1: Pre-validation of the Reconstructed 3D Human Skin Micronucleus and Comet Assay

Authors
Fautz R.1, Curren R2, Krul C.3, Reisinger K.4, Ouedraogo G.5, Corvi R.6, Aardema M.7, Reus A.3, Barnett B.8, Downs T.8, Faquet B.5, Hoffmann S.9, Hewitt N.10, Barroso J.11, Pfuhler S.8

Name of presenter:
Dr Rolf Fautz, Kao Germany GmbH, Darmstadt, Germany
e-mail address for further correspondence:
Rolf.Fautz@kao.com

Institutions/Companies
1 Kao Germany GmbH, Pfungstädter Straße 92-100, 64297 Darmstadt, Germany. Telephone: +49 6151 39 600, Fax: +49 6151 502485
2 Institute for In Vitro Sciences, Inc., Gaithersburg, MD, USA
3 TNO, Zeist, The Netherlands
4 Henkel AG & Co KgaA, Düsseldorf, Germany
5 L’Oreal Life Sciences Research, Aulnay sous Bois, France
6 Institute for Health and Consumer Protection, European Commission Joint Research Centre, Ispra, Italy;
7 Marilyn Aardema Consulting, LLC, Fairfield, OH, USA;
8 Procter & Gamble Co, Cincinnati, OH, USA
9 seh consulting+services, Koln, Germany
10 Erzhausen, Germany
11 Cosmetics Europe, Brussels, Belgium

Abstract
In vitro clastogenicity assays have a high rate of positive results when compared with rodent carcinogenicity data. To address this, Cosmetics Europe (formerly COLIPA) initiated a multi-laboratory project to establish in vitro genotoxicity assays combining reconstructed human (RS) skin tissues with the micronucleus (MN) and Comet assays. These models reflect the relevant route of exposure to many products, skin, and may be used to follow-up on positive results from the current in vitro genotoxicity battery.

Pre-validation studies on the RSMN assay using EpiDerm™ skin models showed strong intra- and inter-laboratory reproducibility with 8 coded chemicals tested in at least 2 laboratories. The number of coded chemicals was extended to 28 and these results demonstrate excellent specificity: >80% of the in vivo non-genotoxic non-carcinogens were predicted correctly. Five of the 7 genotoxic chemicals were correctly predicted. More coded compounds will be tested with a focus on genotoxic carcinogens to draw a final conclusion on the sensitivity of the RSMN assay.

Phase 2 results for the Comet assay, using EpiDerm™ models, showed good reproducibility. All 3 genotoxic carcinogens were correctly predicted in all laboratories and both non-carcinogens were negative, except for one false positive in one laboratory. Phase 3 testing has merged with a German project with the aim to test 30 coded chemicals among 5 laboratories in 2 reconstructed human skin models, including full-thickness tissues.

Our data support the use of 3D skin models in combination with the MN and Comet assays as relevant tools for genotoxicity testing of dermally-applied chemicals.

ACKNOWLEDGEMENTS: Work by TNO was co-funded by ECVAM, UK NC3Rs and by Cosmetics Europe
# 2: Safer products and less animal distress at a lower cost

## Authors

Oberg Mattias¹, Ringblom Joakim¹, Johanson Gunnar¹.  
E-mail: mattias.oberg@ki.se, Phone: +46 8 524 875 17

## Institutions/Companies

¹ Institute of Environmental Medicine, Karolinska Institutet, PO Box 210, SE-171 77 Stockholm, Sweden,

## Abstract

For the first time, unequally sized dose groups are combined with modern dose-response modeling and evaluated in relation to experimental animals. In regulatory testing, toxicity in animals is often required to define the threshold of toxicity, e.g. NOAEL or Benchmark dose. Such studies are typically carried out following OECD guidelines with four dose groups (control, low, medium, high) with an equal number of animals per group. In this project Monte Carlo simulations were used to investigate if the aggregated animal distress and the total number of animals can be decreased, while maintaining or increasing the informative value of the study. Toxicity studies were simulated using dose-effect curves and variability usually seen in experimental studies. Different study designs with unequally sized dose groups were compared with the standard design to evaluate the efficacy.

Our simulations suggest that it is advantageous to have fewer animals in the high dose group and more animals in the low dose group. Such designs also seem to reduce animal distress by about 50%, still getting better data than with the standard design. The total number of animals as well as the cost of the test compound may thus be significantly reduced by this testing strategy. International collaboration in this field, linking theoretical research with ongoing regulatory testing and guideline development, might result in development of new operating procedures, improved risk assessments and animal welfare.

**ACKNOWLEDGEMENTS:** Financial support from the Swedish Research Council, the Research without Animals Foundation, and the Junior Faculty Programme at the Institute of Environmental Medicine.

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### 3: EPAA Science Award 2010:
Observing reversibility of ocular damage within the Ex Vivo Eye Irritation Test

**Authors**

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<tr>
<td>Felix Spöler¹, Stefan Kray¹, Oya Kray¹, Susanne N. Kolle², Tzutzuy Ramirez², Claudia Panfil³, Norbert F. Schrage³</td>
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Email: spoeler@iht.rwth-aachen.de

**Institutions/Companies**

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<tr>
<td>¹Institute of Semiconductor Electronics, RWTH Aachen University, Aachen, Germany</td>
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<tr>
<td>²BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany</td>
</tr>
<tr>
<td>³Aachen Centre of Technology Transfer in Ophthalmology (ACTO), Aachen, Germany</td>
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**Abstract**

Classification of severe eye irritation/corrosion within the globally harmonized system (GHS) includes persistence of ocular damage observed within animal experiments. Typically *in vitro* assays do not allow about the assessment of the reversibility of damage. Strategic combinations of several alternative test methods within a tiered testing strategy are currently proposed to address the full range of ocular irritation by separately testing severity/corrosion and non-irritancy.

The Ex Vivo Eye Irritation Test (EVEIT) strives for direct observation of reversibility after ocular damage by refining common organotypic assays in two different aspects. First, corneal cultures are used and second, test substance related effects monitoring is enhanced from a single time point analysis to in time dynamic observation using optical coherence tomography and macroscopy. The EVEIT is based on rabbit corneas from animals slaughtered for food production which are cultured using specifically adapted organ culture methods. This allows for an *in vitro* observation of the metabolic stability of the living tissue over 5 days including direct evaluation of recovery after chemical trauma.

The EVEIT was awarded the EPAA Science Award 2010 to demonstrate the applicability of the method in a routine testing laboratory. During the EPAA Science Award 2012 project, a preliminary prediction model was refined as an extension of the available data basis. Further corneal culture preparation was improved but the transferability of the procedures could not be further evaluated during the project. During the project, transportability of the equipment and corneal cultures has been demonstrated within the project. Further within in a one week training period it was possible to set up the equipment and train an experienced technician on the procedural aspects. Hence, full transferability of the method seems to be achievable, while the generated dataset is still too small to predicate inter-laboratory reproducibility.

In summary the results suggest that the EVEIT remains promising tool to improve eye irritation evaluation in testing strategies in particular in the light of the assessment of reversibility of effects.
4: Bridging the gap between validation and implementation of non-animal potency testing methods

Authors

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<th>Authors</th>
<th>Dozier S.¹, Brown J.¹ and Stoddart G.²</th>
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<td>Name of presenter</td>
<td>Gilly Stoddart</td>
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<td>e-mail address for further correspondence</td>
<td><a href="mailto:GillyS@peta.org.uk">GillyS@peta.org.uk</a></td>
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<tr>
<th>Institutions/Companies</th>
<th>¹People for the Ethical Treatment of Animals, 501 Front St., Norfolk, VA 23510, USA. <a href="mailto:JeffreyB@peta.org">JeffreyB@peta.org</a>. Telephone: (757) 793-8941</th>
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<tr>
<td>²People for the Ethical Treatment of Animals Foundation, Society Building, 8 All Saints Street, London N1 9RL UK</td>
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Abstract

In recent years, technologically advanced high-throughput techniques have been developed that replace, reduce or refine animal use in vaccine quality control tests. Following validation, these tests are slowly being accepted for use by international regulatory authorities. Because regulatory acceptance itself has not guaranteed that approved humane methods are adopted by manufacturers, various organisations have sought to foster the preferential use of validated non-animal methods by interfacing with industry and regulatory authorities.

We present a paradigmatic approach that seeks to ensure, quicken and confirm implementation of new replacement, refinement or reduction guidance. A systematic analysis of our experience in promoting the transparent implementation of validated non-animal vaccine potency assays has led to the refinement of our paradigmatic process, presented here, by which interested parties can assess the local regulatory acceptance of methods that reduce animal use and integrate them into quality control testing protocols, or ensure the elimination of peripheral barriers to their use, particularly for potency and other tests carried out on production batches.
5: COSMOS: An International Cooperative Project
Developing Computational Models for Repeated
Dose Toxicity

Author(s)
Richarz A-N.1, Neagu D.2, Yang C.3, Fioravanzo E.4, Pery A.R.R.5, Berthold M.R.6 and M.T.D. Cronin1

Name of presenter: Andrea-N. Richarz
E-mail address for further correspondence: a.richarz@ljmu.ac.uk

Institutions/Companies
1 School of Pharmacy and Chemistry, Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF, England, a.richarz@ljmu.ac.uk
2 School of Computing, Informatics and Media, University of Bradford, Richmond Road, Bradford, BD7 1DP, England
3 Altamira LLC, Candlewood Drive 1455, Columbus OH43235-1623, United States
4 Soluzioni Informatiche srl, Via Ferrari 14, Vicenza, 36100, Italy
5 Unit METO, INERIS, Parc Alata BP2, 60550 Verneuil-en-Halatte, France
6 KNIME.com AG, Technoparkstr. 1, 8005 Zurich, Switzerland

Abstract
The COSMOS (Integrated In Silico Models for the Prediction of Human Repeated Dose Toxicity of COSMetics to Optimise Safety) Project is a unique international collaboration developing computational approaches for the prediction of repeated dose toxicity. The project comprises 15 partners from academia, industry, regulatory agencies and SMEs from across Europe and the US. Moreover, COSMOS is part of a cluster of six research projects within the SEURAT -1 (Safety Evaluation Ultimately Replacing Animal Testing) Research Initiative.

Organ level toxicity involves complex mechanisms, thus it cannot be predicted by a single simplified in silico model. Therefore COSMOS is taking an innovative approach integrating different technologies, e.g. the threshold of toxicological concern approach, grouping of chemicals, (Q)SARs for toxicity prediction and modelling of biokinetics. All are being developed with a special emphasis on the mechanistic basis of the models considered. Computational workflows as well as a new comprehensive database with reliable structures and repeated dose toxicity data will be freely available to support safety assessment without the use of animals and will thus contribute to the 3Rs.

The international dimension is important for the development and especially regulatory acceptance of the models proposed, the international companies having to assure the safety of their products on a global scale. Therefore industry, regulatory agencies and NGOs in Europe and the US are involved either as project partners or as external experts.

ACKNOWLEDGEMENTS

The funding from the European Community's Seventh Framework Program (FP7/2007-2013) COSMOS Project under grant agreement n°266835 and from Cosmetics Europe is gratefully acknowledged.
### Authors

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<td>e-mail address for further correspondence: <a href="mailto:GillyS@peta.org.uk">GillyS@peta.org.uk</a></td>
</tr>
</tbody>
</table>

### Institutions/Companies

| ¹Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia BG |
| ²People for the Ethical Treatment of Animals Foundation, Society Building, 8 All Saints Street, London N1 9RL UK |
| ³International QSAR Foundation, Two Harbors MN USA |

### Abstract

The 21st-Century shift to more prospective hazard identification and hypothesis generation requires greater strategic application of systems biology, QSAR and archived toxicological data in the form of adverse outcome pathways (AOPs). AOPs describe the causal linkages among biological responses to chemicals over time. The complexity of integrating science can be a barrier to progress in terms of the toxicity pathways and networks involved as well as the need to organize knowledge from many disciplines.

Effectopedia is an open-knowledge aggregation and collaboration tool for delineating AOPs in an encyclopedic and predictive manner. It includes discrete cause-effect studies and critical reviews that are relevant to toxicology. To achieve human and machine interpretability, Effectopedia uses an ontology-enhanced, natural language interface that offers clarifying questions and special tags to define the semantic knowledge while preserving the natural language description of the AOP's elements.

Effectopedia serves as a graphical editor to delineate causal linkages at any level of biological organization and species. It creates a common organizational space that (1) helps experts identify gaps in knowledge of causal linkages of biological responses and (2) acts as a web-based conference room for dialogue and synthesis by experts with interest in specific AOPs. Effectopedia's live documents are instantly open for discussions and feedback, whilst giving credit to original authors and reviewers. New contributions are immediately distributed to interested parties. Uncoupling the contribution and review processes permits organizations to define their own seals of approval and associate them with special interest pathways without slowing down the Wiki-inspired contributions.
7: EUROECOTOX – European Network for Alternative Testing Strategies in Ecotoxicology

Authors

H. Witters¹, E. Sela², M. Garcia-Franco³, M. Galay-Burgos³, T. Braunbeck⁴, N. Klüver⁵, L. Blaha⁶, K. Schirmer⁶, S. Scholz⁷, K. Tanneberger⁷, M. Tobor-Kaplon⁸, J. Guinea²

Name of presenter: Witters Hilda
e-mail address: hilda.witters@vito.be

Institutions/Companies

¹ VITO, CARDAM, Mol, Belgium, ² ZF-BIOLABS, Tres Cantos (Madrid), Spain, ³ ECETOC, Brussels, Belgium, ⁴ University of Heidelberg, Heidelberg, Germany, ⁵ Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany, ⁶ RECETOX, Brno, Czech Republic, ⁷ eawag, Dübendorf, Switzerland, ⁸ NOTOX B.V. s’ Hertogenbosch, The Netherlands

Abstract

Animal experiments play an integral role in current environmental risk assessment for the registration of chemicals, pesticides, biocides, pharmaceuticals, feed additives and testing of whole effluents. As with human risk assessment, there is a strong societal demand to replace, reduce or refine the animal experiments performed in order to protect the environment. However, 3R efforts to human risk assessment are relatively more advanced and international OECD guidelines based on alternative methods are already available for some endpoints.

EUROECOTOX (European Network for Alternative Testing Strategies in Ecotoxicology) aims at reviewing the current status of alternative testing approaches for environmental risk assessment and identifying the gaps and limiting steps for reduction, replacement and/or refinement of animal experiments used in environmental risk assessment. The network has launched a website (www.euroecotox.eu) which provides a resource centre and a database on primarily European activities on development and validation of alternative methods for ecotoxicological testing. The consortium has organised an expert meeting (Leipzig, October 2011), and a scientific conference (Dübendorf, June 2012) with academics, industrial and governmental stakeholders in order to address issues on practices and regulatory requirements for ecotoxicity testing, novel strategies and approaches to reduce animal testing, potential bottlenecks for validating new methods and measures to accelerate development and validation of alternatives. An inventory and analysis of currently available approaches and their limitations is being developed, supplemented by experiences from inside the consortium and published work in order to make recommendations for advancing the development and acceptance of alternative methods in environmental risk assessment.

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