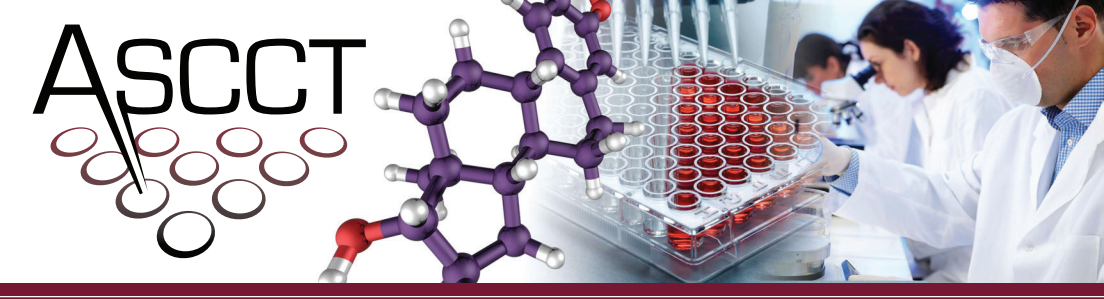
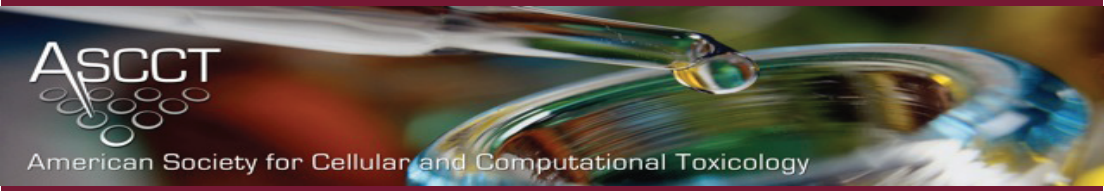


1st Annual Meeting of the American Society for Cellular & Computational Toxicology

September 21, 2012
Lister Hill Auditorium, NLM, NIH
Bethesda, MD



President's Welcome

On behalf of the entire Board of Directors of the American Society for Cellular and Computational Toxicology (ASCCT), I'd like to welcome everyone to our first annual meeting. The "first" of anything is always very exciting, and we hope that everyone here shares a feeling of pride in being present for the first of what I'm sure will be a line of annual meetings stretching far into the future.

It isn't every day that a new scientific society is formed. There have to be individuals who recognize that a need exists and – probably most important – are willing to dedicate their time and effort to literally piece the nuts and bolts of the society together. For that I'd like to thank once again Erin Hill and Kristie Sullivan, two of your officers who not only envisioned the value of an ASCCT, but have been responsible for planning the majority of the activities over the first two years.

Of course, I also need to thank each one of you for becoming an ASCCT member, and in many cases also convincing your company management to contribute to the society. There are now just over 100 individual members of the society, and we have 12 institutional sponsors. Can we double both by our second annual meeting? Our webinar program alone has to be worth many times the price of membership!

Today's program should be very exciting. First we have Dr. Mel Anderson as our plenary speaker, followed by Dr. Suzanne Fitzpatrick of the U.S. Food and Drug Administration speaking on new FDA initiatives and partnerships in computational and cellular toxicology. In addition there will be a poster session in which many of you are spotlighting your latest research efforts.

One final agenda item will be the annual election of officers and board members. Some nominations have already been received, but we will also take nominations from the floor before the election.

Once again, thank all of you for becoming ASCCT members and for joining your friends at this first annual meeting. Take the time to introduce yourself to someone new and initiate the scientific networking that is so important to each of our professional successes. The ASCCT was envisioned as a platform where scientists from the computational and cellular sides of toxicology could freely exchange ideas. Please do it!!

Your president,
Dr. Rodger Curren

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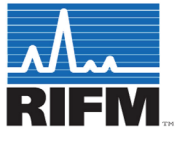

1st Annual Meeting of the American Society for Cellular and Computational Toxicology

September 21, 2012
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9:00 AM	Welcome *Rodger Curren, ASCCT President*
9:15 AM	Plenary Lecture: Computational Cellular Pathway Modeling: Combining Key In Vitro and In Silico Tools to Enhance Modern Safety Assessment *Melvin Andersen, The Hamner Institutes for Health Sciences*
10:15 AM	Overview of the FDA-DARPA-NIH Collaboration on Human/ Organ on a Chip *Suzanne Fitzpatrick, US Food and Drug Administration*
11:00 AM	Coffee Break and Poster Viewing
12:00 PM	The Role of Adverse Outcome Pathways in Streamlining Hazard and Risk Assessment *Catherine Willett, Humane Society of the United States*
12:30 PM	Applying Real Time, Label Free Impedance Technology For Human Cell Based Assays to Detect Endocrine Disruptor Chemicals *Can Jin, ACEA Biosciences Inc.*
1:00 PM	Lunch in the Cafeteria
2:00 PM	Qualification of a 96-well High Throughput In Vitro Micronucleus Assay in CHO Cells Using Flow Cytometry *Rohan Kulkarni, Bioreliance by SAFC*
2:30 PM	ASCCT Business Meeting *Outline of Activities* *Finances* *Election of Board Members* *Any Other Business*
4:30 PM	Cocktail Reception and Presentation of William and Eleanor Cave Award
5:30 PM	Close of Meeting

Presentation Abstracts

The Role of Adverse Outcome Pathways in Streamlining Hazard and Risk Assessment

C. Willett

Humane Society of the United States

The "adverse outcome pathway" (AOP) approach is based on the concept that toxicity results from the chemical exposure by molecular interaction with a biomolecule – the initiating event, followed by a description of the sequential progression of events through to the *in vivo* result, or adverse outcome. The AOP concept can be used in the short-term to enhance hazard and risk assessment at multiple levels, for example, by informing chemical category and structure activity relationships, by increasing certainty of interpretation of both existing and new information and by facilitating the development of integrated testing strategies that maximize useful information gained from minimal testing. Ultimately, AOPs can be used to identify key events for which non-animal tests can be developed, thereby facilitating mechanism-based, non-animal chemical assessment. This concept has been embraced by the Organization for Economic Coordination and Development (OECD) as well as some regulatory authorities around the world. AOP development is exemplified by 3 current examples: sensitization developed (OECD), thyroid hormone-related effects (US EPA), and pathway driven approaches to carcinogenicity assessment (International QSAR Foundation). A critical aspect of pathway development and use is the creation of a standardized and publicly available knowledge-base such as Effectopedia. Broad application of the AOP concept will facilitate the emergence of an internationally-harmonized predictive toxicological framework.

Applying Real Time, Label Free Impedance Technology For Human Cell Based Assays to Detect Endocrine Disruptor Chemicals

A. Foster, C. Jin, Y. Abassi, M. Stampfl, X. Xu, X. Wang

ACEA Biosciences Inc.

Endocrine disruptor chemicals (EDCs) are widely distributed in human daily life and environments. They can be persistent substances affecting hormone synthesis and functions with very low concentrations, at various life stages and after multiple generations. Predicting EDCs is difficult because diverse structures have shown to have EDC activities. *In vivo* testings are slow and expensive, while most *in vitro* testings are based on a single enzyme activity/DNA sequence, therefore, missed the important physiological consequences of exposure. Here we report the application of an impedance based Real Time Cell Analysis (RTCA) technology to monitor native endocrine signaling pathways, for the development of human cell based assays to identify EDCs.

Human cell lines expressing steroid hormone receptors such as estrogen receptor (ER), androgen receptor, progesterone receptor (PR) and glucocorticoid receptors (GR), are selected. Stimulation of candidate cell lines with respective endocrine agonists leads to increases in cell number or morphology which can be detected by gold microelectrodes embedded in the bottom of specialized microelectronic plates. We have identified cell lines with specific response profiles to cover cellular effects from estrogen (17β-estradiol), androgen(DHT), progesterone and glucocorticoid (dexamethasone). Further testing and assay optimization with additional agonists and antagonists, together with data analysis and extrapolation modeling development, would help to assemble a human cell panel to predict unknown EDCs.

Qualification of a 96-well High Throughput In Vitro Micronucleus Assay in CHO Cells Using Flow Cytometry

L. Stankowski[1], Jr., T. Lawlor[1], R. Kulkarni[1] and M. Aardema[1,2]

[1]BioReliance by SAFC
[2]Marilyn Aardema Consulting, LLC

To reduce animal usage and minimize cost and time, *in vitro* genotoxicity screening is conducted early in the process of developing many new products/drugs. To facilitate high throughput testing, we qualified a 96-well flow cytometric *in vitro* micronucleus assay in CHO cells using MicroFlow™ kits (Litron Laboratories) and ten reference compounds identified in OECD TG 487. All compounds were evaluated in ten independent experiments, in duplicate cultures and at ten concentrations, using a 4-hour treatment with S9 and a 24-hour treatment without S9, to assess inter- and intra-experimental variability, as well as sensitivity and specificity. In these ten trials, the frequencies of micronucleated cells in the pooled vehicle and untreated controls was 1.35 ± 0.49 and 1.66 ± 0.53, while the % hypodiploid cells were 0.30 ± 0.15 and 0.68 ± 0.29 (average ± 1 SD; n = 862 or 861; with or without S9, respectively). Using an empirical analysis of this data set, it was possible to reduce the criteria for a positive response for micronuclei and hypodiploidy to 2- and 6-fold concurrent vehicle control values, respectively, thereby increasing sensitivity without any loss of specificity. Mitomycin C and cytosine arabinoside were reproducibly positive without S9, as were benzo(a)pyrene and cyclophosphamide with S9; all four compounds produced a clastogenic signature. Colchicine and vinblastine were positive with and/or without S9, and both produced a significant increase in micronuclei and hypodiploid cells, indicative of an aneugenic mechanism of action. In contrast, di(2-ethylhexyl)phthalate, nalidixic acid, pyrene and sodium chloride were reproducibly negative in all trials and at all dose levels with and without S9. Additional analyses of these data are ongoing as ated to modification of evaluation criteria and threshold effects. This assay works well for screening a large number of compounds and is in use by BioReliance for the EPA ToxCast screening program.

Poster Abstracts

An Analysis of Skin Sensitization Data: Integrating *In Vivo, In Vitro, In Chemico*, and *In Silico* Findings

H. Sharma, K. Norman

Institute for In Vitro Sciences, Inc.

Skin sensitization is classified as an allergic reaction resulting in contact dermatitis and is a critical toxicological endpoint in the evaluation of new chemicals. The importance of accurately predicting skin sensitization potential based upon non-animal based methods, as opposed to the traditional *in vivo* methods, guinea pig maximation tests (GPMT) and murine local lymph node assays (LLNA), has never been greater - most notably following international legislation to ban animal testing of cosmetics. Although *in vivo* testing methods (particularly LLNA) are currently considered the gold standard, new *in silico*, *in chemico*, and *in vitro* tools aim to reduce/replace the use of animals in skin sensitization evaluation. In this study we performed a comparative analysis between the published *in vivo* data and the recent *in silico, in chemico*, and *in vitro* data for a data set of >50 chemicals. The latter category includes KeratinoSens, direct peptide reactivity assay (DPRA), human cell line activation test (h-CLAT), myeloid U937 skin sensitization test (MUSST), and TIMES SS and DEREK analysis. These comparisons provide an analysis of the accuracy of the individual methods and may serve as a basis for developing integrated testing strategy to assess skin sensitization potential of chemicals.

Validation of Computational Models of Skin Sensitization

R. Brown[1], T. Lee[1], K. Maxile[1,2], S. Merrill[1,3]

[1]US FDA Center for Devices and Radiological Health
[2]Mississippi Valley State University,
[3]Marquette University

Allergic contact dermatitis (ACD) has been reported in patients following the release of skin sensitizers from medical devices, including wound care dressings and orthopedic devices. Development of ACD can have serious clinical consequences for patients being treated with medical devices; as a result, it is important to identify potential skin sensitizers as part of the preclinical safety assessment of new devices. In the absence of experimentally derived data, it may be possible to predict the potential for a compound to produce skin sensitization using QSAR models. Nevertheless, before model-derived estimates of skin sensitization potential can be used for regulatory purposes, the models should be validated using data from known human skin sensitizers and compounds known to not produce skin sensitization. In this project we evaluated the predictive ability of two publically available computational models of skin sensitization, Toxtree 2.50 (Ideaconsult, Ltd.) and OECD QSAR Toolbox 2.3 using data on human skin sensitization potential in the ICCVAM report on validation of the LLNA. We then used the models to predict the skin sensitization potential of 135 compounds known to be released from device materials. The models were able to predict the skin sensitization potential of compounds in this data set with a sensitivity of 0.8 and a specificity of 0.2. The preliminary results suggest that these QSAR models show promise as a means to identify potential skin sensitizers in the absence of experimental data.

IVSA: In Vitro Sensitization Assay

M. Troese, G. DeGeorge, L. F. Pratt, and D. Cerven

MB Research Laboratories

Regulatory agencies worldwide are seeking non-animal or *in vitro* testing methods that embrace the 3Rs philosophy. Strong support now exists in the consumer products, pharmaceutical and cosmetics industries to develop and validate alternative assays for use in safety testing programs. Our group has adapted the Sens-it-iv *in vitro* sensitization NCTC IL-18 response assay of Corsini, et al. (Toxicol In Vitro 23 (5):789-796, 2009) to use in the MatTek EpiDerm™ model. This Sens-it-iv testing paradigm was from a monolayer culture system to a 3D tissue with IL-18 secretion into the media to identify dermal sensitizers. In intra-lab validation tests, IL-18 release was measured after a 24-hour topical exposure of twelve sensitizers and five irritants/non-sensitizers. Either distilled water or EtOH was used as solvent vehicles. A Stimulation Index (SI) was calculated relative to the solvent vehicle for each test material. An SI > 2.0 result was considered biologically significant and best fit the data for identification of a positive sensitizer using a 2x2 contingency table and Cooper statistics. The overall Accuracy of the assay was 94%, with no false positives. The Sensitivity, Specificity, Positive and Negative Predictivity were 100%, 83%, 92% and 100%, respectively. IVSA produced a dose-response with dinitrochlorobenzene and 4-nitrobutylbromide. In addition, IVSA correctly identified two photosensitizers (+UVA): chlorpromazine and olaquindox. Thus, IVSA is a useful and highly predictive tool for identifying dermal sensitizers without the use of animals.

Development of the Replacement Ocular Battery – Tiered Testing Strategy of Alternative Toxicology Tests to Replace the Need for Rabbit Eye Tests

D. Cerven, M. Piehl, G. DeGeorge

MB Research

Using a series of non-animal assays in a tiered approach, the Replacement Ocular Battery (ROBatt) accurately predicts categories of acute ocular irritation corresponding to OECD, EPA and GHS guidelines.

At present, no single alternative assay has been accepted by regulatory agencies to completely replace the use of live animals. The BCOP (Bovine Cornea Opacity/ Permeability) test has been accepted by OECD as a screen for severe and corrosive materials. EpiOcular™ and other ocular tissue models are in various stages of review or acceptance. The Cytosensor Microphysiometer has been accepted for sub-severe testing but only aqueous-based materials. The ROBatt approach uses a series of up to three non-animal assays to categorize aqueous and non-aqueous materials.

An FDA-NIH Grant has been awarded to develop the ROBatt decision tree criteria. Initially screening will use the Chorioallantoic Membrane Vascular Assay (CAMVA) to discriminate slight or non-irritants from moderate to severe irritants. Slight or non-irritating materials will be categorized using the Porcine Cornea Confocal Assay (PorFocal). 3D human reconstructed tissue models and/or the Bovine Cornea Opacity/Permeability test (BCOP) will be used for discriminating between moderate and severe to corrosive materials. Lastly, the Porcine Cornea Opacity Reversibility Assay (PorCORA) will be used to categorize severe irritants and corrosives.

Fifty validation chemicals from the ECETOC database of ocular irritation will be initially tested. Having performed over 6,700 CAMVA, 5,700 BCOPs, 3,000 MatTek EpiOcular™ / SkinEthic HCE™, and nearly 100 PorCORA assays, the researchers are confident of the ability of ROBatt to properly categorize any material to international standards.

Development of the Replacement Ocular Battery: Tier 1 – Chorioallantoic Membrane Vascular Assay

D. Cerven, D. Hall, G. DeGeorge

MB Research

The initial tier of the Replacement Ocular Battery (ROBatt) – a tiered testing strategy for regulatory classification of ocular irritation without the use of live animals – the Chorioallantoic Membrane Vascular Assay (CAMVA) was used to screen ocular irritation potential of 52 chemicals of known severity (non-irritant to corrosive). Changes to the vasculature of the Chorioallantoic Membrane (CAM) were evaluated and a reference value (RC_{50}) computed for each test chemical. Individual animal data from the ECETOC database were available for 37 of the 52 chemicals. Fifteen chemicals were selected after consultation with representatives of the EPA and FDA. Basic EPA classification information was available for these materials; individual animal data were not.

Based on the CAMVA screen results, 30 chemicals with irritating potential will be further evaluated in the Bovine Cornea Opacity and Permeability Assay (BCOP). These chemicals are expected to be in the moderate to corrosive range of ocular irritancy. Twenty-two chemicals with slight to non-irritating potential will be evaluated in the Porcine Confocal Assay (PorFocal). These chemicals are expected to be in the non-irritating to slightly irritating range.

Comparison of CAMVA screen results with ECETOC and FIFRA data indicated evidence of over-prediction (5 of 30) for moderate - corrosive chemicals and under-prediction (3 of 22) for slight - nonirritating chemicals. Since the ROBatt is a multi-tiered test system, all test chemicals will be evaluated in at least two models before being classified into an appropriate regulatory corrosive category.

ROBatt is a two-year research grant funded by the NIH and FDA.

Development of the Replacement Ocular Battery (ROBatt) Tier 2 – BCOP

D. Cerven, D. Wolfinger, G. DeGeorge

MB Research

The Replacement Ocular Battery (ROBatt) is being developed as a Tiered Testing Strategy to replace Draize *in vivo* ocular irritation testing. It comprises (1) Chorioallantoic Membrane Vascular Assay (CAMVA), mimicking the vascular reaction of the conjunctiva; (2) Bovine Cornea Opacity/Permeability Test (BCOP), to assess mild to moderate corneal damage; (3) Porcine Corneal Opacity/Reversibility Assay (PorCORA), to discriminate severe irritants from ocular corrosives; and (4) PorFocal Assay, to separate non-irritants/slight irritants.

Initially, CAMVA segregated 52 chemicals with known levels of ocular irritation into two groups. The 30 most irritating were tested by BCOP, which measures reduction in corneal light transmission following insult. Fluorescein permeability detects corneal changes that may not be associated with opacity. Corneal damage was calculated as In Vitro Score (IVS) = Opacity Score + (15 x Permeability Score). A BCOP IVS threshold of 12.0 was chosen to assign 17 chemicals as severe/corrosive to be tested in PorCORA, and 13 chemicals as moderate irritant category (HMIS 2, GHS 2A and EPA III). ROBatt categorizations were compared with those obtained from the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) database and information provided by the NIH and FDA. Of the 13 materials in the moderate category, 5 classified Category III (USEPA FIFRA guidelines), 5 classified Category IV, and 3 were Category II. Results indicate a need to establish an IVS range below which the test material would be evaluated by PorCORA to establish Category IV classification.

ROBatt is a 2-year research grant funded by the NIH and FDA.

Bridging the Gap Between Validation and Implementation: Replacing Animal Use in Vaccine Batch Potency Testing

S. Dozier[1], J. Brown[1], A. Currie[2]

[1]People for the Ethical Treatment of Animals Regulatory Testing Division
[2]PETA Foundation UK

As technologically advanced high-throughput techniques are developed that replace, reduce or refine animal use, harmonization of validated protocols between international regulatory authorities is necessary to foster wide-reaching implementation. As regulatory acceptance does not guarantee that an approved non-animal method will be adopted by manufacturers, interfacing with industry to disseminate information regarding exemptions from *in vivo* regulatory standards is necessary to encourage the use of validated non-animal methods. Company and government policies must be in place to take advantage of new scientific advances that reduce the use of animals for vaccine testing, and continued efforts are necessary to ensure that available exemption processes allowing for the use of replacements for animal-based tests are not hindered. By engaging with regulators and manufacturers, PETA helps promote 3Rs approaches to vaccine batch potency testing. This promotion process is customized to the needs of each project and aims to confirm the acceptability of data from novel methods by regulatory authorities, to distribute information on available and accepted non-animal approaches, to involve the press in publicizing accepted non-animal techniques, and to confirm manufacturer implementation of these methods. This poster examines detailed case studies of PETA's approach to fostering regulatory and industrial integration of *in vitro* erysipelas and leptospirosis vaccine batch potency methods, as well as TABST waivers, while also providing examples of additional cases in which the organization was able to facilitate the implementation and distribution of protocols that allow for reduced animal use across multiple regulatory agencies and within the biologicals industry.

Animal Protection Perspectives on the Proposed Animal Testing Policy Revision to the Federal Hazardous Substances Act Enforced by the Consumer Product Safety Commission

A. Birdie[1], S. Leary[2], V. Katrinak[3], K. Willett[4], J. Sandler[5], K. Sullivan[1]

[1]Physicians Committee for Responsible Medicine
[2]Alternatives Research & Development Foundation
[3]American Anti-Vivisection Society, [4]Humane Society of the United States
[5]People for the Ethical Treatment of Animals

The Consumer Product Safety Commission (CPSC) has proposed two rules. The first will codify its statement on animal testing and the other will amend and clarify CPSC definitions of toxicity. CPSC policy is designed to give manufacturers subject to the Federal Hazardous Substances Act advice on how to obtain data to appropriately label products as toxic, flammable, corrosive, or irritating. CPSC has proposed these changes "in order to make the ICCVAM recommendations and Commission's animal testing policy more accessible and transparent." CPSC does not require animal testing, and the proposed language reinforces this by stating a preference for methods that "use none or fewer animals while maintaining scientific integrity." Each section of the policy includes a reference to "valid" and/or accepted methods listed on the CPSC website. By not listing methods in the policy and referring to an outside list, CPSC would allow for continual update of accessible methods. Although the revised definitions of toxicity provide more clarity than data from nonanimal methods, the CPSC regulations maintain an emphasis on animal- data-derived definitions of toxicity that do not fully capture current, or leave room for future, scientific advances. A more appropriate approach would be to uncouple definitions of toxic effects from specific animal test results. The CPSC-proposed amendments to this animal testing policy and definitions of toxicity improve CPSC policy with respect to reducing the reliance on animal testing; however, we offer specific suggestions that, if implemented, will make acceptable nonanimal methods easier to identify and use.

Elements of A "Fast Track" Approach to Validation of Non-Animal Methods

E. Janus

Steptoe & Johnson LLP

As recently stated in the June 2012 EPA Endocrine Disruptor Screening Program (EDSP) *Comprehensive Management Plan*, "it is likely a new validation framework would need to be developed to rapidly evaluate the new high-throughput methods and computer models" that are necessary to actualize the new toxicity testing paradigm laid out in the now-classic 2007 National Research Council report. In addition to the needs of the EDSP, there may be specialized testing needs for emerging technologies (i.e. nanoscale materials) and reviews of existing chemicals (i.e. Australia's IMAP effort, EPA's IRIS program). This presentation will describe elements of a successful "fast track" framework to approve new non-animal methods for use in regulatory programs. This new framework would still need to meet the mandate of FFDCA for use of "validated test systems" yet would need to do so in a much more focused way that leverages prior approvals and reviews by other validation groups. In addition, the framework would need to be quite flexible to meet the needs of regulatory programs that need to incorporate the best available and most modern scientific discoveries. Lastly, greater international coordination of non-animal testing needs should drive the prioritization of the development and validation of new non-animal testing methods.

Development of Computational Models and Workflows for the Prediction of Human Repeated Dose Toxicity for Cosmetics

C. Yang[1], M.T.D. Cronin[2], A.-N. Richarz[2], S Enoch[2], A.P. Worth[3] J.F. Rathman[1], E. Fioravanzo[4], D. Neagu[5], J.-M. Zaldívar-Comenges[3], M. Berthold[6]

[1]Altamira LLC, [2]School of Pharmacy and Chemistry, [3]European Commission, Joint Research Centre, [4]Soluzioni Informatiche srl, [5]School of Computing, Informatics and Media, University of Bradford, [6]KNIME.com AG,

The COSMOS (Integrated *In Silico* Models for the Prediction of Human Repeated Dose Toxicity of COSMetics to Optimise Safety, www.cosmostox.eu) Project addresses the safety assessment needs of the cosmetics industry, without the use of animals. The main aim is to develop freely available tools and workflows to predict safety to humans following the use of cosmetic ingredients and related chemicals. COSMOS is part of a cluster of seven projects (the SEURAT-1 Cluster, www.seurat-1.eu) funded by the European Commission and the European Cosmetics Association (Cosmetics Europe).

The integrated suite of computational workflows being developed within COSMOS includes models based on the threshold of toxicological concern (TTC) approach, innovative chemistry such as quantitative structure-activity relationship (QSAR) models and multi-scale modelling based on physiologically-based pharmacokinetics (PBPK). In particular COSMOS aims to compile new sources of toxicological data and create an inventory of known cosmetic ingredients and their associated chemical structures; assess the extension of the current TTC approach to cosmetics ingredients; develop innovative toxicity prediction strategies based on chemical categories, read-across and QSAR related to key events in adverse outcome pathways (AOP) or mode-of-action (MoA) pathways; develop a multi-scale modelling approach to predict target organ concentrations and extrapolate from *in vitro* to *in vivo* exposure scenarios. The databases and modelling approaches are being integrated into flexible computational KNIME workflows forming a set of building blocks that allows users to incorporate their own data and search existing data compilations. These workflows will be made freely available to assist in the prediction of human repeated dose toxicity and the safety assessment of cosmetic ingredients and related chemicals.

First results achieved include establishing the COSMOS database for repeated dose toxicity data with a customised data entry tool, developing the COSMOS chemical inventory and datasets for the TTC analysis of cosmetics, as well as the KNIME platform including workflows to identify MoA chemotypes and properties associated with particular mechanisms of toxicity. Application of MoA approaches to QSAR and compound categorization is also being pursued by a cross-cutting project across the consortium.

The authors are grateful to the US Food and Drug Administration (FDA) and US Environmental Protection Agency (EPA) for the oral repeated dose toxicity data provided for the cosmetic ingredients. The research leading to these results has received funding from the European Community's Seventh Framework Program (FP7/2007-2013) COSMOS Project under grant agreement n° 266835 and from Cosmetics Europe.

Comparison of Different Skin Models to the 3T3 NRU PT for Chemically Induced Phototoxicity

L. Pratt, G. DeGeorge, D. Cerven

MB Research

The 3T3 neutral red uptake assay for phototoxicity (3T3 NRU PT) is currently the only *in vitro* assay approved for the screening of potentially phototoxic compounds. The model uses a mouse fibroblast monolayer instead of human-derived cells/cell lines, which raises questions as to its predictivity for human exposure to phototoxins. While OECD guideline 432 is based on 3T3 cell results, it states that other cells can be used, if equivalency is demonstrated. These studies report a side-by-side comparison of 3T3 results to the HaCaT keratinocyte cell line, primary adult human epidermal keratinocytes (HEKa) and a 3D human skin model using the same panel of chemicals to determine if a human-based system is as predictive as the 3T3 test model. The chemicals tested are a subset of those used to validate the 3T3 system. The 3T3 negative chemicals (Hexachlorophene, SDS and L-histidine) show similar negativity in the three human-derived skin systems. Of the 3T3 positives, Chlorpromazine and Norfloxacin were judged equal in responsiveness in the monolayer human cell lines to the 3T3 system, while the 3D human skin model was less sensitive by 30-100 fold in effective chemical concentration. All EC_{50} concentration values for the 3D skin model are much higher than the monolayer cell systems. Amiodarone, a weak positive in the 3T3 system, is also very weakly positive for the HaCaT cells and 3D skin model, but fails to be positive in HEKa. Necessary modifications to the 3T3 protocol for the specific culture conditions of each alternative model are described.

Use of Confocal Microscopy to Examine Ultra-Mild Ocular Irritation in Cultured Porcine Corneas

M. Piehl, M. Carathers, G. DeGeorge, D. Cerven

MB Research

Confocal microscopy allows “optical histological” sectioning of living tissue (e.g. cornea) to determine viability within corneal epithelium without traditional histology. We have developed a novel assay, PorFocal, which uses cultured waste porcine corneas to assay individual corneal cell death with high sensitivity due to a confocal microscopy endpoint. In PorFocal, test substances are placed directly onto living corneal tissue in culture; therefore, solubility of the test substance is irrelevant. PorFocal cultured corneas are maintained in a living state for 7 days with daily application of the test substance. This exposure schedule allows for detection of extremely mild ocular toxicity of additive effects over time, measured by quantification of individual stained dead cells within the corneal tissue by confocal microscopy. Corneal tissue is imaged in an “optical histological” manner where a series of image “slices” are acquired at increasing depths into the corneal tissue. The images can then be digitally reconstructed to display the entire corneal tissue volume imaged. Replicate corneas were treated with Phosphate Buffered Saline (PBS, negative control), or 10-fold dilutions of Benzalkonium chloride (BAC; 0.1%, 0.01%, and 0.001%) or Sodium Dodecyl Sulfate (SDS; 0.5%, 0.05%, and 0.005%). Corneas were dosed topically with 50-µL of test substance daily for 7 days. On day 8, corneas were stained with dead cell stain and imaged. Dead cells per volume and statistical analysis was performed using ANOVA. Test substances dosed at 10-fold dilutions were statistically significant ($p<0.001$) in a dose dependant manner. These data demonstrate the potential sensitivity of the PorFocal assay.

Using TRPV1 Channel Activity to Predict the Ocular Stinging Potential of Baby Shampoos

A. Forsby[1], K. Norman[2], J. Andaloussi-Lilja[1], J. Lundqvist[1], V. Walczak[3], R. Curren[2], K. Martin[3], N. Tierney[3]

[1]Department of Neurochemistry, The Arrhenius Laboratories for Natural Science, Stockholm University
[2]Institute for In Vitro Sciences
[3]Johnson & Johnson Consumer and Personal Products Worldwide

The Transient Receptor Potential Vanilloid type 1 (TRPV1) channel is one of the most well characterized pain-inducing receptors. The purpose of this study was to predict human eye stinging of 19 baby bath and shampoo formulations by studying TRPV1 activity, as measured by increase in intracellular free Ca2+. The NociOcular test, a novel recombinant neuronal *in vitro* model with high expression of functional TRPV1 channels was used to test formulations containing a variety of surfactants, preservatives, and fragrances. TRPV1-specific Ca2+ influx was abolished when the TRPV1 channel antagonist capsazepine was applied to the cells prior to shampoo samples. The positive control, an adult shampoo that contains coco mono ethanolamide, a known stinging ingredient, was the most active sample tested in the NociOcular test. The negative control, a marketed baby shampoo, was negative in the NociOcular and human tests. Seven of the formulations induced stinging in the human test, and of those six were positive in the NociOcular test. Twelve formulations were classified as non-stinging in the human test, and of those ten were negative in the NociOcular test. There was no correlation between the clinical stinging results for the baby formulations and the data generated from other *in vitro* eye irritation assays (cytosensor microphysiometer, neutral red uptake, Epiocular, trans-epithelial permeability). Our data support that the TRPV1 channel is a principle mediator of eye stinging sensation induced by baby bath and shampoo formulations and that the NociOcular test may be a valuable *in vitro* tool to predict human eye stinging sensation.

An NGO's Role in Nanomaterials Regulation: A Focus on In Vitro, Ex Vivo, and In Silico Methods

S. Dozier

People for the Ethical Treatment of Animals

PETA became involved in aiding in the development of nanomaterial toxicity testing guidance in 2005 when most regulatory agencies were in the early stages of defining nanomaterials. A PETA specialist in nanomaterials participated in these initial meetings both in the United States and Europe, as regulators around the world were grappling with many of the same issues relating to regulating this novel chemical type.

The global nanotoxicology community struggled to determine the most effective assessments of nanomaterial toxicity. A top priority was to address concerns relating to the many scientific problems associated with animal-based toxicology in general and pulmonary toxicity testing specifically. We worked closely with regulators and top toxicologists in the field to steer regulators and industry away from instillation testing and identified the most promising suite of *in vitro* pulmonary toxicity test methods to replace reliance on rat inhalation tests[1].

We have presented *in vitro* and *ex vivo* barrier testing methods and contributed to international standards that cite scientifically valid, *in vitro* methods. These endpoints include: developmental and embryotoxicity[2], the blood-brain-barrier[3], as well as nano-specific concerns (e.g., inflammation and oxidative stress, among others).

PETA's seat on national and international standards-making bodies will further contribute to overcoming regulatory hurdles that prevent the use of sophisticated non-animal methods in the emergent field of nanotechnology.

[1]Rothen-Rutishauser B, et al. A newly developed in vitro model of the human epithelial airway barrier to study the toxic potential of nanoparticles. ALTEX. 2008;25(3):191-6. And, MatTek's EpiAirway model: http://www.mattek.com/pages/products/epiairway, among others.

[2]Myllynena, P, et al. Kinetics of gold nanoparticles in the human placenta. Reproductive ToxicologyVolume 26, 2008, p130-137

[3]Vandenhaute, E., et al., Case study: adapting in vitro blood–brain barrier models for use in early-stage drug discovery. Drug Discovery Today. Volume 17, 2012, p285–290

Metabolomics and Transcriptomics for the Identification of Pathways of Toxicity: Study of Developmental Neurotoxicity in Rat Primary Aggregating Brain Cell Cultures

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Tox-21c proposed a paradigm shift in the field of toxicology. The report proposes the application of the latest advances in science and technology to develop more relevant test strategies. The concept is that pathways of toxicity (PoT) can be identified using *in vitro* cell systems, high throughput testing, 'omics' approaches, systems biology and computational modeling. "Pathways of toxicity" are defined as changes in normal biological processes, e.g. cell function, communication and adaptation to environmental changes, which are expected to result in adverse health effects. An area of toxicology where Tox-21c could have a significant impact is developmental neurotoxicity (DNT). There is concern that exposures to environmental chemicals contribute to the increasing incidence of neurodevelopmental disorders in children. However, very few substances have been identified as developmental neurotoxicants.

The Center for Alternatives to Animal Testing (CAAT) aims to develop an *in vitro* approach using metabolomics and transcriptomics for DNT assessment. A 3D rat primary neuronal organotypic model is exposed to pesticides, drugs and metals that are well known or suspected (developmental) neurotoxicants. Our goal is to identify critical pathways based on a systems toxicology approach relying on transcriptomics and metabolomics outcomes. Besides quantitative measurement of genes expressed in different cell types, mass spectrometry based metabolomics measurements are performed. The mass spectrometry analysis showed differences in metabolite levels between control and treated cells. Further analysis of the altered metabolites should give mechanistic insight into the DNT of these compounds. This study demonstrates that gene expression and metabolomic analysis can be sensitive endpoints for DNT assessment.

Japanese New Project "ARCH-Tox" for the Future Chemicals Management Policy: Research and Development of In Vitro and In Vivo Assays for Internationally Leading Hazard Assessment and Test Methods

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[2]Hatano Research Institute (HRI), Food and Drug Safety Center (FDSC)
[3]Chromosome Engineering Research Center, Tottri Univ.
[4]Sumitomo Chemical, Co., Ltd., Osaka, Japan, 5Chemical Evaluation and Research Institute (CERI)

In 2011, Japan' Ministry of Economy, Trade and Industry (METI) launched a new 5 years research project, entitled as "ARCH-Tox", with the goal of promoting the 3Rs in 28-day repeated dose oral toxicity studies, which are used to screen for compliance with Japan's Chemical Substances Control Law. This project includes the following two sub-projects.

1. Tox-Omics Project: Development of methods to detect the possibility of multiple-toxic effects using gene expression analysis.
Tox-Omics project will attempt to analyze changes in gene expression in animals tested in 28-day repeated dose studies. This result contributes to establish methods for prediction or detection of carcinogenicity, immunotoxicity, or other effects of chemical substances in major organs.

2. In Vitro Project: Development of *in vitro* assays to detect toxicities, including target organ toxicity and metabolic function.
This sub-project will attempt to establish *in vitro* test methods simulated *in vivo* toxic effects for the speedy and efficient assessment of hepatotoxicity, nephrotoxicity, and other endpoints in repeated dose studies.

We believe that the successful completion of these projects will help further worldwide application of the 3Rs to safety evaluation of chemicals in systemic toxicity testing.

The 3D Human Skin Micronucleus Assay: A Novel In Vitro Approach for Genotoxicity Testing of Dermal Exposures

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[2]Marilyn Aardema Consulting, LLC Fairfield OH USA

Skin is the site of the highest exposure to many chemicals, drugs and other products, and an assay for the assessment of genotoxicity in skin is a valuable addition to a testing approach. This is especially applicable to cosmetic ingredients that can no longer be testing in *in vivo* genotoxicity assays due to the 2009 EU 7th Amendment ban. To meet these needs, the 3D reconstructed human skin micronucleus assay (RSMN) in EpiDerm™ was developed (Curren et al Mut Res 607 192-204, 2006). This assay offers a more biologically relevant *in vitro* approach to assess genotoxicity compared to standard 2D in vitro genotoxicity assays since it provides a functional stratum corneum that takes into account permeability, appears to have normal dermal metabolic capability and is expected to have more normal DNA repair and cell cycle control unlike p53 deficient cell lines used in most standard genotoxicity assays. To meet the increasing interest in this assay, we have developed a GLP RSMN assay that also incorporates CREST labeling of micronuclei for identification of aneugenic versus clastogenic mechanisms. Qualification studies with chemicals of various modes of action were conducted including mitomycin C (cross-linker), vinblastine sulphate (aneugen), methylmethane sulfonate (clastogen). Results show dose-dependent increases of MN and demonstrated good reproducibility and comparability to previously published results. Non-carcinogens such as 4-nitrophenol are negative in the assay. Chemicals like vinblastine sulphate induce a large increase in micronuclei with positive CREST labeling indicative of aneugenicity. The GLP RSMN assay is a promising new *in vitro* assay for genotoxicity testing.

The Syrian Hamster Embryo (SHE) Cell Transformation Assay: Results with Three Dietary Ingredients – Menadione, Curcumin and Quercetin Hydrate

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[3]Marilyn Aardema Consulting, LLC

The Syrian hamster embryo (SHE) cell transformation assay (CTA) is an *in vitro* test for potential carcinogenicity that has been approved for development into an OECD guideline (EURL-ECVAM recommendation, In-vitro Carcinogenicity Testing 2012). In the present study, three chemicals were tested in the SHE CTA, menadione (Vitamin K3, CAS number: 58-27-5), curcumin (CAS number: 458-37-7) and quercetin hydrate (CAS number: 849061-97-8). Menadione is a synthetic chemical compound sometimes used as a nutritional supplement because of its vitamin K activity. Curcumin and quercetin hydrate are dietary flavonoids; quercetin is found in citrus fruit, buckwheat and onions and curcumin is part of the Indian spice turmeric. Both exhibit antioxidant effects at low dose and pro-oxidant effects at higher concentrations. Accordingly, these materials show adverse effects in some *in vitro* genotoxicity tests that do not manifest *in vivo*. The purpose of this study was to investigate the predictivity of the SHE CTA as a means to inform consumer safety cancer risk assessment. The SHE CTA was performed using a seven-day exposure regimen following the ECVAM (European Center for the Validation of Alternative methods) recommended protocol. Concentrations for the SHE CTA were selected based on initial dose range finding data. The relative plating efficiency for menadione (2.5 µg/ml), curcumin (2.5 µg/ml) and quercetin (5.0 µg/ml) at the highest concentration scored was 41.3%, 49.3% and 43.6%, respectively. None of these test article concentrations induced a statistically significant ($p < 0.05$) increase in the number of morphologically transformed colonies in comparison to the vehicle control. Thus, these three dietary ingredients do not cause cell transformation in the SHE CTA under the conditions described.

The In Vitro Comet Assay Using Human TK6 Cells: Qualification of a High Throughput 96-Well Screening Format

K. Pant[1], S. Bruce[1], D. Albert[1], S. Springer[1], Y. Xu[1], T. Lawlor[1], M. Aardema[2]

[1]BioReliance by SAFC
[2]Marilyn Aardema Consulting, LLC

There is increased interest in the field of genetic toxicology to use p53 competent human cells in *in vitro* assays instead of transformed rodent cell lines that are associated with misleading positive results. To this end, we have qualified a 96 well screening Comet assay in human p53 proficient TK6 cells. Following the protocol used in the JaCVAM (Japanese Center for Validation of Alternative Methods) Comet validation studies (version 6.2), six reference compounds were tested: ethyl methanesulfonate, cycloheximide, triton-X, 9-aminoacridine, 2-amino anthracene and methyl methanesulfonate. Four independent experiments were conducted using duplicate cultures, and seven to ten test article concentrations, using a 4-hour treatment with and without exogenous metabolic activation to assess inter- and intra-experimental variability, as well as sensitivity and specificity. The number of clouds (hedgehogs) on the slides was used as the toxicity measure. The median % tail DNA was used as the parameter to evaluate the DNA damage Reproducible positive results were obtained with ethyl methanesulfonate, 9-aminoacridine, 2-amino anthracene and methyl methanesulfonate as expected and reproducible negative results were obtained with cycloheximide and triton-X. This assay works well for screening a large number of compounds and is in use by BioReliance for the EPA ToxCast screening program.

Supplemental Information

The William and Eleanor Cave Award

The William and Eleanor Cave Award is presented to recognize achievements in alternatives to the traditional use of animals in testing, research or education.

It is presented biannually by the Alternatives Research & Development Foundation and carries a $5,000 prize.

Past recipients of the award have included:

- Ruy Tchao, University of the Sciences, Philadelphia
- George Russell, Professor Emeritus of Biology at Adelphi University
- John Sheasgreen, President of MatTek Corporation
- Leon Bruner, The Procter & Gamble Company
- Daniel Smeak, Colorado State University
- Rodger Curren, President of Institute for In Vitro Sciences
- ATLA, Accepted by Michael Balls

William and Eleanor Cave were devoted officers of The American Anti-Vivisection Society for decades. They recognized the opportunities in developing new technologies and alternative methods to address the problems of animal experimentation. They dedicated resources to fund research, eventually resulting in the establishment of the Alternatives Research & Development Foundation.

ARDF's mission is to fund and promote the development, validation and adoption of non-animal methods in biomedical research, product testing and education. ARDF has awarded over two million dollars in grants to investigators developing alternative test methods at major universities across the U.S. and sponsors scientific meetings such as the World Congresses on Alternatives and Animal Use in the Life Sciences. Information is available at www.ardf-online.org.

Alternatives Research & Development
FOUNDATION

The American Society for Cellular and Computational Toxicology (ASCCT)

Mission:

The ASCCT is a new scientific society which will provide an organized forum for discussion of cellular and computational toxicology approaches, especially as replacements for animal-based toxicology methods. Through its meetings and activities, the Society will facilitate the development, acceptance, and routine use of cellular and computational methods through open dialog between industry, academic, advocacy, and regulatory scientists. The Society strives to include the participation of young scientists to promote their contributions to the field.

Goals:

- Facilitate the development, acceptance, and routine use of cellular and computational methods
- Increase the routine application and use of computational and *in vitro* methods for prioritization, classification, and risk assessment purposes
- Foster open dialog between industry, academic, advocacy, and regulatory scientists throughout North America
- Include the participation of young scientists to promote their contributions to the field
- Strengthen cooperation between stakeholders

All Members will receive:

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