present and former projects in the field of animal experimentation and alternatives include:

– Abolition of *in vivo* tests for Botox:
  Already in 2004 the Academy published an extensive article on the cruel mouse test, the so called LD50 test, for the batch testing of botulinum toxin and its increasing use as an anti-wrinkle substance (Ruhdel, I., *ALTEX 21*, 23-25). Various activities, including participation in international efforts to look at 3Rs in this field followed.
– Phasing out the use of mice for the characterization and quality control of stem cells in the teratoma assay:
  The Academy initiated and co-organized a workshop of the German platform SET in 2010.
– Promoting the 3Rs within the European Biocidal Products Regulation:
  Together with the German Animal Welfare Federation's European umbrella organization, Eurogroup for Animals, and our partner organization, the Humane Society International (HSI), the Academy compiled extensive amendments to bring data requirements more into line with the scientific state-of-the-art in safety testing and replace duplicated or unnecessary animal tests.
– Analysis of EU legislation:
  In a recent study funded by the German platform SET, the Animal Welfare Academy analyzed data requirements regarding the state-of-the-art of science and technology. The report, to be published in ALTEX, gives a detailed analysis of shortcomings of individual EU legislation and also compares the different legal acts.

Other projects the Animal Welfare Academy undertook in the past included EU statistics; 3Rs databases; animal testing specifically of: cosmetics, GMO crops, medical devices, shellfish toxin; alternatives in education; the use of primates in research and testing; the use of transgenic animals in biomedical research; clinical relevance of animal experimentation in biomedical research; ethics committees; the process of licensing animal experiments authorities in Germany, and others.

For further information contact:
Roman Kolar
Deutscher Tierschutzbund e.V.
Akademie für Tierschutz
Spechtstr. 1
D-85579 Neubiberg
Tel.: +49(0)89-600291-0
Fax: +49(0)89-600291-15
roman.kolar@tierschutzakademie.de
www.tierschutzakademie.de

News from NICEATM and ICCVAM

We are pleased to provide this update on recent and planned activities of the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and its Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). ICCVAM is a committee composed of representatives from 15 U.S. Federal regulatory and research agencies that require, use, or generate toxicological and safety testing information. The purpose of ICCVAM is to reduce, refine, and replace the use of animals in testing. To accomplish this, ICCVAM is charged with evaluating the usefulness and limitations of new, revised, and alternative safety testing methods and integrated testing strategies with regulatory applicability. ICCVAM provides recommendations on the scientific validity of evaluated methods and strategies to U.S. Federal agencies, which must respond to ICCVAM within 180 days. NICEATM, which is located within the National Institute for Environmental Health Sciences (NIEHS), administers ICCVAM and provides scientific and operational support for ICCVAM-related 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 safety testing methods that more accurately assess the health hazards of chemicals and products while reducing, refining (enhancing animal welfare and decreasing or eliminating 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.

Federal agencies respond to ICCVAM recommendations on use of the murine local lymph node assay for potency categorization

ICCVAM recommended to Federal agencies that the murine local lymph node assay, or LLNA, may be used to categorize the potency of chemicals causing allergic contact dermatitis (ACD) in humans. Specifically, ICCVAM recommended that the LLNA may be used to categorize some substances as strong sensitizers, thus identifying those substances considered to have a significant potential for causing skin hypersensitivity resulting in ACD. U.S. Federal agencies have responded to the ICCVAM recommendations. Regulatory agencies, including FDA, EPA, CPSC, and OSHA, have indicated that they will take actions in response to the ICCVAM recommendations to encourage use of the LLNA for this purpose where appropriate.

Skin diseases are the most common type of occupational illness in the United States, according to the U.S. Bureau of Labor Statistics. Many skin disease cases arise from repeated exposures to skin-sensitizing substances, which can lead to ACD, an immunologically mediated hypersensitivity reaction. Studies have shown that ACD has a significant adverse impact on quality of life in affected individuals.

For more than 10 years, the LLNA has been accepted worldwide as a valid alternative to traditionally accepted guinea pig test methods for assessing ACD hazard potential for most testing applications. Substances with the potential to cause ACD can also be categorized with the traditional test methods using guinea pigs. However, the LLNA uses fewer animals than guinea pig test methods, requires less time to perform, provides dose-response information, and, in most cases, eliminates pain and distress in the test animal.

The new ICCVAM recommendation provides guidance on how to use the LLNA to categorize some chemicals and products as strong skin sensitizers. However, since only half of the known strong human skin sensitizers can be identified as such in the LLNA (52% or 14 out of 27), additional testing or information is necessary to conclude that substances are not strong skin sensitizers.

The ICCVAM evaluation is detailed in a report entitled *ICCVAM Test Method Evaluation Report: Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans* (NIH Publication No. 11-7709). In June 2011, ICCVAM forwarded recommendations to Federal agencies and made these recommendations available to the public (76 FR 18639). In accordance with the ICCVAM Authorization Act of 2000 (42 U.S.C. 285l-3), agencies have notified ICCVAM in writing of their findings, and ICCVAM is making these responses available to the public.

The Federal agency responses to the ICCVAM recommendations and more information about the ICCVAM evaluation of the LLNA for potency categorization can be found on the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/methods/immunotox/LLNApotency.htm

The ICCVAM Test Method Evaluation Report is available on the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-pot/TMER.htm

NICEATM and ICCVAM are also currently evaluating several *in vitro* and *in chemico* methods for their potential to further reduce and eventually replace the use of animals for ACD safety testing.

ICCVAM recommends non-animal *in vitro* method to identify potential endocrine-active substances

ICCVAM recently recommended to Federal agencies that a non-animal test method can be used as a screening test to identify substances with *in vitro* estrogen agonist and antagonist activity. This test method, the BG1Luc estrogen receptor (ER) transactivation (TA) test method (also known as the LUMI-CELL® ER test method) uses cultured human cells to identify substances that can induce or inhibit human ER activity *in vitro*.

Xenobiotic Detection Systems, Inc. (XDS, Durham, NC, USA) developed the LUMI-CELL® ER test method and nominated the method to ICCVAM for an interlaboratory validation study. ICCVAM and its advisory committee, the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) recommended the study as a high priority. NICEATM subsequently coordinated an international validation study with counterparts in Japan (the Japanese Center for the Validation of Alternative Methods) and Europe (the European Centre for the Validation of Alternative Methods).

ICCVAM's Interagency Endocrine Disruptor Working Group (EDWG), composed of scientists from ICCVAM member agencies, worked with NICEATM to carry out relevant evaluation activities following completion of the international validation study. A background review document, test method performance standards, and ICCVAM test method recommendations were reviewed by an independent international peer review panel. ICCVAM considered the Panel report and comments from the public, the EDWG, and SACATM in preparing the final test method recommendations.

ICCVAM recommends that the accuracy and reliability of the BG1Luc ER TA test method support its use as a screening test to identify substances that can induce or inhibit human ER activity *in vitro*. ICCVAM concludes that the accuracy of this assay is at least equivalent to the only ER TA test method currently in a U.S. regulatory test guideline, the Environmental Protection Agency's "OPPTS 890.1300: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903))." The BG1Luc ER TA test method was found to offer several advantages over the existing ER TA method, including (1) validation for use over a wider concentration range of test substances, (2) potential to detect a wider range of ER-active substances, (3) ability to identify both substances that induce and and those that inhibit the estrogen receptor, and (4) availability of the cell line used for the test from more than one source.

The ICCVAM evaluation is detailed in a report entitled *ICCVAM Test Method Evaluation Report: The LUMI-CELL® ER (BG1Luc ER TA) Test Method: An In Vitro Assay for Identifying Human Estrogen Receptor Agonist and Antagonist Activity of Chemicals* (NIH Publication No. 11-7850). The report also provides (1) performance standards that can be used to evaluate functionally and mechanistically similar test methods, (2) recommended test method protocols, (3) a final background review document describing the current validation status of this test method, and (4) recommendations for future studies.

The ICCVAM report and recommendations have been transmitted to Federal agencies for their review and response to ICCVAM in accordance with the provisions of the ICCVAM Authorization Act of 2000, which requires agencies to review the recommendations and respond to ICCVAM within 180 days.

The BG1Luc ER TA test method was adapted to a high-throughput format using 1536-well plates by the National Institutes of Health (NIH) Center for Translational Therapeutics (NCTT; formerly the NIH Chemical Genomics Center). Preliminary results are promising, and it is expected that this method will be incorporated into the Tox21 screening paradigm in 2012.

The ICCVAM Test Method Evaluation Report is available on the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/methods/endocrine/ERTA-TMER.htm

More information about the ICCVAM evaluation of the use of the BG1Luc ER TA test method for identification of potential endocrine-active substances can be found on the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/methods/endocrine/end_eval.htm

Hold the date: NICEATM and ICCVAM International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing, September 19-21, 2012

NICEATM and ICCVAM will convene an "International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing: State of the Science and Planning the Way Forward" on September 19-21, 2012. The workshop will be hosted by the U.S. Department of Agriculture (USDA) Center for Veterinary Biologics at the National Centers for Animal Health in Ames, Iowa. NICEATM and ICCVAM are organizing the workshop in collaboration with partner organizations in the International Cooperation on Alternative Test Methods.

Leptospirosis is an emerging and widespread bacterial zoonotic disease caused by spirochetes of the genus Leptospira. Worldwide more than 500,000 human cases of leptospirosis are reported 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 the World Health Organization, leptospirosis is a global research and public health priority. Leptospirosis also affects many animal species including livestock, pets, and wildlife. In the United States, vaccines are used to protect cattle, swine, and dogs. Vaccines for people are available in some other countries, and NIH is supporting the development of new human vaccines.

Regulatory authorities require potency testing prior to release of each production lot of *Leptospira* vaccine to ensure that it will be effective. However, such testing currently involves large numbers of laboratory animals and many experience significant unrelieved pain and distress without the benefit of pain-relieving drugs, accounting for over one-third of the animals reported to the USDA in this pain category. Accordingly, NICEATM, ICCVAM, and its international partners recently identified Leptospira vaccines as one of its top three priorities for development, validation, and implementation of improved alternatives.

While *in vitro* potency assays are now approved by the USDA and are available for four Leptospirosis serovars, these new assays have not yet been widely implemented. This workshop will bring together international scientific experts from government, industry, and academia to review available methods and approaches that can reduce, refine, or replace animal use for veterinary *Leptospira* vaccine potency testing and to develop an implementation strategy to achieve global acceptance and use of available alternatives. Participants will review recent advances in science and technology and new methods and approaches that are more humane, that use fewer or no animals, and that may provide improved accuracy, efficiency, and worker safety. The workshop will also address global acceptance and implementation of scientifically valid alternative methods. A poster session will feature presentations on current research, development, and validation of alternative methods for *Leptospira* vaccine potency testing.

Registration information and a workshop program will soon be available on the NICEATM-ICCVAM website. NICEATM and ICCVAM also invite the submission of abstracts for scientific posters to be displayed during this workshop; abstracts should be submitted by August 13, 2012. Online registration, instructions for poster authors, and other information will be available at: http://iccvam.niehs.nih.gov/meetings/LeptoVaccWksp-2012/LeptoVaccWksp.htm

If you have questions about the workshop or would like more information, please contact NICEATM at: niceatm@niehs.nih.gov

NICEATM and ICCVAM presentations at the 51st Annual Meeting of the Society of Toxicology

NICEATM and ICCVAM presented seven scientific posters describing recent activities at the 51st Annual Meeting of the Society of Toxicology, which took place on March 11-15, 2012 in San Francisco, CA, USA.

Two posters focused on the ICCVAM evaluation of the BG1Luc ER TA test method. This test method uses human cells to identify substances with *in vitro* estrogen agonist and antagonist activity (see article above). One poster summarized the ICCVAM recommendations on the BG1Luc ER TA test method, and the second poster described performance standards that have been developed for the test method.

Three posters described NICEATM evaluations of methods to identify substances with the potential to cause allergic contact dermatitis (ACD). One poster described an updated evaluation of the reduced LLNA, and another presented an evaluation of the use of two nonradiolabeled LLNA methods for potency categorization of substances causing ACD in humans. A third poster described a NICEATM analysis comparing use of the direct peptide reactivity assay with a three-test battery for *in vitro* identification of potential sensitizers, and proposes an integrated testing strategy that can significantly reduce animal testing.

A sixth NICEATM-ICCVAM poster presented the results of a NICEATM analysis to determine if acute oral systemic toxicity data can be used to estimate and avoid acute dermal systemic toxicity testing. The final poster presented a summary of conclusions and recommendations from the October 2011 International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing.

Abstracts and poster presentations are available on the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/meetings/SOT12/sotablst.htm

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 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 strategies 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.

On behalf of the National Toxicology Program, NICEATM also invites the nomination of pathway-based in vitro assays that can be considered for incorporation into the Tox21 high-throughput screening initiative.

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

For more information

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

Institute for In Vitro Sciences
Advancing Science & Animal Welfare Together

IIVS News & Views

IIVS is pleased to announce the addition of Dr Brian Jones as Director, Education and Outreach Programs. Dr Jones has extensive knowledge of the development and use of non-animal methods for assessing the toxicity and sensitization potential of cosmetic ingredients and formulations. One of Dr Jones' primary activities at IIVs will be to organize and lead IIVS' International Outreach Program in countries such as China, Brazil, and Russia. Relevant information on these activities can be found below.

For questions regarding IIVS' International Outreach Program please contact Brian at: bjones@iivs.org

IIVS Practical Workshop for Alternative Methods in Brazil November 16-18, 2011 Goiânia, Brazil, Universidade Federal de Goiás

IIVS continued its international *in vitro* methods training programs with the first "Practical Methods" workshop held in Goiânia, Brazil, 16-18 November, 2011. The Workshop *Internacional Prático sobre Métodos Alternativos* was co-organized by Dr Chantra Eskes of SeCam and hosted by the Goiás School of Pharmacy. The purpose of this workshop was to assist in the adoption of non-animal testing methods by Brazilian regulatory authorities by familiarizing both regulatory and industrial scientists with the technical aspects of the *in vitro* methodologies.

The first day consisted of regulatory updates and introductory lectures from prominent stakeholders such as the head of ANVISA's cosmetics division and a representative from the Ministry of Health. The second and third days consisted of technical lectures and laboratory demonstrations of non-animal safety testing methods by IIVS staff. The response from all the attendees to this meeting was extremely positive with 95% of the respondents indicating the workshop either met or exceeded their goals. A second workshop – probably located closer to the major population center of São Paulo – was strongly endorsed by the participants, as were additional training sessions that would highlight advancements in the methodologies.

XVI International Scientific and Practical Conference Cosmetic Industry: Looking into the Future Moscow, Russia, October 25, 2011

The Russian Association of Perfumery & Cosmetics has organized this International Scientific and Practical Conference since 1996. With more than 350 people participating, the conference has become a significant venue for manufacturers, suppliers, registration/certification bodies, and testing laboratories in Eastern Europe. The 16th meeting contained a separate day dedicated to the topic of non-animal alternatives as tools for the safety and efficacy assessment of cosmetics. Brian Jones presented on the routine use of non-animal methods by personal care product manufacturers in the US, with special emphasis on the use of 3-D tissue models for assessing potential gingival irritation from oral personal care products. Other presentations addressed the use of alternatives in the EU, Russia, Belarus, Kazakhstan, and China.

International Outreach Program: China

In November 2011, Drs R. Curren and B. Jones traveled to China to participate in meetings to further IIVS' International Outreach Program. In Guanzhou they participated in a workshop sponsored by the Guangdong (GD) CDC, with direction from the China sFDA, focused on the Qualification of the 3T3 Neutral Red Uptake Phototoxicity Test. Other invited experts included Dr Hajime Kojima (JaCVAM), Dr John Harbell (Mary Kay Cosmetics), Drs Alice Cai and Robert Zhao (L'Oréal) and Dr Jiao Hong (Inspection and Quarantine Technology Center; IQTC).

In Guangdong, Brian and Rodger presented at the Laboratory Animal Association's "Education and Scientific Symposium on Animal Alternatives" held at the Sun Yat-sen University. Their talks covered the current global status of alternatives, the 8th World Congress on Alternatives, use of the Bovine Corneal Opacity and Permeability assay, and non-animal methods for assessing dermal sensitization.

Brian also attended the "Seminar on Ingredient Management and Development" sponsored by the Chinese Association of Fragrance, Flavor and Cosmetic Industry (CAFFCI), European Federation for Cosmetic Ingredients (EFfCI), Association of International Chemical Manufacturers (IACM) and the sFDA. The meeting highlighted the challenges associated with bringing new ingredients and formulations to market in China.