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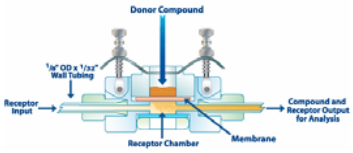
Institute Update

Expert Users Workshop Review of the OECD Guidelines of Percutaneous Absorption (PA) *In Vitro*

When planning *in vitro* percutaneous absorption (PA) studies, there are many considerations that must be addressed to obtain useful results. Specific requirements for study design and data analyses differ across industry sectors (personal care, household, agricultural and chemical products, dermal and trans-dermal pharmaceuticals, etc.), and there is no universal agreement in study design or analyses among the various regulatory agencies.

Although the OECD 428 *in vitro* skin absorption test guidelines (TG-428) were finalized in 2004, there are a number of procedures that are not uniquely defined. A reason for this is to allow for differences in study goals, design and analyses. The Guidance Document (Conduct of Skin Absorption Studies, March 2004) was proposed to provide further technical clarification and guidance, while still allowing for specific requirements. Even though the Guidance Document provides greater detail to support TG-428, there still are procedures common to most PA applications that could benefit from further clarification. Consequently, using these documents to establish a PA program, in the absence of further guidance, could be challenging for laboratories lacking specific expertise.

In order to supplement the guidelines with additional details beneficial to new users, IIVS (with the support of The Colgate Palmolive Company and the PCRM), convened an Expert Users Workshop. The purpose of the workshop was to discuss the TG-428 and Guidance Document and how they may be practically applied to *in vitro* PA protocols currently in use. The Expert Panelists were tasked with presenting resolutions that they have successfully implemented to address common problems, particularly in studies for regulatory submission and review.



Schematic View of a Diffusion Cell

The participants compared and contrasted specific aspects and interpretations of the guidelines relative to their PA programs and made recommendations on protocol components that are essential for obtaining useful pharmacological data from the *in vitro* PA methods. Technical presentations addressed issues common to a wide range of PA applications such as skin model selection and preparation, barrier integrity test methods and their analyses, receptor fluid selection criteria, test formulation preparation issues and test sample analyses. Other presentations addressed issues of data analyses, interpretation and risk assessment. The ensuing discussions were then used as a basis for a review of TG-428 and the respective Guidance Document, culminating in a series of clarifications and recommendations. The report from the Expert Panel Workshop is available on our web-site (www.iivs.org).

13th Congress on Alternatives to Animal Testing

Dr. Rodger Curren, IIVS President , was an invited speaker at LINZ2006, the Annual Meeting of MEGAT (Middle European Society for Alternative Methods to Animal Testing) in Linz, Austria. Rodger presented work conducted in collaboration with Procter & Gamble on the Reconstituted Skin Micronucleus Assay. This is traditionally the second largest meeting on alternatives to animal testing and hopefully will be attended by more American scientists in the future. An important aspect of the program is that presenters addressing the scientific and ethical issues of alternatives speak during the same sessions. This format allows participants access to both perspectives on each topic presented.

Linz2006 was sponsored by the Austrian Alternatives Group, zet .

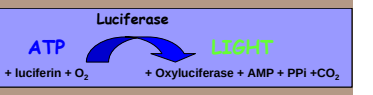
BITS & PIECES

- Look for IIVS News & Views in ATLA (Alternatives to Laboratory Animals)
- Dr. Rodger Curren will participate in a 2 day A-Cute-Tox Program workshop (11-12 September, Ispra, Italy) focused on the further development of a strategy for estimating the acute toxicity of compounds
- INVITOX meeting Oct 2-5, 2006 Ostend, Belgium www.invitox2006.org

Inside this issue:

Use of An Adenosine Triphosphate (ATP) Cytotoxicity Assay in Normal Human Epidermal Keratinocytes (NHEK)to Predict Systemic Toxicity *In Vitro*

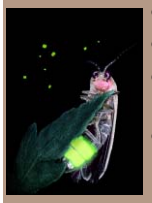
Ekwall *et al.* (ATLA 17:83-100, 1989) have proposed that "80% of chemical-induced systemic toxicity is the result of disruption of basic cellular processes common to most cell types in the body, and that "systemic toxicity for many chemicals could be estimated in *in vitro* cultures." The Multicenter Evaluation of In Vitro Cytotoxicity (MEIC) was established to test this hypothesis. Strickland *et al.* (The Toxicologist, Abs#761, 2003) reported on a validation study to evaluate cytotoxicity assays using NHEK and BALB/c 3T3 with a neutral red uptake (NRU) viability endpoint to predict acute systemic toxicity. Recently, IIVS and Cambrex North Brunswick Inc. have conducted a proof of concept study to evaluate the ATP endpoint to assess viability of NHEK cultures.



Untreated NHEK's divide and proliferate. Exposure to a toxic chemical will interfere with this process and result in a reduction of the viability as reflected by cell number. Since, within each cell type there is a reasonably uniform quantity of ATP, an assay for ATP provides a direct measure of the number of viable cells present. Cytotoxicity is expressed as a concentration-dependent reduction in the bioluminescent measurement of ATP after chemical exposure (Crouch, *et al.,* 1993).

For the current study, 20 chemicals for which human lethal serum data were available were selected to represent a wide acute toxicity range. At least three trials of all 20 blind-coded chemicals were tested in compliance with GLPs at IIVS. A subset of 10 chemicals was tested at Cambrex North Brunswick, Inc. The results of this phase were used to develop the prediction equation to estimate human lethal serum LC_{50} values, and to evaluate reproducibility of the test methods. The results were compared to those reported previously using an NRU endpoint (IIVS in-house results, Curren, 1992; Wallace, 1993).

Conclusions:



- The ATP endpoint is faster and requires fewer steps than the NRU endpoint, hence making this endpoint very appropriate for high throughput screening
- Demonstrated relationship between *in vitro* IC_{50} values and human lethal serum LC_{50} concentrations (r^2 = 0.887)
- Very high interlab reproducibility of mean IC_{50} values (differences of <1/3 log for the 10 blind-coded chemicals)
- Considering the dynamic range of the assay, the difference in positive control reproducibility between labs is considered biologically insignificant
- Very high correlation (r^2 =0.979) between ATP IC_{50} and NRU_{50} values indicates that for these 20 chemicals the ATP endpoint identified essentially the same IC_{50} as the neutral red uptake endpoint in NHEK cultures treated according to the protocol In Vitro Cytotoxicity Validation Study (ICCVAM, 2003)
- Results show that the NHEK assay with the ATP endpoint shows promise in the early evaluation of potential systemic toxicity

Future Activities

Cambrex Corporation has sponsored the current testing of an additional 50 chemicals primarily selected from those used in the international validation of the *in vitro* normal human keratinocytes (NHK) NRU cytotoxicity test (Strickland, *et al.*, 2002).

Practical Methods Workshop

Hans Raabe presents the development of the long term corneal culture assay.

The Practical Methods Workshop (most recently held June 13-15, 2006) continues to be a successful forum for sharing information and ideas. Lectures were given by IIVS staff and invited speakers. Topics included discussion of established models such as the Bovine Corneal Opacity and Permeability Assay (presented by Dr. Joseph Sina of Merck Research) and the In Vitro Percutaneous Absorption Assay (presented by Dr. Robert Bronaugh, USFDA) as well as new methods, such as the In Vitro Reconstituted Skin Micronucleus Assay (presented by Greg Mun, IIVS).

Instruction turned practical in the afternoon when participants were teamed with IIVS laboratory personnel to work hands-on with specific assay systems. This year's comments and reviews confirmed the exceptional skill set of the IIVS technical staff.

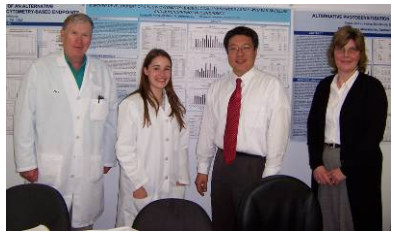
The ambitious agenda filled the day with lectures and laboratory instruction. Evenings were filled with opportunities for participants to relax over food and cocktails with members of the IIVS staff, resulting in a mutual exchange of ideas and a greater understanding of the role both groups play in the advancement of alternative methods.

Valerie Deoudes of IIVS works with Cristi Gomez of Mary Kay on the performance of the Neutral Red Release Assay.

The Institute would like to take this opportunity to thank everyone who contributed to making this years' course a great success! We invite those who haven't had an opportunity to take advantage of the course to join us next year. Please contact Amanda Ulrey at aulrey@iivs.org if you would like to learn more.

Reproducibility Through Information Exchange

Interlaboratory reproducibility is a key component in defining the usefulness and validity of an alternative assay system. Recognizing this fact, IIVS and MB Research Laboratories are engaged in an exchange of technical experience and best practices for the Bovine Corneal Opacity and Permeability Assay. Over the past few months, the two laboratories have cooperated to review documentation, compare protocols and equipment, observe the performance of the assay at each site, and perform concurrent evaluations of blinded test materials. Based on data generated and discussions between the scientists from both facilities, a harmonized protocol will be developed and made available for use. The protocol will standardize many procedures and equipment requirements between the two labs, assuring that data generated provide a consistent evaluation of the ocular irritation potential of materials regardless of the facility performing the assay. IIVS and MB invite other facilities performing the BCOP assay to participate in evaluating the harmonized protocol. Contact jluczak@iivs.org for updates on protocol availability.



Daniel Cerven and Allison Ball (MB Research) and Greg Mun and Janet Luczak (IIVS) pause for a photo during their collaboration on BCOP protocol standardization.

FRAME

IIVS is fortunate that there are several international organizations that have programs complementary to ours. One of the better known is the UK's Fund for the Replacement of Animals in Medical Experiments (FRAME), founded in 1969 to promote the concept of alternatives to the use of live animals in medical research and toxicity testing. Like IIVS, FRAME is able to maintain awareness of the performance characteristics of many alternative methods since it operates an Alternatives Laboratory at the University of Nottingham. IIVS and FRAME have cooperated on numerous laboratory activities in the past and will continue to look for ways to help each other advance alternatives in the future. For more information about FRAME, please visit www.frame.org.uk

Publishers of FRAME News and ATLA

SAP Member Highlight—Prof. Michael Balls

Professor Michael Balls, a member of the IIVS Scientific Advisory Panel since its inception, has had a long and distinguished career in the protection of animals and the advancement of alternative methods. Prof. Balls is probably best known for his tenure as the first Head of ECVAM from 1993 until his retirement in 2002. During that time not only did he oversee major advancements in the regulatory acceptance of non-animal methods, he still found time to give great support to the formation of IIVS. In 1997 we were honored when he graciously presented the keynote speech during the IIVS Grand Opening Celebration.

Professor Balls has been the recipient of many awards for his contributions to the field, including the first Marchig Animal Welfare Award, The Russell and Burch Award from the HSUS, and the Michael Kay Award of the Royal Society for the Prevention of Cruelty to Animals. In 2002 Prof. Balls was appointed a Commander of the Most Excellent Order of the British Empire (CBE) by Queen Elizabeth II.

This year Prof. Balls celebrates his 25th year as the Chairman of the Trustees of FRAME. During much of his time as Chairman, he has also acted as Editor of ATLA. Prof. Balls continues to be a vital member of the IIVS SAP, supplying information and a European insight on the progress of alternative methods.

IIVS would like to congratulate Professor Balls as he celebrates his 25th year as Chairman of the FRAME Trustees!

Institute for In Vitro Sciences, Inc.

Advancing Science & Animal Welfare Together

21 Firstfield Road
Suite 220
Gaithersburg, MD 20878

Phone: 301-947-6523
Fax: 301-947-6538
Email: info@iivs.org

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The IIVS water bottle attends an afternoon lecture in Linz.

Micronucleus Manuscript Accepted for Publication

A manuscript co-authored by P&G and IIVS scientists, "Development of a Method for Assessing Micronucleus Induction in a 3-D Human Skin Model (EpiDerm™)", has been accepted for publication in the journal Mutation Research, Genetic Toxicology and Environmental Mutagenesis. Drs. Marilyn Aardema (P&G) and Rodger Curren (IIVS) have developed the assay to measure genetic toxicity in skin, an organ with high exposure to many chemicals and products. We feel the Reconstructed Skin Micronucleus Assay (RSMA) will be very helpful in complying with the animal-testing prohibitions of the 7th Amendment to the Cosmetics Directive.

Send us photos of you and your IIVS water bottle. You could be featured in our next newsletter!

"What's New at Our House"

Meet the newest member of the IIVS Board of Directors; Peter Theran, DVM.

As VP of Animal Science for the MSPCA, President of the Center for Laboratory Animal Welfare, and national consultant in animal welfare, Dr. Theran's primary focus is the welfare of animals in biomedical research, consumer product safety testing, and education.

Dr. Theran has served on numerous committees and boards of directors of organizations related to both animal welfare and research. Dr. Theran is a member of the Board of Directors of Chimp Haven, a non-profit organization dedicated to providing homes for chimpanzees no longer needed in research, and of Public Responsibility in Medicine and Research (PRIM&R), a research ethics organization. From 1998 to present he has been a member of the Scientific Advisory Committee on Alternative Toxicological Methods which advises ICCVAM and NTP. In 2004, Dr. Theran was appointed to the Institute of Laboratory Animal Research Council of the National Academy of Science. We are pleased to have Dr. Theran sit on our Board of Directors where we look forward to his experience to help guide the development and growth of IIVS.

Hans Raabe has been appointed to the position of **Director of Laboratory Services** at IIVS. He is responsible for the overall direction of lab activities and is pleased to be serving IIVS and its clients in this expanded capacity.

Gregory Moyer, Laboratory Supervisor at IIVS, received his M.B.A. in May from the University of Phoenix. We would like to congratulate Gregory on his accomplishment and look forward to his continued success at IIVS.

Raymond Tice, Ph.D. (NIEHS/NTP) uses his IIVS water bottle to quench his thirst after a hard day of hiking in Zion and Bryce Canyon National Parks in Utah.