Draize Test-Related EpiOcular™ Technical References

(In descending order by MatTek Corp. Technical Reference Number)

285. EVALUATING THE OCULAR IRRITATION POTENTIAL OF 54 TEST ARTICLES USING THE EPIOCULAR HUMAN TISSUE CONSTRUCT MODEL (OCL-200). [1]Blazka, M.E., [2]Harbell, J.W. , [3]Klausner, M. , [2]Merrill, J. , [3]Kubilus, J. , [1]Kloos, C. and [1]Bagley, D.M. [1]Colgate-Palmolive Company, Piscataway, NJ; [2]The Institute for In Vitro Sciences, Inc., Gaithersburg, MD; [3]MatTek Corporation, Ashland, MA. Society of Toxicology Meeting, Poster # 1070, (2003).

Colgate-Palmolive is sponsoring a research program to validate the use of the EpiOcular Model in evaluating the eye irritation potential of surfactants. Previously, in a study that demonstrated the reliability of the EpiOcular Model, four laboratories using a formal and detailed study protocol tested 19 test materials. In the current study, two laboratories (Institute for In Vitro Sciences, Inc. and MatTek Corp.) have tested 54 test articles using the same study protocol. EpiOcular is a commercially available three-dimensional in vitro model of the human corneal epithelium composed of normal human-derived epidermal keratinocytes. Test articles included a shampoo formulation and 30 different surfactants (10-cationic; 11-anionic; 7-nonionic; 1-amphoteric; 1-zwitterionic) which were liquids, powders or creams. Multiple concentrations of 11 of the surfactants were tested, to evaluate the model's ability to predict dose-related differences in a test article's potential for ocular irritation. Testing was conducted in compliance with FDA GLPs. The laboratories were blinded to the identities of the test articles. Test results were compared to previously published animal eye irritation studies. In terms of reliability, the results were reproducible within and between the laboratories. In terms of relevance, the EpiOcular model correctly predicted the Draize score for a majority of the samples tested. The model also correctly predicted increasing irritation potential of surfactants with increased concentrations. These data provide additional evidence that the EpiOcular model meets the validation criteria, as defined by the Interagency Coordinating Committee on the Validation of Alternative Methods (NIH Publication No. 97-3981), for assessing the ocular irritation potential of certain classes of surfactant and surfactant-based formulations.

259. ASSESSMENT OF OCULAR IRRITATION RANGES OF MARKET-LEADING COSMETIC AND PERSONAL-CARE PRODUCTS USING AN IN VITRO TISSUE EQUIVALENT. McCain, N.E., Binetti, R.R., Gettings, S.D., Jones, B.C. Cell Biology & In Vitro Toxicology, Avon Products, Inc., Suffern, NY. The Toxicologist, 66 (1-S), 243, Soc. of Toxicol. (Reston, VA), Abstract #1191, (2002).

The cosmetics and personal-care industry has focused considerable effort on the search for replacements for traditional animal-based safety tests. *In vitro* models have been found to be particularly useful for the assessment of eye irritation potential. We have used the MatTek EpiOcular™ OCL-200 tissue model (composed of human epidermal keratinocytes that differentiate and form a stratified squamous epithelium similar to corneal tissue) to establish ranges of *in vitro* ocular irritation scores for several categories of cosmetic and personal-care products. A significant advantage of the EpiOcular model is that it can be used to discriminate between the irritation potential of extremely mild products (most cosmetic and personal care products are formulated for inherent mildness). Materials were topically applied to the EpiOcular tissue equivalents (167L/cm2). Surfactant-based products were tested at a concentration of 10% to simulate "rinse-off" exposures; all other products were tested without dilution. Materials were tested using either the standard (4 hours) or extended (20 hours) exposure protocol based on their expected irritation potential (ie consideration of formula composition, product type, etc.). Cellular viability was used as a marker for irritation potential and measured at various time points by a MTT metabolism colorimetric assay. MTT was quantitated spectrophotometrically at 570nm and an ET50 (time to 50% loss of viability) calculated for each product. A range of *in vitro* ocular ET50 scores was then determined for each product category. The results obtained from this study provide a reference database of *in vitro* ocular irritation scores for a cross-section of currently marketed cosmetic and personal-care products. Comparison of the ET50 for new formulations with the range of ET50 scores established for currently marketed products of similar type is a useful benchmark of anticipated consumer acceptability under conditions of actual use.

248. COMPARATIVE EVALUATION OF FIVE *IN VITRO* TESTS FOR ASSESSING THE EYE IRRITATION POTENTIAL OF HAIR-CARE PRODUCTS. Jones[1], P.A., Budynsky[2], E., Cooper[1], K.J., Decker[2], D., Griffiths[1], H.A., and Fentem[1], J.H. [1]Safety & Environmental Assurance Centre (SEAC), Unilever Colworth Laboratory, Sharnbrook, Bedfordshire MK44 1LQ, UK; [2]Unilever Home & Personal Care, 3100 Golf Road, Rolling Meadows, IL 60008, USA. ATLA 29, 669-692, (2001).

This study compared five methods, the isolated rabbit eye (IRE), bovine corneal opacity and permeability (BCOP), EpiOcular™, fluorescein leakage (FL) and neutral red release (NRR) assays, for predicting the eye irritation potential of hair-care formulations. Ten shampoo and seven conditioner formulations of known ocular irritation potential were tested. Each group included a market-acceptable formulation as a comparative benchmark. Predictions of ocular irritation were made by using classification models (IRE, BCOP and EpiOcular assays) or by direct comparison with benchmarks (IRE, EpiOcular, FL and NRR assays). The BCOP assay was less sensitive than the IRE test in discriminating between formulations of different irritation potentials, and did not perform as well as the other assays in identifying mild formulations. All of the assays appeared to be better at discriminating correctly between the shampoos than between the conditioners. The EpiOcular assay showed the closest concordance between the *in vivo* results and the *in vitro* data from cell-based assays (particularly for shampoos). The FL assay also showed a high concordance (particularly for conditioners). There was a tendency for these *in vitro* assays to over-predict eye irritation potential, but there was no under-prediction and they were particularly successful at identifying mild formulations. The NRR assay was less predictive with both shampoos and conditioners. The results from this comparative evaluation fully support the continued use of the IRE test as a suitable alternative to *in vivo* eye irritation testing in rabbits, although it also over-predicted the irritancies of several of the formulations. The value of using concurrent benchmarks (reference standards), appropriate to the materials being tested, in interpreting the data obtained from *in vitro* tests, was also demonstrated. Overall, the results indicate that further comparisons of the IRE, EpiOcular and FL assays are warranted using much larger numbers of test materials.

191. THE EPIOCULAR TISSUE MODEL*: IN VIVO* VERSUS *IN VITRO* DRAIZE SCORES FOR CONSUMER PRODUCTS. Klausner[1], M., Osborn[1], M., Bellavance[1], K., Breyfogle[1], B., Kubilus[1], J., Cerven[2], D.R., and DeGeorge[2], G. [1]MatTek Corporation, Ashland, MA and [2]MB Research Laboratories, Spinnerstown, PA. Toxicological Sciences, 54, (1), 188 Abstr. #884 (2000).

EpiOcular™ (OCL-200) is an organotypic tissue model of the human corneal epithelium (HCE) cultured from normal human keratinocytes using serum free medium. H&E stained histological cross-sections show that the structure of EpiOcular closely parallels that of the HCE. Previously, a prediction equation for eye irritation, Draize score (MMAS) = -4.74 + 101.7/ √ET-50, was developed by correlating the *in vitro* ET-50 with Draize rabbit eye scores for 19 water-soluble chemicals from the ECETOC database and 41 cosmetic or personal care products/ingredients (ET-50 refers to the time of exposure which reduces the tissue viability to 50%, as determined by the MTT assay, in minutes). The current study reports *in vitro* results for an additional 24 consumer products, including shampoos, hand soaps, laundry detergents, dish-washing liquids, and skin lotions. A plot of the *in vivo* and calculated *in vitro* Draize scores, when correlated to the line *In vivo Draize (MMAS) = In Vitro Calculated Draize,* gave the correlation coefficient, r = 0.85; when a single outlier was excluded r = 0.93. The useful range of the EpiOcular test was evaluated by testing surfactants at concentrations at which the Draize test is insensitive (MMAS scores < 2.0). EpiOcular was able to distinguish between surfactants at concentrations 3-10 fold below this point. Thus, the EpiOcular tissue model appears to be a sensitive, accurate *in vitro* means of predicting *in vivo* ocular irritancy for a range of consumer products and raw materials.

190. COLGATE-PALMOLIVE'S PROGRAM - VALIDATE THE EPIOCULAR™ HUMAN TISSUE CONSTRUCT MODEL (OCL200). Blazka[1], M.E., Harbell[2], J.W., Klausner[3], M., Raabe[2], H., Kubilus[3], J., Hsia[4], F., Minerath[5], B., and Bagley[1], D.M. [1]Colgate-Palmolive Co., Piscataway NJ, [2]Institute for In Vitro Sciences, Gaithersburg, MD. [3]MatTek Corp., Ashland, MA, [4]3M Co., St. Paul, MN and [5]Kimberly Clark, Neenah, WI. Toxicological Sciences, 54, (1), 188, Abstr. #883 (2000).

The objective of this corporate-based program was to formally validate the EpiOcular™ model's ability to predict the eye irritation potential of surfactants and surfactant-based formulations, As defined by the Interagency Coordinating Committee on the Validation of Alternative Methods (NIH Publication No.97-3981), a validated method is one for which the reliability and relevance for a specific purpose have been established. EpiOcular™ is a commercially available three-dimensional *in vitro* model of the human corneal epithelium composed of normal human-derived epidermal keratinocytes. Four laboratories (Institute for In Vitro Sciences, MatTek, 3M and Kimberly Clark) conducted a range finding and three definitive assays on each of 20 blinded materials (6 formulations, 14 surfactants) in compliance with FDA GLPs using a formal and detailed test protocol. EpiOcular results for the test materials were compared to previously published eye irritation studies in animals, In terms of reliability, the results were reproducible within and between the laboratories. In terms of relevance, the EpiOcular™ model correctly predicted the Draize score for a majority of the samples tested. Results from five of six formulations were in agreement with the *in vivo* data. In addition, the eye irritation potential for anionic and cationic surfactants was correctly predicted. For those materials not predicted, the majority seemed to be alcohol ethoxylate surfactants that were under-estimated or amphoteric surfactants that were over-estimated for eye irritation. A complete statistical analysis of the data will be presented. These data indicate that, for certain classes of surfactant and surfactant-based formulations, the EpiOcular™ model has met the critical validation criteria for assessing ocular irritation.

183. THE APPLICATION OF REFERENCE STANDARDS IN THE PREVALIDATION AND VALIDATION OF *IN VITRO* TESTS FOR EYE IRRITATION. Brantom, P., Fentem[1], J., Balls[2], M., and Cassidy[3], S. BIBRA International, Woodmansterne Road, Carshalton, Surrey SM5 4DS, UK; [1]SEAC Toxicology, Unilever Research, Colworth, Sharnbrook, Bedfordshire MK44 1LQ, UK; [2]ECVAM, Institute for Health & Consumer Protection, European Commission Joint Research Centre, 21020 Ispra (Va) Italy; and [3]Dow Corning, Rue General de Gaulle 62, 1310 la Hulpe, Belgium. ATLA, 27, 332, (1999).

The present project arose out of the proposals of a working group on reference standards established by ECVAM in 1997. The primary objective of this pilot study was to determine whether the current approach of using *in vitro* data to make safety assessment decisions, employed in-house by many companies, could be accepted in a regulatory context as scientifically valid and applicable. Reference standards are used in many areas of science to provide a scale against which an unknown is judged. It is considered likely that the value of existing *in vitro* methods can be significantly improved by the use of reference standards appropriate both to the limitations of the assay and to the unknown substances under test. The methods selected for investigation in this study are: isolated chicken eye (ICE); bovine cornea opacity and permeability (BCOP); HET-CAM/NRU; EpiOcular®; and red blood cell (RBC). The study is divided into two main phases. The first phase establishes results for five known chemicals in each assay. The chemicals will be selected by a small team of people as being the best reference standards for that assay and will form the basis of a prediction model that will be used in phase 2. The second phase will test a further set of five chemicals, together with the reference standards. The test set will be provided as coded "blind" samples. Each laboratory will be required to predict the eye irritation of the five test chemicals using the prediction model developed in phase 1. The study is expected to be completed in June 2000. Further information on study design and early stages of the study will be incorporated into the poster.

178. COLGATE-PALMOLIVE'S PROGRAMME TO VALIDATE THE EPIOCULAR™ HUMAN TISSUE CONSTRUCT MODEL (OCL-200). Blazka, M.E., Harbell[1], J.W., Klausner[2], M., Raabe[1], H., Kubilus[2], J., Hsia[3], F., Minerath[4], B., and Bagley, D.M. Colgate-Palmolive, Piscataway, NJ, USA; [1]Institute for In Vitro Sciences, Gaithersburg, MD, USA; [2]MatTek, Ashland, MA, USA; [3]3M, St Paul, MN, USA, and [4]Kimberly Clark, Neenah, WI, USA. ATLA, 27, 331, (1999).

Currently, no alternative to the Draize eye test has been validated and accepted by any regulatory body. The objective of this corporate-based programme was to formally validate the EpiOcular™ model's ability to predict the eye irritation potential of surfactants and surfactant-based formulations. Developed by MatTek, EpiOcular is a three-dimensional organotypic model of the human corneal epithelium composed of normal human keratinocytes. The EpiOcular model uses the exposure time required to reduce the tissue viability to 50% (ET50) to predict the Draize Modified Maximum Average Score (MMAS) using the algorithm: Draize MMAS = -4.74 + 101.7 ET50, where ET50 is 1 ET50 240 minutes. The validation programme was developed in accordance with criteria established by the Interagency Coordinating Committee on the Validation of Alternative Methods (NIH Publication No.97-3981). During the prevalidation phase of the programme, the prediction model was developed, the protocol was finalised, test materials meeting programme criteria were obtained, and participating laboratories were qualified. Testing was conducted in compliance with FDA Good Laboratory Practice regulations. Laboratories that participated in the programme included the Institute for *In Vitro* Sciences, MatTek, 3M and Kimberly Clark. Criteria for test material inclusion were that good quality historical animal data were available, and that the materials were either surfactants or surfactant-based formulations. During the validation phase, test sites conducted a rangefinder and three definitive assays on each of 20 blind tested materials (6 formulations, 14 surfactants).

176. THE EPIOCULAR PREDICTION MODEL: A REPRODUCIBLE *IN VITRO* MEANS OF ASSESSING OCULAR IRRITANCY POTENTIAL. Klausner, M., Sheasgreen, J., Breyfogle, B., Sennott, H., Liebsch[1], M., and Kubilus, J. MatTek Corporation, Ashland, MA 01721, USA; and [1]ZEBET, 12277 Berlin, Germany. ATLA, 27, 299, (1999).

The EpiOcular™ tissue model (OCL-200) is an organotypic model of the human corneal epithelium (HCE) cultured from normal human keratinocytes using serum-free medium. Haematoxylin and Eosin stained histological sections show the structure of EpiOcular™ to closely parallel that of the HCE. During 1998, 85 lots of OCL-200 were produced and monitored using the MTT assay. The common surfactant, Triton X-100 (0.3%), was used as a positive control and the Effective Time-50 values (time of exposure after which viability is reduced to 50% [ET50]) were determined for each lot of tissue. The 1998 ET50 average of 25.2 ± 5.6 minutes (± 1 standard deviation) was not significantly different ($p > 0.75$) than the 1996 average of 24.9 ± 6.3 minutes (n = 47 lots), the first year of commercial production. ET50 values were measured for 19 water-soluble chemicals from the ECETOC database and 41 cosmetic or personal care products/ingredients. Draize data for these materials were correlated to ET50 values and a prediction model was constructed: Draize = -4.74 + 101.7/ (ET50) ^ 0.5 (correlation coefficient, r = 0.90). Stability of the EpiOcular™ model for intemational shipment was probed by shipping the tissue to ZEBET in Germany. A plot of the predicted Draize in the two laboratories gave r = 0.94; plotting predicted versus actual Draize scores gave r = 0.90 and r = 0.94, for MatTek and ZEBET, respectively. Thus, the EpiOcular™ tissue model is reproducible and appears to be an *in vitro* means of accurately predicting *in vivo* ocular irritancy.

146. EVALUATION OF THE EPIOCULAR™ TISSUE MODEL AS AN ALTERNATIVE TO THE DRAIZE EYE IRRITATION TEST. [1]Stern, M., [2]Klausner, M., [1]Alvarado, R., [1]Renskers, K., [1]Dickens, M. [1]Avon Products, Inc.,Suffern, NY; [2]MatTek Corporation, Ashland, MA. Toxicology In Vitro, 12, 455-461, (1998).

Cosmetic ingredients were tested to determine the ability of the EpiOcular™ tissue model to predict eye irritation potential. *In vitro* results were compared with historical Draize eye irritation records. Forty-three samples, consisting of 40 cosmetic raw ingredients of different type and physical form (i.e. liquids, powders, gels) were evaluated. Using the MTT cytoxicity assay, an ET_{50} value (effective time of exposure to reduce tissue viability to 50%) was determined for each sample. ET_{50} values were categorized into four irritation groups: (a) non-irritating/minimal; (b) mild; (c) moderate; or (d) severe/extreme. Comparison of *in vitro* EpiOcular™ and *in vivo* Draize classifications showed that 63% (27 of 43 samples) were classified identically. Assay performance improved to 95% (41 of 43 samples) with the addition of samples overpredicted by a single irritation class. This evaluative exercise represents a conservative safety assessment. There were no underpredictions of eye irritation for any material in this study. Based on these results, use of the EpiOcular™ tissue model shows promise as an *in vitro* assay to assess the ocular irritation potential of cosmetic ingredients.

141. DEMONSTRATING THE OCULAR SAFETY OF AN EYE COSMETIC PRODUCT USING ALTERNATIVES TO ANIMAL EYE IRRITATION TESTS. Ghassemi[1], A., Osborne[1], R., Kohrman[1], K. A., Roddy[1], M. T., Harbell[2], J.W., and Kanengiser[3], B.E. [1]The Procter & Gamble Company, Hunt Valley, MD and Cincinnati, OH; [2]Microbiological Associates, Inc. Rockville, MD. [3]Clinical Research Laboratories, Inc., Piscataway, NJ. Sponsored by L.D. Lehman-McKeeman**.** The Toxicologist, 36 (1), 42, Soc. of Toxicol. (Reston, VA), Abstract #221, (1997).

Previous studies have reported the use of the Tissue Equivalent Assay (TEA) and clinical eye irritation tests for assessing the ocular safety of various consumer products. In lieu of rabbit eye irritation testing, a tiered approach was employed to asses the ocular safety of a new mascara formula (A). After review of available ingredient eye irritation data, several new formulas and marketed benchmark mascaras were evaluated in an extended TEA (maximum 24 hr exposure). The TEA identified those new formulas which would have low eye irritation potential in humans, as compared to a broad range of prototype and marketed mascaras previously evaluated in the extended TEA. One mascara prototype (A) with a low eye irritation potential as determined by the TEA and two safely marketed benchmark mascaras (X and Y) were then evaluated in a 28-day safety in-use test with self-assessed sensitive and non-sensitive human subjects as well as contact lens wearers under the direction of a Board Certified ophthalmologist. A novel grading system (scale of 0-13) was employed for fluorescein staining of conjunctival, corneal, and caruncular tissues. Additionally, the tear film break up time was measured for each subject. Sensory reaction assessments were provided by the subjects at each exam time and via diaries. The results demonstrated that mascaras A, X, and Y were equally safe for consumer use. The minimal, transient eye effects observed were non-product related and clinically non-significant. Consumer exposure data further supported the *in vitro* and clinical results. This research provides additional confirmation that a combination of the TEA and clinical tests can be used successfully for ocular safety assessment of cosmetic products.

139. PREDICTION OF OCULAR IRRITATION POTENTIAL OF AIR CARE PRODUCTS USING *IN VITRO* TOPICAL APPLICATION ASSAYS. Manderfield[1], C.E., Swanson[1], J.E., Kennedy[1], O., Lee[2], M.K., Bauernschub[2], M.A., Harbell[2], J.W. [1]S. C. Johnson & Son, Inc., Racine, WI; [2]Microbiological Associates, Inc., Rockville, MD. Sponsored by R. D. Curren. The Toxicologist, 36 (1), 44, Soc. of Toxicol. (Reston, VA), Abstract #223, (1997).

Air care products contain high concentrations of fragrance materials which are generally aqueous insoluble. Predicting the ocular irritancy potential of this product class has been difficult and unreliable using dilution-based assays. This problem suggested that to properly model *in vivo* exposures, the neat test material must be applied directly to the test tissue. This study sought to determine the utility of the EpiOcular™ human tissue construct (MatTek Corporation) for prediction of ocular irritancy potential of air care formulations and ingredients. The responses of the EpiOcular construct were also compared to those of the Skin[2] ZK1200™ construct (Advanced Tissue Sciences). Sample formulations were prepared which spanned the expected irritancy range for this product class (Draize MMAS ranging from 4.7 to 52.3). Tissues were treated topically with 100 µl/tissue (EpiOcular) or 25 µl/tissue (ZK1200) of neat test material. Tissue viability was measured against time of exposure to determine the exposure time required to reduce viability to 50% of controls (ET_{50}). MTT reduction was used to determine tissue viability in both cases. In general, the EpiOcular construct proved to be more robust that the ZK1200 and gave ET_{50} values which better resolved the toxicity over the full range of irritancy potential. When the EpiOcular log ET_{50} values were plotted against the log MMAS values the r^2 value was 0.73. The slope and intercept of this regression were very similar to the prediction model (log ET_{50} vs. predicted MMAS) proposed by the MatTek Corporation.