Workshop 5.14
Meeting the challenge of the 7th Amendment to the EU Cosmetics Directive

Poster

Addressing animal testing concerns: A novel micronucleus assay using the human 3D skin model, EpiDerm™

Marilyn Aardema[1], D. Gibson[2], G. Mun[2] and R. Curren[3]
[1] The Procter and Gamble Co, Cincinnati Ohio, USA; [2] Institute for In Vitro Sciences, Gaithersburg, MD, USA

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. Skin is one of the target areas of interest for many cosmetic products because it is generally the tissue with the highest exposure. Therefore we have begun development of a micronucleus assay using a commercially available 3D engineered human skin model, EpiDerm™ (MatTek Corp, Ashland, MA, USA). We first evaluated whether a population of binucleated cells sufficient for a micronucleus assay could be obtained by exposing the tissue to 1-3 ug/ml cytochalasin B (Cyt B). The frequency of binucleated cells increased both with time and with increasing concentration of Cyt B. Cyt B at 3 ug/ml allowed us to reliably obtain 40-50% binucleated cells at 48 h and was used in future studies. The background frequency of micronuclei in this model is low (~0.1%) and reproducible. Studies with model genotoxins including mitomycin C, vinblastine sulfate and methylmethane sulfonate demonstrated that micronuclei can be reproducibly induced in this 3D skin model. This is the first step in developing a routine "*in vivo*-like" assay for chromosomal damage in human tissue.

Poster

Differential effects of irritants and allergens in an epidermoid cell line

Gautam Banerjee[1], Smitha Upadhyaya[1], Purvi Mehrotra[1], Archana Kasala[1], Vilas Sinkar[1], Gavin Maxwell[2] and David Basketter[2]*

[1] Hindustan Lever Research Cent re, Environmental Safety Laboratory, Mumbai, India;
[2] Unilever, Safety and Environmental Assurance Centre, Bedford, UK

Experience to date has suggested that dendritic cells are the most likely to respond in a differential manner to chemicals which are either irritant and/or allergenic to the skin. However, the mechanism underlying such a differential response is unknown. Furthermore, there have recently been observations suggesting epidermal keratinocytes may also display a differentiated response. Accordingly, we have measured the response in an epidermoid cell line (A431) to a range of irritants and allergens. The effect of exposure to two or more sub-cytotoxic concentrations of each chemical on the elevation of MHC class II expression (RT-PCR) and the release of interleukin-12 (IL-12) (ELISA) were investigated. The irritant sodium dodecyl sulphate had no effect on either MHC-II expression or IL-12 release. All the allergens tested showed at least 20% increase in IL-12. For MHC II, allergens induced a modest increase in mRNA expression, whereas several irritants failed to induce the MHC. However, these changes in MHC need to be confirmed by quantitative real time PCR. Further work is required to both test this assay and develop the prediction model, but it serves to remind us that in an *in vitro* test approach for the assessment of irritants and allergens, it is appropriate for us to remember that keratinocytes also play an important role.

Poster

The COLIPA *strategy for the development of in vitro* alternatives

Odile de Silva[1], Rolf Fautz[2], Stuart J. Freeman[3], Carsten Goebel[4], Irene Manou[5], Pauline McNamee[6], Thierry Oddos[7], Wolfgang Pape[8], Winfried Steiling[9], Hidekazu Toyoda[10] and Carl Westmoreland[11]

[1] L'Oréal, Paris, France; [2] Kao Professional Salon Services, Darmstadt, Germany; [3] GlaxoSmithKline, Surrey, UK; [4] Wella, Darmstadt, Germany; [5] COLIPA, Brussels, Belgium; [6] The Procter and Gamble Company, Egham, Surrey, UK; [7] Johnson and Johnson, Val de Reuil, France; [8] Beiersdorf, Hamburg, Germany; [9] Henkel, Düsseldorf, Germany; [10] Shiseido Research Center, Yokohama-Shi, Japan; [11] Unilever, Safety and Environmental Assurance Centre, Sharnbrook, UK

The cosmetics industry's commitment to phase out animal tests is long standing: since 1992, SCAAT (Steering Committee on Alternatives to Animal Testing) is co-ordinating activities towards development of non animal alternative methods, contributing to their validation. *In vitro* tests are needed to identify relevant aspects of the complex interactions of a chemical with human skin, eye and other target tissues. COLIPA's Task Forces (TF) undertake the work necessary to develop *in vitro* alternatives for toxicological endpoints of key interest, i.e. skin sensitisation, skin irritation, eye irritation and genotoxicity. The TF Skin Tolerance currently runs projects to develop *in vitro* test systems to identify potential allergens and irritants. The TF Eye Irritation is focussed on the underlying physiological mechanisms of eye irritation and recovery to identify *in vitro* endpoints more predictive of the *in vivo* human response to chemicals and is working in close collaboration with ECVAM. For all TFs the current challenge is developing an appreciation of how to use their data output for risk assessment in addition to hazard identification.

Risk assessment for chemicals used as ingredients also covers systemic exposure. The acceptance of a method to assess percutaneous absorption *in vitro* (OECD 428) was a major success for our TF. As a further step, the TF Genotoxicity plans work to adapt *in vitro* genotoxicity testing to dermal exposure. In co-operation with academia, industry, scientists and regulators, our strategy tries to combine the best scientific approaches resulting in alternative methods for the most appropriate safety assessment of cosmetics.

Lecture

The challenges of the 7th Amendment

Odile de Silva
L'Oréal, Paris, France

The cosmetic industry has a pro-active approach on the issue of alternatives to animal testing through its voluntary SCAAT research programme since 1992. It has recognised the specific challenges set in the 7th Amendment and responds with an increased engagement in R&D programmes. Domains of toxicity currently covered are eye irritation, skin irritation and allergy, mutagenicity. Research projects are investigating mechanisms of toxicity, however SCAAT teams are also helping the validation process through ECVAM and ICCVAM.

Fundamental scientific questions are raised: the appropriate basic knowledge of key biological mechanisms needs to be understood, what should be the balance between *in vitro* tests versus *in silico* or chemistry-based tests, how should the data be integrated and interpreted. This also leads to the fact that although industry is already working in partnership with academia, regulators, ECVAM, etc. What we really need is a critical number of scientists from academia attracted by the challenge of developing "non-animal alternatives" in view of replacing regulatory tests, and who would lead the research in new and bold areas.

Industry together with other stakeholders are currently investigating new and pragmatic thinking: read-across, TTC, analytical methods, etc. There is a need to come up with different approaches, stemming from the fact that we shall have to integrate all kinds of new data, new ways of combining, extrapolating, of dealing with data gaps. Some of these tools will be applicable to "known" chemistry, however new methods should be developed for future chemistry, where no references or similarities can be of help.

Poster

Strategy for *in vitro* alternatives to inhalation toxicology: Where do we begin?

Leona Greenwell[1], Erwin Roggen[2], Julie Holder[3], Philip Carthew[1], Carl Westmoreland[1] and Julia Fentem[1]

[1] Unilever – Safety and Environmental Assurance Centre, Colworth Laboratories, Sharnbrook, Bedfordshire, UK;
[2] Novozymes A/S, Bagsvaerd, Denmark; [3] GSK Research and Development, Ware, UK

The 7th Amendment to EU Cosmetics Directive stipulates that alternatives to repeat-dose toxicity animal studies must be in place by 2013. Addressing the challenges of developing a suitable battery of tests to replace *in vivo* inhalation testing is no simple task. A multitude of questions must be answered, ranging from the technically challenging "how do we emulate representative inhalation exposure *in vitro*?" to the more ethereal "what exactly defines a NOAEL *in vitro*?". In order to maximise the chances of developing a complete strategy by the EU deadline avoiding replication of effort, cross-industry co-operation is essential. Attempts are being made to formally establish an "International Partnership for Alternatives to Animal Testing" (IPAAT). Meanwhile, we (Unilever, Novozymes and GSK) have established a collaboration to undertake the necessary research and development in the area of respiratory toxicology.

We are currently investigating a range of approaches encompassing both 'top down" bridging studies and "bottom up" investigative studies. These include the use of various models (i.e. lung slice, air-liquid interface and co-cultures), *in silico* systems (i.e. deposition, exposure) and leading edge technologies (i.e. 'omic markers, stem cell derived models, raman spectroscopy).

Progress is being made – markers of specific endpoints are being characterised *in vitro*, and efficacy and limitations of different models are being assessed, along with their comparability to observed results *in vivo*. Research is being initiated into representative aerosol exposure *in vitro* and the development of mutually available databases.

Lecture

7th Amendment to the Cosmetics Directive

Thomas Hartung
ECVAM, Institute for Health and Consumer Protection, Joint Research Center, Ispra, Italy

The political expectations of the 7th Amendment to the Cosmetics Directive are high with regard to the phasing out of animal experiments: For two years already, a testing ban for finished products is in place. Testing bans for ingredients enforced by marketing bans are approaching in 4 and 8 years, notably, independent of the availability of validated alternative methods. This political pressure has resulted in the targeted development and validation of methods required in a new dimension. However, the question has to be raised, how realistic it is to meet the deadlines for the different animal tests? A review of the efforts and achievements in areas like topical and systemic toxicities, reproductive toxicology or carcinogenicity shall provide an interim analysis of the state of the art.

Poster

Bergamot oil intended for topical use – attempts for a risk assessment of phototoxicity

Dagmar Jirova[1], Kristina Kejlova[1], Hana Bendova[1], Helena Kandarova[2], Zdenek Weidenhofer[3] and Manfred Liebsch[2]

[1] National Institute of Public Health, National Reference Center for Cosmetics, Prague, Czech Republic; [2] Federal Institute for Risk Assessment BfR, ZEBET at BfR, Berlin, Germany; [3] National Institute of Public Health, National Reference Laboratory for Mass Spectrometry, Prague, Czech Republic

Bergamot oil is a widely used aromatic ingredient, e.g. in food and cosmetics. Its use is often limited due to reported phototoxicity, usually attributed to bergapten content.

The aim of this study was to clarify the differences in the phototoxicity of several bergamot oils obtained from different suppliers. The phototoxicity of the samples was evaluated *in vitro* in the 3T3 NRU Phototoxicity Test (PT) and a phototoxicity test on reconstructed human skin model (EpiDerm™, Mattek). In addition, in case of non-phototoxic classification in the EpiDerm phototoxicity assay, photo-patch testing in a limited group of human volunteers was performed.

Amongst 4 different samples, two phototoxic and two non-phototoxic oils were classified by 3T3 NRU PT, however, only on the basis of borderline phototoxicity results. Surprisingly, even samples classified borderline proved to be clearly phototoxic in the EpiDerm test. In general, the skin model test and human patch test provided concordant results. In both cases, it was estimated that bergamot oils (classified as non-phototoxic by 3T3 NRU PT) were safe for use up to 1%. The skin model test therefore seems to be a useful tool in the risk assessment, since it enables to set a margin of safety before any testing in humans.

Analytical analysis (applying capillary GC/MS) enabled identification and quantification of photoactive compounds present in the test samples. Besides bergapten, differences in citropten, bergamottin, geranial and neral content were identified. We conclude, that the different phototoxic effect depends also on the amount of these components.

Lecture

Animal testing and alternative methods relating to cosmetic products

Georgette Lalis
European Commission, Directorate F – Consumer Goods, Brussels, Belgium

The European cosmetics industry is an example of an internationally competitive industry which has established itself as a market leader. With 35 billion €, the European cosmetics industry has an ex-factory output which is twice that of Japanese companies and one third higher than U.S. companies. It is estimated that around 5 billion cosmetic products are consumed by Europeans every year. The cosmetics industry is a dynamic industry, characterised by innovation and a high rate of product development. On average, major cosmetics companies replace or reformulate around 25% of their products each year.

The Cosmetics Directive 76/768/EEC has been adopted in order to ensure the free circulation of cosmetic products in the internal market and the safety of cosmetic products placed on it. It also establishes a prohibition to test finished cosmetic products and cosmetic ingredients on animals (testing ban), and a prohibition to market in the European Community, finished cosmetic products and ingredients used in cosmetic products which were tested on animals (marketing ban).

A number of initiatives have been launched at the European level to promote alternative methods to animal testing, such as funding under the 6th Framework Programme on Research and Development amounting to 39 Mio Euros. Research in the development of alternatives is not only beneficial for animals but also encourages the development of new markets for these methods.

DG ENTR and DG Research will hold a conference on animal tests and alternative methods, "Europe Goes Alternative", on November 7, 2005, in Brussels to demonstrate that the European Commission keeps animal welfare high on the political agenda. Given that the 5th World Congress covers most of the scientific issues, we will pursue a more policy oriented approach for the conference in Brussels to identify further possibilities to improve development, validation and legal acceptance of alternative methods.

Lecture

Japanese challenge to develop alternative methods for safety evaluation of cosmetics

Yasuo Ohno
National Institute of Health Sciences, Setagaya, Tokyo, Japan

Research group supported by Japanese Ministry of Health, Labor, and Welfare have already evaluated *in vitro* eye irritation test (several cytotoxicity tests), 3T3-NRU phototoxicity tests by the co-operation of JSAAE and Japan Cosmetic Industry Association. These methods are useful for the toxicity evaluation of cosmetics ingredients if they are combined with *in vivo* method in case of ambiguous prediction or utilisation of positive chemicals. We have conducted validation of *in vitro* skin corrosivity tests (VitroLife Skin) and *in vitro* phototoxicity test battery using yeast and red blood cell. These methods are in the process of evaluation. We are going to conduct validation of modified LLNA that do not use radioisotope labelled compounds. The research group is now conducting research to develop alternative methods for *in vitro* acute toxicity tests with metabolic activation steps, *in vitro* skin sensitisation tests, *in vitro* photo-sensitisation tests, and appropriate data collecting and processing procedures for the evaluation of alternative methods. We are expecting our results will contribute to correspond to the 7th Amendment to the EU Cosmetics Directive.

Lecture

Good science must be the key factor in the development and use of alternative methods for safety assessment of cosmetics

Vera Rogiers
Department of Toxicology, Vrije Universiteit Brussel (VUB), Brussels, Belgium

Background/Aims: With the implementation of the 7th Amendment (2003/15/EC) into the EU cosmetic legislation a testing and marketing ban have been introduced. In practical terms this means that all toxicological testing on animals must be replaced by validated alternatives in a time span of 6 years, with the exception of repeated-dose toxicity, toxicokinetics and reproductive toxicity (10 years). The question arises now whether this is scientifically feasible.

Discussion: ECVAM provided an objective overview of the current status of alternative methods and strategies and the prospects for their validation and regulatory acceptance (30/4/2004). The SCCNFP was asked for its comments (SCCNFP/0834/04). The clear message was given that total abolishment of animal tests within 10 years was considered to be not feasible from a scientific point of view, in particular, seen the fact that only replacement methods would be allowed. In a joint document, experts from three committees, advising the commission on toxicological matters, came to the same conclusion (CSTEE 2004). Nevertheless, the optimistic time frame was retained by the Commission. Recently, several EU research projects have been initiated, including ReProTect, AcuteTox, Predictomics, Sensitive, etc. which give hope for future new developments. However, seen the timeframe to scientifically elaborate such complex studies, to pre-validate and validate potential successes and to implement these into the EU legislation, it becomes evident that the deadlines cannot be met. It is high time to realise that science follows its own rules and cannot be driven by a political agenda.

Poster

EU Cosmetics Directive: Failures and challenges from the animal welfare point of view

Irmela Ruhdel
German Animal Welfare Federation, Animal Welfare Academy, Neubiberg, Germany

EU Directive 2003/15/EC lays down deadlines for bans in the cosmetics sector: an animal experimentation ban for 2009 and a sales ban on animal tested cosmetics for 2013. The Directive has to be regarded a minimum compromise, but even some of its basic provisions have not been implemented by the European Commission so far. This concerns for example an immediate ban on animal experiments where animal free methods are scientifically validated or accepted also when they are not included into Annex V of the EU Dangerous Substances Directive. The Commission failed to list several animal free methods that have been endorsed by ECVAM or that have been accepted by the OECD or individual EU Member States in the new Annex IX of the Directive. In its timetables of October 2004 the Commission also ignored the time-limits for the sales ban on three additional endpoints of the safety evaluation as given in the Cosmetics Directive. This is counterproductive as the deadlines for the bans were intended to give a fresh impetus for new animal free tests. The Commission must ensure optimal conditions for the replacement of animal experiments such as sufficient funding. Additionally, all types of industries should be involved actively in this process because all of them profit from new animal free tests and testing strategies. In any case the animal experimentation ban and at least part of the sales ban have to come into force 2009 and 2013 irrespective of the availability of animal free methods.

Lecture

Sound science: A prerequisite for advancing alternative methods and protecting public health

William S. Stokes
National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC, USA

Current laws and regulations protect human health by requiring the safety assessment of new products such as cosmetics prior to their marketing and subsequent human exposure. Such laws were enacted in response to public outrage following blindness, severe injuries and deaths caused by untested cosmetics and other products. Subsequent safety evaluations of cosmetic ingredients and products using animals and *in vitro* methods have now largely eliminated adverse health effects. Nevertheless, public pressures have led to recent adoption of the 7th Amendment to the European Union Cosmetics Directive, which now bans the use of animals for testing finished cosmetic products and will ban the use of animals for most testing for ingredients in 2009. In order for *in vitro* methods to gain acceptance as complete replacements for animals, there must be scientific evidence that the use of these methods will provide for equivalent or improved protection of human health. ICCVAM, which is charged by law with evaluating the scientific validity of new, revised, and alternative test methods, has evaluated several alternative test methods applicable to cosmetics testing that have now achieved regulatory acceptance and is currently evaluating several other applicable methods. These methods have or will significantly reduce animal numbers and animal pain and distress; however, none have been found to be scientifically valid as complete replacements for animals. Despite the legislated testing bans, alternative methods will only be able to fully replace animal use and ensure adequate protection of the public when supported by sound science.