News

EU: EPAA offers Prizes

EPAA is offering a € 3,000 3R Communication Prize for an essay or article that broadens awareness of the role of safety assessment and regulatory compliance in product development, and that promotes replacement, reduction and refinement (3Rs approaches). As the EPAA's lead theme this year is unveiling the potential of reduction and refinement, the focus should be on these 2Rs.

EPAA is also offering a 3Rs Science Award to support the development and regulatory acceptance of 3Rs alternative methods. Financial support of up to € 100,000 will be provided to the institution of origin of the successful applicant to allow the extension of an existing post-doctoral contract for a period of up to 1 year. The candidate together with its current institution should submit a joint proposal of a research and development project with the potential to be conducted in collaboration with EPAA partners and to significantly contribute to the 3Rs.

Further information on both awards can be found on the EPAA website (www.epaa.eu.com).

EPAA newsletter
June 2010

EU: 45 days to save animals from REACH tests

The EU chemical legislation REACH demands updated and expanded safety assessments for thousands of chemicals already on the market. These assessments will require tests on 8 to 54 million animals.

A 45 day commenting period has been included in the REACH process upon pressure from animal protection organisations. Companies need to submit suggested testing strategies for their chemicals to the European Chemicals Agency (ECHA) in Helsinki for approval. The ECHA will publish these strategies on its website (http://echa.europa.eu/consultations/test_proposals/test_prop_cons_en.asp) for a period of 45 days. During this time Doctors Against Animal Experiments Germany plans to investigate whether the required data already exists or whether the information may instead be obtained by alternative methods. The decision on whether the experiments must be performed is made by ECHA after this commenting period.

For this purpose, the toxicologist Wolfgang Stengel, M.D., joined the team in May 2010. He will prepare the comments in cooperation with the British Union for the Abolition of Vivisection (BUAV) and the European Coalition to End Animal Experiments (ECEAE), which has also employed a toxicologist for this purpose.

A similar strategy was followed by US animal protection groups at the beginning of the century when a similar risk assessment programme for high production volume chemicals was conducted there. Toxicologists' comments compiled within the 90 day comment period regarding the testing strategies for about 1,400 chemicals led to a reduction in animal numbers from 1,034,000 to 158,000.

Whether such a great success can also be achieved for REACH is questionable. It is especially problematic that, next to the shorter commenting period and the higher number of chemicals, the ECHA will not publish as much information on the chemicals as was done in the US. However, every comment on the proposed testing strategies may save animals. The three cooperating organisations aim to use this opportunity as best they can to prevent as many animal tests as possible.

Adapted from www.aerzte-gegen-tierversuche.de

EU: Public consultation on alternatives for cosmetics testing

The Directorate-General for Health and Consumers launched a public consultation on a draft "Report on alternative (non-animal) methods for cosmetic testing: current status and future prospects – 2010".

The draft report has been drawn up by working groups of experts nominated by different stakeholders and by the European Centre for the Validation of Alternative Methods (ECVAM), hosted by the EC-JRC's Institute for Health and Consumer Protection.

Comments from the public are solicited on five chapters covering repeated dose toxicity, skin sensitisation, carcinogenicity, toxicokinetics and reproductive toxicity. The report shall provide the state of the art with regard to the scientific basis for alternative testing for these and an estimate when the scientific knowledge will be available to have test methods that could enter ECVAM (pre-) validation. Factual information, amending/complementing the information contained in the reports is sought as are justified comments on the experts' time estimates.

Based on the final report, the European Commission will inform the European Parliament and the Council on the availability of alternatives in relation to testing for the three types of health-related effects (repeated-dose toxicity, reproductive toxicity and toxicokinetics) covered by the 2013 deadline in the Cosmetics Regulation concerning the marketing ban of cosmetics containing substances tested on animals, and on the future prospects for the development of such alternatives.

Target group:
All citizens and organisations can contribute
Period of consultation:
23 July-15 October 2010
Expected publication date of the final report:
end of 2010/early 2011
Documentation access:
Draft Report on Alternative Methods for Cosmetics Testing

In Vitro Methods Unit/ECVAM
Institute for Health & Consumer Protection (IHCP)
European Commission – Joint Research Centre (EC_JRC)

EU: New animal protection Directive

After many years of bartering between animal protectionists, experimental scientists and EU bodies the text for the new Directive for the protection of animals used for scientific purposes was accepted on EU level on 8th September 2010. Doctors Against Animal Experiments Germany stated their disappointment that even minimal improvements for the protection of animals were not adopted.

They stated that the new Directive is a great disappointment for the EU citizens, who clearly demonstrated their aversion to animal experiments in public opinion polls commissioned specifically by the EU. Between 84 and 94 percent of 40,000 questioned citizens called for a more effective protection of apes, cats, guinea pigs as well as mice and fish in a survey commissioned by the EU Commission in 2006.

The original draft of the Directive submitted by the EU Commission in 2008 did incorporate some improvements of animal protection, but these were softened or stricken in subsequent versions. Applications by the Greens to reinstate these improvements were not accepted.

The new Directive does not offer special protection for apes, the need for an ethical evaluation of a proposed animal experiment is not required and alternative methods only become compulsory once they are officially recognized, a process that can take decades. A further dramatic change for the worse is the specification that Member States may not introduce stricter animal protection standards.

The Member States must implement the Directive into national law within two years.

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EU: CAAT-Europe and t⁴ host workshop on teaching and education

The Center for Alternatives to Animal Testing – Europe (CAAT-Europe) and the Transatlantic Think Tank for Toxicology (t^4) are bringing together experts in the teaching of different areas of the 3Rs for exchange of knowledge, definition of the *status quo* and design of a common strategy for improved knowledge dissemination in the field. The workshop aims to identify target groups and their specific educational needs, compile and analyse existing 3Rs courses and identify gaps to be filled, identify concepts for teaching new scientific aspects of 3Rs approaches (integrated testing strategies, computer modelling, toxicology for the 21st century, etc.) and outline a strategy to collaborate in creating 3Rs knowledge and educational material. The workshop (25-27 October 2010) is by invitation only; a joint expert report shall be published in ALTEX.

Press release CAAT-Europe
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EU: CAAT-Europe Information day

The Center for Alternatives to Animal Testing – Europe (CAAT-Europe) is starting a series of high level Information days. The first event on 28th October 2010 will focus on the *status quo* of the REACH Directive, the US Toxic Substance Control Act (TSCA) reauthorisation and the implementation of the US National Research Council strategy for *Toxicity Testing in the 21st Century*.

REACH in the EU and TSCA reauthorisation in the US are large-scale programs to obtain toxicological data for old compounds that prompt testing needs and changes in methods. This Information day aims to give a status update on REACH and TSCA to a European audience. Moreover, CAAT-EU will introduce the report of the US National Research Council, *Toxicity Testing in the 21st Century*, which puts forward the vision on advanced toxicity testing and human health assessment. The authors describe in this report that the explanatory power of toxicological risk assessment can be maximised by employing high throughput *in vitro* screening assays, tests in lower organisms, systems biology, functional genomics and transcriptomics as well as predictive *in silico* approaches. This Information day aims to introduce this vision to a European audience by inviting some of the key US players as speakers.

The complete program will be available for viewing mid-September on the CAAT websites http://cms.uni-konstanz.de/leist/caat-europe and www.caat.jhsph.edu.

Press release CAAT-Europe
University of Konstanz, Germany
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GER: Doctors Against Animal Experiments Germany award € 10,000 for animal-free cancer research – call for applications

For the second time Doctors Against Animal Experiments Germany are awarding a € 10,000 science prize for animal-free cancer research. The prize was established specifically for this purpose by a will. The goal of the award is to motivate especially younger scientists to conduct animal-free research and to promote scientific work carried out without the use of animal materials, including clinical or epidemiological studies. The prize will be awarded for outstanding scientific work in these areas. It may be awarded retrospectively for completed or current research or as an incentive for a planned project. Scientists who have already received the prize may not apply a second time.

Application criteria:

- work in the field of cancer research
- research without animal experiments and without animal material (e.g. *in vitro* or *in silico*) or
- clinical or epidemiological study
- research located in Germany or other German-speaking countries

Application documents:

- detailed description of research targets, research methods and the specific progress that will result for cancer research
- declaration of the significance of this work for animal welfare

curriculum vitae

The prize may be awarded in total to or shared by more than one recipient.

Please send applications by 30 March 2011 to:

Doctors Against Animal Experiments
Landsbergerstr. 103
80339 Munich, Germany

info@aerzte-gegen-tierversuche.de
www.doctors-against-animal-experiments.org

IND: Restructuring the zoology curriculum to reduce animal use

Following the recommendation of I-CARE, a directive was issued in 2006 by the University Grants Commission to restructure the zoology curriculum so as to avoid the use of animals and adopt alternative methods. An expert commission was set up at national level, which also held a brain-storming session with representative senior teachers, scientists, higher education planners and executives. The recommendations of the expert commission are:

- Undergraduate students will not do any dissection themselves, but one species, preferably a laboratory-bred animal, may be used for demonstration by the faculty.
- Post-graduate students may choose between dissection of a few species, again preference to laboratory-bred animals, as specified in the curriculum, or a project related to biodiversity or bio-systematics.
- Colleges and universities must strictly adhere to the Wildlife Protection Act, 1972, and the Prevention of Cruelty to Animals Act, 1960. No frogs may be dissected as these are protected under the Wildlife Protection Act, 1972.
- Colleges and universities must establish "Dissection Monitoring Committees" to control the use of animals.

To allow the adoption of alternatives to dissection, zoology and life science departments should be provided with appropriate information and communication technology.

The number of science undergraduate students in India exceeds 1.5 million. The recommendations made by the committee therefore stand to reduce the number of dissected animals per year by 10-20 million.

Mohammad A. Akbarsha,
Mahatma Gandhi Doerenkamp Center (MGDC)
for Alternatives to Use of Animals in Life Science Education,
Bharathidasan University,
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INT: OECD publishes TG 439: In Vitro Skin Irritation

The Reconstructed Human Epidermis Test Method was adopted as Test Guideline 439: In Vitro Skin Irritation on 22nd July 2010 and was published the following day by OECD Publishing (www.oecdbookshop.org).

According to the description on the website, the Test Guideline describes an *in vitro* procedure that may be used for the hazard identification of irritant chemicals (substances and mixtures) in accordance with the UN Globally Harmonized System of Classification and Labelling (GHS) Category 2. It is based on reconstructed human epidermis (RhE), which in its overall design closely mimics the biochemical and physiological properties of the upper parts of the human skin. Cell viability is measured by enzymatic conversion of the vital dye MTT into a blue formazan salt that is quantitatively measured after extraction from tissues. Irritant test substances are identified by their ability to decrease cell viability below defined threshold levels. The Test Guideline also includes a set of Performance Standards for the assessment of similar and modified RhE-based test methods. There are three validated test methods that adhere to this Test Guideline. Depending on the regulatory framework and the classification system in use, the procedure may be used to determine the skin irritancy of test substances as a stand-alone replacement test for *in vivo* skin irritation testing, or as a partial replacement test, within a tiered testing strategy.

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SUI: ALTEX Prize 2010 for Costanza Rovida

Costanza Rovida was awarded the ALTEX Prize 2010 for her article *Re-evaluation of animal numbers and costs for in vivo tests to accomplish REACH legislation requirements for chemicals – a report by the transatlantic think tank for toxicology*, which was co-authored by Thomas Hartung, at the banquet of the Linz Congress.

ALTEX editorial staff and the scientific advisory board annually choose the best main article of the previous year. The 15 jury members awarded one, two or three points to one article each. The winning article received 17 points. Second place went to Klaus Peter Rippe for *Weighing of interests in justifying animal experiments – Comments on a shift in ethical pradigms* and third place to Anett Ullrich, Donna B. Stolz, Ewa C. Ellis, Stephen C. Strom, George K. Michalopoulos, Jan G. Hengstler and Dieter Runge for their article *Long term cultures of primary human hepatocytes as an alternative to drug testing in animals*.

The prize and the certificates were awarded by Stefanie Schindler, the new president of ALTEX Edition. Apart from the prize money of CHF 2,000 the winner's membership fees for EUSAAT are paid for three years by ALTEX Edition and travel expenses to the Linz Congress were also reimbursed.

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SUI: Doerenkamp-Zbinden Prize 2010 for Michael Balls

For his life's work, Michael Balls, zoologist and emeritus professor for Medical Cell Biology, editor of the journal ATLA since 1983 and former director of ECVAM was chosen to receive the Doerenkamp-Zbinden Prize 2010 unanimously by the Doerenkamp-Zbinden foundation board. Like no other Michael Balls has shaped the field of alternative methods. He studied zoology at Oxford, worked later at the University of Geneva and the University of California, Berkeley, Reed College, Portland, OR, and the University of East Anglia. In 1985 he moved to the University of Nottingham Medical School where he was professor of medical cell biology from 1990-1995. Balls became a trustee of FRAME (Fund for the Replacement of Animals in Medical Experiments) in 1979 and has been chairman of the trustees since 1981. 1993-2002 he was the first director of ECVAM (European Centre for the Validation of Alternative Methods).

Balls drove the development of the principles of validation of alternative methods and contributed to their first successful outcomes. He is well known for his quick intellect and provocative manner, having stimulated many discussions and given many impulses to advances in the replacement of animal experiments.

Michael Balls was invited by the DZF to the social evening of the Linz Congress, where he received the prize from President Franz P. Gruber and Vice President Thomas Hartung.

On the same evening, which was also his birthday, an honorary medal was awarded to Emeritus Professor Walter Pfaller of the University of Innsbruck. He studied and worked at the University of Innsbruck continually with only one interruption in 1976-1977, during which he was at the University of Maryland, Baltimore, and later became Professor of Physiology. With his involvement in a series of EU projects, his contributions to EUSAAT and set, the organisation of the Linz Congresses, the ECVAM Scientific Advisory Committee and the board of CAAT-EU, he has contributed much to the advances of alternative methods in the last two decades.

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SUI: OpenVirtualToxLab™

The VirtualToxLab™ is an *in silico* tool for predicting the toxic potential (endocrine and metabolic disruption, interference with the hERG ion channel) of drugs, chemicals and natural products. Its automated protocol – accessible through the Internet – simulates the binding of any molecule of interest towards a series of 16 proteins, known trigger adverse effects (androgen, aryl hydrocarbon, estrogen α/β, hERG, glucocorticoid, liver X, mineralocorticoid, thyroid α/β, peroxisome proliferator-activated receptor γ; cytochrome P450 1A2, 2A13, 2C9, 2D6 and 3A4), calculates the associated binding affinities and estimates the resulting toxic potential. In contrast to other approaches in the field, the technology allows to verify a prediction at the molecular level by interactively inspecting the binding mode of the tested compound with all target proteins in real-time 3D. Further information can be found on http://www.virtualtoxlab.

org where the detailed documentation can be downloaded and the results for more than 2,500 tested compounds can be viewed.

By October 1, 2010, a free version – the OpenVirtualToxLab™ – will become acessible to any scientifically oriented organization. The only difference to the version liable to pay costs is that the Biographics Laboratory 3R reserves the right to publish the results obtained with the OpenVirtualToxLab™. Registration will be available on-line. We trust that the OpenVirtualToxLab™ will further contribute to the reduction of animal experiments as it can be applied to hypthetical compounds, i.e. before chemical synthesis and *in vitro*/*in vivo* screening.

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SUI/GER/USA: New president of ALTEX Edition

The atmosphere at the board meeting of the society ALTEX Edition hosted by CAAT-EU at the University of Konstanz on 5th July 2010 was very relaxed. Not only the positive financial balance, which Managing Director Franz P. Gruber presented, but also the society's solid future perspectives were the reason for the good mood of the delegates from Animalfree Research (Stefanie Schindler), CAAT-EU (Mardas Daneshian), Doerenkamp-Zbinden Foundation (Thomas A. Hartung), Ligue suisse contre la vivisection (Daniel Favre) and Zurich Animal Protection (Claudia Mertens). Owing to the drop of membership and ensuing financial restrictions EUSAAT can no longer sit on the board.

After the unanimous acceptance of the annual financial statement the vote for a new president took place. The former president, Nina Schweigert, left the foundation board of Animalfree Research at the beginning of the year. Her successor, Stefanie Schindler, won the vote and became the new president with one abstention and no dissenting votes. Carol Howard of CAAT was unanimously voted vice president. She will replace Helmut Appl of EUSAAT.

As for the Abstract book for the Linz Congress 2010, ALTEX Edition will seek sponsorship of the production and printing costs for the Abstract book for the World Congress in 2011 in Montreal.

Stefanie Schindler studied veterinary medicine at the Justus-Liebig-University Giessen, Germany. From 2000 to 2006 she worked at the Chair of Biochemical Pharmacology (Prof. Wendel), University of Konstanz. She completed her PhD thesis in veterinary medicine to the Free University of Berlin (2006) and also received a PhD in biology at the University of Konstanz (2006, International validation of an *in vitro* alternative to animal testing). Stefanie has been working as a scientific assistant at the foundation Animalfree Research in Zurich since 2008 and is a member of the scientific advisory board of the Foundation Research 3R in Berne, Switzerland.

Carol Howard has been a science writer for more than 20 years and has worked at CAAT for the past 9 years. She is responsible for content development for a wide range of CAAT publications, including Altweb, the Alternatives to Animal Testing Website (http://altweb.jhsph.edu).

Carol holds a Master's degree in biology and a Bachelor's degree in psychology. She has completed a one-year graduate program in science communication. She is author of the non-fiction book *Dolphin Chronicles* (Bantam Books, 1996). Her writing also has been published in National Geographic Books, Time Life Books, *Psychology Today, Reader's Digest* and various literary journals.

A life-long love of animals and long-standing interest in science attracted Carol to CAAT and the field of alternatives.

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UK: First decline in laboratory animal numbers in a decade

The number of experimental procedures on live animals dropped by 37,000 in 2009 in comparison to the previous year. According to the report of the British Home Office, 3.6 million procedures were performed in total. FRAME, the Fund for the Replacement of Animals in Medical Experiments, welcomed the modest reduction and voiced hope that this is the beginning of a long-term downward trend.

Although the number of primates used for experimental procedures dropped by 7%, FRAME remains deeply concerned about the continued use of monkeys in UK research, giving the reason that these animals are highly intelligent and social and are likely to suffer more than others in laboratory situations.

The breeding of genetically modified animals rose by 10% over 2008. In fact, the number of genetically engineered animals used in laboratories exceeded the number of "normal" animals for the first time. FRAME calls for the publication of further information on the types of procedures used in the course of breeding programmes to evaluate how the persistent rise can be halted using 3R principles.

Overall numbers of experimental procedures fell from the late 1980s until around 2000 but returned to the levels of 1987 last year. According to FRAME, the decline appears to be a reflection of the reduction in biomedical research caused by the economic downtrend and some changes in general scientific trends.

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USA: IIVS News & Views

IIVS receives first Ethical Science Award

In recognition of its leadership in the optimization, validation, and routine use of non-animal testing methods, IIVS has been awarded the first *Mark Twain Ethical Science Award*. This honor, presented by PETA, recognizes IIVS for its successes in helping hundreds of companies worldwide utilize testing strategies that minimize the use of animals, while at the same time maintaining or improving the current level of safety for the public.

"We are honored to be the first recipient of this prestigious award from PETA," says Rodger Curren, President of IIVS. "Their continued support of our work to expand the application of non-animal methods for products testing has been significant for many of our successes." A recent example of such collaboration was the partial funding provided by PETA for IIVS to participate in an international study for non-animal methods to detect skin irritants. This resulted in the EU accepting certain reconstructed human skin models for regulatory submissions. The award is named for Mark Twain in recognition of his staunch opposition to the abuse of animals in laboratory experiments. PETA recently sponsored Twain's hometown museum exhibit and hailed the author as America's first animal advocate.

2010 In Vitro Alternatives Forum October 18 & 19 Hotel Monaco – Old Town Alexandria

The beautiful Hotel Monaco in historic Old Town Alexandria, Virginia, USA, provides a superb setting to learn about the factors driving the increased need for alternative test methods and the international activities designed to promote their use and acceptance. The program includes overview and commentary on the reauthorization of the Toxic Substances Control Act (TSCA), advancements in the area of 21st Century Toxicity Testing, approaches to identify skin sensitizers, utilization of 3D tissue models, and more. Speakers include international experts from industry, regulatory agencies, and the animal protection community. Register soon at www.iivs.org.

American Society for Cellular and Computational Toxicology (ASCCT)

IIVS and PCRM encourage your support for the newly established scientific society, ASCCT, dedicated to the advancement of *in vitro, in silico*, and other toxicological testing methods, especially as replacements for animal-based tests. A key goal of the society is to provide regular meetings during which experts can share their knowledge of such methods with those seeking to implement non-animal testing strategies. It is hoped that these meetings will also provide an opportunity for scientists from industry and the regulatory community to discuss the best practice of these methods as they are applied to regulatory toxicology situations. An open information session about the goals of the society will be held during the *2010 In Vitro Alternatives Forum*. If you are interested in participating in the Society or learning more about proposed activities, please visit www.assctox.org or contact Erin Hill at ehill@iivs.org or Kristie Sullivan at ksullivan@pcrm.org

The Toxics Substances Control Act (TSCA): an opportunity to improve the science behind risk assessment

The IIVS Summer newsletter contains an article contributed by Kate Willett, PhD (Science Policy Advisor, Regulatory Testing Division, PETA), regarding current TSCA reform in the US. In the article, Dr. Willett describes the two bills introduced into both the House and Senate of the US Congress and PETA's efforts to include animal protection meas-

ures such as the requirement for the use of alternative test methods when they exist. "While these bills are headed in the right direction, further elements need to be strengthened or clarified to ensure that animal use is minimized and eventually eliminated. A major improvement would be to require the use of non-animal methods where they exist, along the lines of Directive 86/609 and REACH legislation which states that animal testing should be performed 'only as a last resort.'" Willett also suggests that the bill should include stronger language requiring, rather than allowing, the use of integrated strategies, and that experts in alternative methods should be included in the mandated Science Advisory Board. To view the article in its entirety, please visit www.iivs.org or contact ehill@iivs.org.

Workshop Report: Mutagenicity/ genotoxicity testing without animals? German Federal Institute for Risk Assessment (BfR) June 24-25, Berlin, Germany

Since March 2009, the 7th Amendment to the EU Cosmetics Directive (76/768/EEC) has banned the marketing of cosmetics or ingredients that have been tested *in vivo* after this date for acute toxic effects. For the assessment of genotoxic/mutagenic effects, a battery of well-established and regulatory accepted in vitro assays are available. However, long experience with these *in vitro* assays has revealed low specificity of the methods resulting in a high percentage of false positive results. Combining the *in vitro* assays in a battery approach increases the overall sensitivity while the specificity is even further reduced. Thus, various regulations accept negative results from the battery as a valid predictor of the absence of a genotoxic/mutagenic hazard, while positive outcomes in any of the assays in the battery generally require confirmatory *in vivo* testing.

Rodger Curren, IIVS President, was invited to participate in a BfR Expert Meeting focused on identification of the current status of development or prevalidation of new, complex *in vitro* models (e.g. human 3D epidermal or full thickness skin models, embryonated hen's egg test, etc.) for genotoxicity. Models like these are believed not only to better demonstrate biokinetic aspects of barrier and metabolism, but also to exhibit more normal DNA repair systems. The participants also considered whether modifications of existing tests, their test data interpretation procedures, or modifications of testing strategies might result in better predictions of the risk of genotoxic events *in vivo*. The general conclusion was that several of the scientific solutions proposed will eventually allow adequate predictions of genotoxic risk without the use of animals. A consensus document of the proceedings of the workshop is expected to be published before the end of the year. For more information on the BfR and its activities, please visit www.bfr.bund.d

In Vitro OECD Test Guideline for Skin Irritation

In another major advance for *in vitro* methods, OECD Draft Test Guideline 439 (TG439) – In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method – was approved by the Working Group of National Coordinators (WNT) on 23-25 March 2010. The TG is expected to be adopted by the Council in July and subsequently published for use by the international toxicology community. TG439 allows any of three reconstructed human epidermis models (EpiSkin™ , EpiDerm™ SIT (EPI-200), and the SkinEthic™ RHE) to be used to classify materials as either GHS 2 or non-irritant (UN GHS No Category). The WNT also agreed to develop a Guidance Document on Skin Irritation/ Corrosion. This project will start with an Expert Meeting to review the different types of tools that can be used in a testing strategy and their applicability. Hans Raabe, Vice President and Director of Laboratory Services at IIVS, will participate in the Experts Meeting scheduled in October at the German Federal Institute for Risk Assessment (BfR) in Berlin, Germany.

BASF Now Offers Equipment for the BCOP Assay

OECD Test Guideline 437 describes the BCOP test as a method that can be used to classify substances as ocular corrosives and severe irritants as defined by the EPA (Category 1), the EU (Category R41), and the GHS (Category 1). During the in-house validation of the BCOP assay, BASF experienced difficulty in obtaining reliable, state-of the-art, and commercially available equipment. Therefore, they designed and optimized instrumentation for the BCOP which they now offer commercially. The BASF BCOP Opacitometer Kit includes an opacitometer with certified light meter, PC interface with data transfer program, corneal holder set for 8 test substances, glass filter with holder for calibration, validation data set, and instruction manual. The complete Opacitometer Kit is available for 9,900 €. Individual components may be purchased separately. A lead time of approximately 6 weeks is required. To receive a brochure please contact ToxOffice@basf.com.

Erin Hill, IIVS
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USA: ICCVAM recommends new versions of murine LLNA

ICCVAM recently forwarded final recommendations to U.S. agencies for two new nonradioactive versions of the murine local lymph node assay, as well as recommendations for expanded applications of the LLNA (ICCVAM 2010a, b, c; announced June 29 in the *Federal Register*, 75 FR 37443). These recommendations are expected to result in up to 50% fewer animals being used to identify chemicals and products that could cause allergic contact dermatitis, compared to the original LLNA. They will also allow more institutions to take advantage of the reduction and refinement benefits afforded by the LLNA compared to the traditional guinea pig methods.

ICCVAM recommended the LLNA in 1999, and it has since been accepted in the U.S. and internationally for use in identifying substances with the potential to cause allergic contact dermatitis. ICCVAM originally recommended limitations for the LLNA with regard to the range of substances for which it was applicable. After evaluating an updated database for the use of the LLNA to test pesticide formulations, metals, substances in aqueous solutions, and other products, ICCVAM has expanded its recommendation on the applicability domain of the LLNA. ICCVAM now recommends that the LLNA may be used to test any chemical or product for allergic contact dermatitis hazard potential unless the chemical or product to be tested has properties that may interfere with the ability of the LLNA to detect sensitizing substances.

The traditional LLNA uses radioisotopes; therefore, its use is limited to laboratories qualified to handle and properly dispose of radioactive materials. The current ICCVAM recommendations support the use of two modified nonradioactive versions of the LLNA to assess allergic contact dermatitis hazard potential of chemicals and products, with certain limitations. The modified nonradioactive versions of the LLNA recommended by ICCVAM are:

- The LLNA: BrdU-ELISA, which uses the nucleotide analog bromodeoxyuridine (BrdU) to assess lymph node cell proliferation instead of radiolabeled substances. The LLNA: BrdU-ELISA was developed by Dr. Masahiro Takayoshi at the Chemicals Evaluation and Research Institute in Japan (Takeyoshi et al., 2001). Validation studies for the test method were completed in coordination with the Japanese Center for the Validation of Alternative Methods (JaCVAM).
- The LLNA: DA, which measures adenosine triphosphate content to assess lymph node cell proliferation instead of radiolabeled markers. This test method was developed by Dr. Kenji Idehara at Daicel Chemical Industries, Ltd., in Japan (Idehara et al., 2008; Yamashita et al., 2005). The validation studies for the LLNA:DA were also completed in coordination with JaCVAM.

Compared to traditional guinea pig methods used to test products for allergic contact dermatitis hazard potential, the LLNA uses fewer animals and eliminates animal pain and distress. The updated applicability domain for the LLNA and the availability of the nonradioactive LLNA methods should allow more institutions to take advantage of the animal welfare benefits of the LLNA, while avoiding the environmental hazards of the radioactive materials used in the traditional LLNA.

In March of this year, U.S. Federal agencies accepted ICCVAM recommendations on an updated LLNA protocol and modified procedure called the reduced LLNA that uses 40% fewer animals (see following article). The agencies also accepted performance standards for the LLNA that can be used to more efficiently evaluate the validity of proposed modified versions of the LLNA. Information on the ICCVAM evaluation of the LLNA can be found on the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/methods/immunotox/immunotox.htm

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ICCVAM/NICEATM
press release
6th July 2010

USA: ICCVAM proposals for international test guidelines approved

The National Coordinators of the Test Guidelines Programme of the Organisation for Economic Co-operation and Development (OECD) met on March 23-25, 2010. The Coordinators approved several new international test guidelines and a guidance document that NICEATM and ICCVAM developed in cooperation with their partners in the International Cooperation on Alternative Test Methods (ICATM). The test guidelines and guidance document include the following alternative methods evaluated and recommended by ICCVAM:

- An updated Test Guideline 429 for using the LLNA to assess whether chemicals and products may cause allergic contact dermatitis. The revised test guideline provides an updated protocol that reduces animal use by 20% compared to the previous version of the guideline. It also incorporates an optional reduced LLNA procedure that allows for an additional 40% reduction in animal use.
- A new test guideline for the LLNA: BrdU-ELISA. This nonradioactive version of the LLNA uses the nucleotide analog bromodeoxyuridine (BrdU), instead of radiolabeled substances, to assess lymph node cell proliferation. The LLNA: BrdU-ELISA was developed by Dr. Masahiro Takayoshi at the Chemicals Evaluation and Research Institute in Japan.
- A new test guideline for the LLNA: DA. This version of the LLNA assesses lymph node cell proliferation by measuring adenosine triphosphate content instead of radiolabeled markers. This test method was developed by Dr. Kenji Idehara at Daicel Chemical Industries, Ltd., in Japan.
- A guidance document on using cytotoxicity tests to estimate starting doses for acute oral systemic toxicity tests. The approach uses an IC_{50} value from an in vitro basal cytotoxicity test to estimate the *in vivo* LD_{50} value. The estimated LD_{50} value can then be used as a starting dose for assessing acute oral systemic toxicity using the up-and-down procedure, the acute toxic class test method, or the fixed dose procedure. This approach can reduce animal use as much as 50% for some substances. The guidance document is based on the results of a NICEATM international validation study and ICCVAM recommendations that were endorsed by U.S. Federal agencies in 2008.

ICCVAM/NICEATM
press release
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CAAT*feed*

CAAT Director Thomas Hartung Receives Agilent Thought Leader Award for Research into Novel Toxicity Pathways for Embryonic Brain Development Using Metabolomics

On August 18th, Agilent Technologies Inc. (NYSE: A) and the Agilent Foundation announced that Dr. Thomas Hartung has received an Agilent Thought Leader Award in support of his research for the use of toxicity pathways to predict developmental neurotoxicity. This work could help identify possible contributions of chemicals to disorders such as autism and attention hyperactivity disorders. Dr. Hartung recently was named a leading toxicologist by the science journal *Nature*.

The award includes Agilent Foundation funding for research and a company donation of instruments worth more than $ 500,000 to the Center for Alternatives to Animal Testing (CAAT), part of the Johns Hopkins Bloomberg School of Public Health. Dr. Hartung will focus his research on the identification of novel toxicity pathways by combining two promising cell culture models with emerging metabolomics technology.

"This award makes cutting edge technology available for a project in the core of implementing the vision of a new

regulatory toxicology," says Hartung. "Problems of the 21st century can only be solved with 21st century technologies."

"The information we need to fully understand the toxic effects of chemicals on humans cannot be obtained using traditional animal models. We are not 70-kilogram rats," adds Hartung.

The identified pathways will be annotated in a public database the scientific community can use for further mechanistic studies. This will be of particular benefit to the pharmaceutical and chemical industry to assist with identifying how drug compounds and chemicals interact with human biochemical pathways. In the case of drug development, this information will allow for better toxicological assessment of promising drug lead compounds at early preclinical stages, reducing costs and time.

The field of toxicology is undergoing rapid change prompted by the 2007 National Research Council's report, 'Toxicity Testing in the 21st Century: A Vision and a Strategy.' The report aims to move toxicity testing to work with human cells and computer modeling instead of animal tests to improve the prediction of human adverse effects. This is considered the way forward to assess the tens of thousands of yet untested chemicals in the environment and consumer products. The study supported with the award is piloting this approach.

"The research will have great impact on the field of toxicology by performing a proof of principle study for the use of toxicity pathways to predict developmental neurotoxicity," said Mark Vossenaar, senior director of strategic marketing for Agilent's Life Sciences Group. "Working closely with one of the leading research institutions in the world, we believe the results will demonstrate the feasibility of LC/MS based metabolomics in toxicity studies."

Agilent's new Thought Leader Program promotes fundamental advances in the life sciences by contributing financial support, products and/or expertise to the research of influential thought leaders. Agilent Technologies Inc. (NYSE: A) is the world's premier measurement company and a technology leader in chemical analysis, life sciences, electronics and communications. The company's 18,500 employees serve customers in more than 100 countries.

CAAT-EU Information Day: Toxicology in the 21st Century, TSCA Reauthorization, and REACH

CAAT-EU will host the first in a series of high level Information Days on October 28, 2010, in Konstanz, Germany. The event will focus on the status quo of REACH, the US Toxic Substance Control Act (TSCA) reauthorization, and the implementation of the US National Research Council strategy for *Toxicity Testing in the 21st Century*.

The REACH directive in the EU and TSCA reauthorization in the US are large-scale programs to obtain toxicological data for old compounds, prompting increased testing and the need for new methods. This Information Day will provide a status update on REACH and TSCA for a European audience. Moreover, CAAT-EU will introduce the report of US National Research Council, *Toxicity Testing in the 21st Century*. The authors of the report suggest that the power of toxicological risk assessment can be maximized by employing high throughput *in vitro* screening assays, tests in lower organisms, systems biology, functional genomics and transcriptomics, as well as predictive *in silico* approaches. This Information day aims to introduce this vision to a European audience by inviting some of the key US players as speakers, including representatives from The National Research Council (NRC)/ National Academy of Sciences (NAS), the Environmental Protection Agency (EPA), American Chemistry Council (ACC), BASF, and the Johns-Hopkins Bloomberg School of Public Health.

Full details are available on the CAAT and CAAT-EU websites: http://caat.jhsph.edu and http://cms.uni-konstanz.de/leist/caat-europe/

Critical Evaluation of the Use of Dogs in Biomedical Research & Testing – A CAAT Workshop

On January 12 and 13, 2011, CAAT will hold a workshop bringing together investigators who use dogs as disease models to present their research while fostering dialogue on the implementation of alternative models. Representatives from the US Food and Drug Administration (FDA) will be invited to review data obtained from dogs submitted in support of drug submissions. Veterinarians and experts in dog behavior will be invited to discuss the welfare issues associated with research and testing on dogs.

The workshop will take place at the Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland. Further details will be posted on the CAAT website: http://caat.jhsph.edu

For more information contact Joanne Zurlo, CAAT Director of Science Strategy (jzurlo@jhsph.edu)

Recent Publications

Bunk, S., Sigel, S., Metzdorf, D., Sharif O., Triantafilou, K., Triantafilou, M., Hartung, T., Knapp, S. and von Aulock, S. Internalization and co-receptor expression are critical for TLR2-mediated recognition of lipoteichoic acid in human peripheral blood. *J. Immunol.* in print.

Hartung, T. (2010). Lessons learned from alternative methods and their validation for a new toxicology in the 21st century. *J. Toxicol. Env. Health 13*, 277-290.

Rockel, C., Hartung, T. and Hermann, C. (2010). Different S. aureus whole bacteria mutated in putative pro-inflammatory membrane components have similar cytokine-inducing activity. *Immunobiol.* in print.