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Institute Update

3-D Human Skin Models – The Next Generation of Tools for the In Vitro Toxicologist

For over 15 years *in vitro* toxicologists have been intrigued by the possibility of having an endless supply of affordable reconstructed human skin models to use in product development and safety testing. However, despite good efforts, that goal seemed to be always just out of reach. From the earliest attempts at commercialization of such models, batch-to-batch variability, lack of complete characterization and a high cost of production have conspired to keep 3-D artificial skin tissues from reaching their full potential.

During this time, another phenomenon added uncertainty to the field. It seemed that no sooner had confidence begun to develop in one model when its manufacturer went out of business - only to be replaced quickly by another company selling a different model. The new model then needed time to be characterized and accepted before it could be routinely used. Understandably, some researchers became hesitant to invest heavily in developing performance databases for the models.

However, during the last 6-7 years there has been a growing sophistication in production techniques leading to the routine commercial availability of 3-D skin cultures from both US and European manufacturers. Perhaps one of the reasons that more skin models are appearing is due in some degree to the 7th Amendment to the Cosmetics Directive which prohibits animal testing for a number of skin-related toxicological endpoints by 2009. For example, corrosivity, skin irritation and skin sensitization testing of both cosmetic ingredients and final products will soon have to be conducted in non-animal systems. Other assays are also being developed using the skin models, such as a point of contact genotoxicity assay now being further pursued by COLIPA (see pg 3).

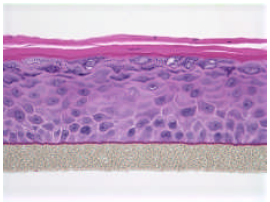

Image of untreated EpiDerm™ tissue.

It is clear that more diverse and sophisticated uses will continue to be developed for these tissues - and the more intricate and expensive the application, the more concern will develop about stability and reproducibility. Typically manufacturers have supplied only basic information concerning the reproducibility of toxicity endpoints (most commonly just a standard cytotoxicity response). However, as these models are utilized in more sophisticated and labor intensive assays, more characterization of each tissue lot will be required to assure suitability for the study.

Part of the maturation of the commercial use of these models will be the requirement by purchasers for a minimum set of information, such as performance with a positive control, histology, and normal growth

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Society of Toxicology Annual Meeting

START YOUR ENGINES… and join us in Charlotte, NC March 25-29th for the annual Society of Toxicology meeting and exhibit. Please stop by our booth (#1425) Monday through Wednesday for a copy of our newsletter, list of services and giveaway items. Study Directors will be available to discuss your program and testing needs.

Visit our Posters:
"Estimating Systemic Toxicity In Vitro Using an Adenosine Triphosphate Cytotoxicity (ATP) Assay in Normal Human EpiDermal Keratinocytes" from 1-4:30 on Tuesday (#1182-504) and
"Further Development of a Reconstructed Skin Micronucleus Assay" from 1-4:30 on Wednesday (#1671-223). Contact us for copies of our posters.

BITS & PIECES

- Workshop for the European Coalition to End Animal Experiments: March 31 - Neubiberg, Germany
- ECVAM announces a database on alternative methods to animal experimentation (DB-ALM). http://ecvam-dbalm.jrc.cec.eu.int
- Save the Date: Practical Methods in In Vitro Toxicology Workshop - October 9-11, 2007
- 6th World Congress on Alternatives & Animal Use in the Life Sciences (WC6) will be held in Tokyo, Japan from August 21-25, 2007

Inside this issue:

Technical Notes

3-D skin models allow for topical application of the test material.

Over the past several years the increase in demand for three-dimensional (3-D) human tissue constructs has been met with the entry of new models into the market. Within the 3-D skin model market there are differences in each of the manufacturer's offerings (e.g., in primary cells used, in tissue barrier function, sensitivity, epidermal-only or full-thickness, etc.) which may result in differences in specific responses under standardized applications. While validation authorities and peer review groups can help determine which models are appropriate for a specific application, it is up to the testing laboratory to maximize the usefulness of these powerful tools. Accordingly, it is important that the testing laboratory develop an understanding of the nature and impact of the functional characteristics of each of the manufacturer's 3-D tissue model offerings. It is the selection of proper test material exposure times, the inclusion of appropriate benchmarks, the consistent use of positive and negative controls, and the execution of the study using sound documentation practices, that establishes a reliable and effective testing program from which product development and safety decisions can be made.

To determine irritancy potential, test materials are typically applied topically to the tissues, rinsed after a specified exposure period, and evaluated for cytotoxicity. To accurately evaluate the time-to-toxicity of a test material, exposure times are typically *not* pre-selected. Instead, an initial assessment of the appropriate exposure time range is made either by a formal exposure time range-finding experiment, or within an experiment by initially evaluating the responses of 1 or 2 exposure times, followed by 2 or 3 additional exposure times. The goal is to select a narrow range of exposure times that represent the full range of toxicity in treated tissues. By increasing the precision of the assay, one can more accurately evaluate the potential irritancy of the test article and compare responses among test articles of similar chemistry.

When available, reference materials or benchmarks may be tested concurrently with the test materials. The benchmarks provide the means for interpreting the cytotoxicity responses of the test materials. Benchmarks should be from the same chemical or product class as the test material, and should include *in vivo* safety data or market history. Since formulation and vehicle effects, as well as synergistic effects from multiple active ingredients play a crucial role in skin penetration and irritation, ideally the benchmark will differ from the test material only in the active ingredient(s) under investigation. Multiple benchmarks may be used to establish ranges for both "acceptable" and "unacceptable" responses. Thus, benchmark test results can be used to set maximum and/or minimum acceptable limits against which test results are judged.

To ensure that the models provide reproducible and reliable results from standardized protocols, positive and negative controls should be run concurrently with the test materials in every assay. The positive control provides the means to evaluate the quality and reproducibility of the 3-D tissue construct, as well as the execution of the assay by the testing laboratory. The positive control response is compared to the acceptable historical response range (mean $\pm$ 2 std. deviation) to assure that it falls within the predetermined limits. The negative control provides the baseline against which the test material and positive control are compared. In addition, the negative control may be used to determine the effect of any solvents utilized. At least 2 to 4 exposure times should be tested to match the test article and positive control exposure times.

SAP Member Highlight - Dr. Marilyn Aardema

IIVS is pleased to welcome Dr. Marilyn Aardema as a new representative to our science advisory panel. Dr Aardema, currently Principal Scientist within the Genetic Toxicology Research Group at P&G, has worked in the area of genetic toxicology for over 20 years. After receiving her Ph.D. in genetics from the University of Tennessee- Oak Ridge National Laboratory, Dr. Aardema joined P&G where she has been able to focus on her interests in non-animal genetox test methods, toxicogenomics, and the use of genetox in risk assessement. In addition to being active on numerous committees and task forces, Marilyn is also a member of the ECVAM Steering Committee on Cell Transformation, a US OECD expert reviewer and a member of the COLIPA SCAAT Mutagenicity Subcommittee. IIVS is pleased to have worked recently with Dr. Aardema on the development of a reconstituted skin micronucleus assay which may one day replace the rodent micronucleus test for cosmetics products.

SkinInVitro 2007 Meeting

In February 2007 a symposium was organized by CellSystems® manufacturers of the EST-1000 skin model (St. Katharinen, Germany), to review the use of *in vitro* skin models for research and testing, and the legal regulations surrounding them. The meeting was titled: Progress in Research, Development and Legal Regulation of *In Vitro* Skin Models. Held in Cologne Germany, the aim of this meeting was to present the most recent findings of the fundamental applications for skin models, new applications (including skin sensitization research, and development of genotoxicity assays), and the development of new models (notably full-thickness skin models). Because these, mostly scientific issues, are strongly interconnected with legal regulations, lectures also included the opinions of regulatory authorities and the *status quo* of validation studies dealing with the use of such models as alternatives to animal testing. Participants included representatives from industry, ECVAM, FRAME, ZEBET and various skin model manufacturers. Hans Raabe of IIVS was invited to present on The Use of *in vitro* Skin Tissues in a GLP-compliant Testing Facility. For more information on the program visit: http://www.cellsystems.de/SkinInVitro/SIVindex.html

3-D Human Skin Models

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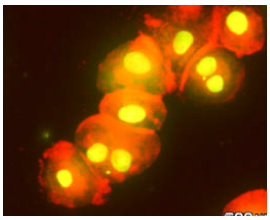
characteristics. However, it is only fair – and certainly more efficient – if users and manufacturers work together to determine what background information is necessary for the tissue to be useful for specific endpoints. Of course, a balance must be sought, because additional work for the manufacturer results in a higher cost for the end product. Nonetheless, this is the inevitable path and the time to begin addressing these issues is now. An excellent example of such multi-stakeholder conversations occurred at a meeting convened by ZEBET (Oct. 2006, Berlin) on the use of skin models for genotoxicity testing. Here representatives of four skin manufacturers, tissue users and regulators were able to discuss at an early stage how these genotoxicity tests might be conducted and how skin tissues used in them should be characterized.

The future of the skin models lies in their use in a regulatory environment which involves rigorous standards that both the users and manufacturers must strive to meet. Only with a spirit of cooperation can all sides develop the models and the assays which work together to meet regulatory needs.

Development of Genotoxicity Assays in 3D Human Skin Models

To meet the requirements of the EU 7th Amendment to the Cosmetics Directive, manufacturers of cosmetics products will need to ascertain the safety of ingredients using non-animal methods. Starting in 2009, *in vivo* genotoxicity tests for cosmetic ingredients will not be allowed. Unfortunately, current *in vitro* genotoxicity tests are known to have a high sensitivity yet very low specificity, the latter resulting in an unacceptable number of false positive results. In a recent analysis of over 700 chemicals it was determined that 75-95% non-carcinogens are positive in one or more of the current *in vitro* genotoxicity assays (Kirkland et al., Mut. Res. 584, 1-256, 2005). Without the current *in vivo* genotoxicity assays to help resolve these false positive results, companies will be limited in their ability to develop products containing new ingredients since a large number of the new compounds will be falsely predicted as carcinogens in current *in vitro* genotoxicity assays and will therefore be limited and/or prohibited for use. To address this problem, COLIPA investigators have outlined a program which aims to replace *in vivo* genotoxicity tests with scientifically valid *in vitro* methods. The primary goal is to predict overall carcinogenic potential of dermally applied substances by using 3D human skin equivalents as a surrogate tissue. The program will encompass three phases: transferability/optimization, reproducibility and prevalidation.



COLIPA is funding investigations of in vitro genotoxicity assays such as the micronucleus assay pictured above.

Comparative studies in EpiDerm™, EpiSkin™, Phenion 3D human skin models, and normal human skin are underway. IIVS is pleased that one assay being investigated is the micronucleus assay developed by scientists at P&G and IIVS (Curren, et al., Mut. Res. 607, 192 – 204, 2006).

Extracted from the project plan of the COLIPA Genotoxicity Task Force.

CELEBRATING 10 YEARS!

In 1997 IIVS was created as a non-profit organization in order to focus on educational and outreach programs involving *in vitro* methods. Using first-hand knowledge of the performance of these methods, IIVS has been able to uniquely advocate for their use and acceptance. These interactions take a variety of forms: from peer-reviewed manuscripts to college seminars to hands-on training sessions for regulatory agency personnel. Unlike our testing services, these educational and outreach programs are funded solely by the generous contributions of our supporters. Without this assistance, IIVS could not provide the extensive programs which we feel are essential to the continued growth of the field. IIVS would like to thank the following who - through their continued monetary and philosophical support – have joined with us to replace animal testing with scientifically valid alternative methods:

Colgate-Palmolive Company
Combined Federal Campaign
The Dial Corporation
Johnson & Johnson
Kimberly Clark Corporation
People for the Ethical Treatment of Animals
POM Wonderful
The Procter & Gamble Company
S. C. Johnson & Son, Inc.

"What's New at Our House"

IIVS will be moving a short distance to a new facility at 30 West Watkins Mill Road in Gaithersburg, MD. We will be the first tenants in the building and will occupy 6,600 sq. feet of newly built office and laboratory space. Construction will begin in March with an estimated move in summer 2007.

Join us in welcoming our newest team member **Matt Hyder**! Matt graduated Magna Cum Laude from Marymount University in May of 2006. He received a Bachelor of Science degree in Biology with a concentration in Cellular and Molecular Biology. Matt performs the Bovine Corneal Opacity and Permeability Assay and is training to conduct the Topical Application Assay for Eye Irritation which utilizes 3-dimenstional tissue constructs.

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POM Wonderful Supports IIVS' Mission to Develop In Vitro Methods to Replace Animal Research.

As the largest US pomegranate grower, POM Wonderful is committed to exploring how pomegranates can benefit human health. For over a decade, POM Wonderful has been at the forefront of medical research on this special fruit and has provided $20 million in research funding to scientists worldwide.

According to POM Wonderful, "The pomegranate has been celebrated in mythology, featured in fine art, and revered for its health benefits since the dawn of time. Today, modern science confirms that the pomegranate is truly a medical marvel.

Recently published clinical studies indicate that pomegranate juice provides unique benefits in promoting cardiovascular and prostate health. Scientists believe that the pomegranate's high levels of natural antioxidants are responsible for these intriguing health benefits." For more information on POM Wonderful, please visit their website at www.pomwonderful.com.

Announcements

ECVAM's Scientific Advisory Committee issued a statement in November 2006 that SkinEthic™ human skin model can be used for distinguishing between corrosive and non-corrosive chemicals within the context of the OECD Test Guideline, TG 431. http://ecvam.jrc.it/index.htm (Publications section; ESAC Statements).

Amendments to the **REACH** directive were published in the Official Journal of the European Union on December 30, 2006. **{"Excerpt from Regulation (EC) No. 1907/2006}**

Article (1) states:
"This Regulation should ensure a high level of protection of human health and the environment as well as the free movement of substances, on their own, in preparations and in articles, while enhancing competitiveness and innovation. **This Regulation should also promote the development of alternative methods for the assessment of hazards of substances."**

This language is new to the regulation and calls not only for the use of validated alternatives, but also the proactive development of new methods. The entire document can be viewed at http://ec.europa.eu/enterprise/reach/index_en.htm.