

November 8–11, 1998

SYMPOSIUM 1

**Human Corneal Epithelial Cell Lines as *in vitro* Models
for Evaluating Eye Irritation**

*J. H. Kruczewski, T. L. Walker, S. L. Ward, and L. C. DiPasquale,
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Stratified cultures of transfected human corneal epithelial cell lines, HCE-T cells, have been grown on permeable membranes to create the "HCE-T Model." This system is a tissue equivalent of the corneal surface that will support the ocular toxicology testing of consumer products. The Model is maintained in a format where it is basally exposed to media and typically exposed to air. In this format, cells stratify into four to six cell layers, express tight junctions, produce an apical layer of mucin, and demonstrate a barrier property, all which are features similar to the corneal epithelium. The barrier function of the HCE-T Model can be demonstrated *in vitro* by its ability to retain sodium fluorescein and to maintain a significant electrical resistance. Transepithelial permeability to sodium fluorescein (TEP) has been used in this model as a physiologically relevant, standard assay to screen for the ocular irritancy potential of water-soluble substances. This protocol involves the generation of concentration-response data for test materials. Groups of 20 diverse chemicals and 25 surfactant-based product formulations have been tested in the HCE-T TEP assay, and *in vitro* data was compared to historical Draize rabbit eye irritation test maximum average scores (MAS). The Pearson's correlation coefficient, *r*, for these comparisons were 0.71 and 0.86 respectively. A separate protocol for evaluating the ocular irritancy potential of water-insoluble substances in the HCE-T Model is in development. The TEP assay is not applicable since these samples generally can't be diluted in aqueous diluents nor reliably washed from the culture surface. This alternate protocol involves the generation of time-response data for test materials. Toxicity endpoints that have been evaluated through assay of basal media were changes in the inflammatory mediator, prostaglandin E_2 , and the metabolite, lactate. Preliminary data from the analysis of four formulations indicated that only the lactate assay results corresponded sufficiently to the historical Draize MAS to suggest the possible utility of this endpoint. Further protocol research and database development will focus on providing a battery of useful assays to support the ocular toxicological

analysis of a wide array of chemicals and with varied physical forms.

**Strategic Use of the Stress Gene Heme Oxygenase-1
as an Index in Evaluating and Screening Drugs
and Chemicals for Ocular Formulations**

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Purpose. Predicting the toxic potential of compounds to the ocular surface has been dependent on the Draize test for the past half century. Alternatives to Draize testing have recently been sought. Stress gene expression has emerged as a means of quantifying cellular reaction and, thus, the toxic potential of the compound in question. This study examines the expression of the major stress response gene heme oxygenase-1 (HO-1) in a human transfected corneal epithelial cell (HCE-T) line following challenges with a number of known ocular irritants and evaluates the effectiveness of using the HO-1 promoter activation as a stress index.

Methods. Irritants tested included hydrogen peroxide, isopropyl alcohol, sodium hydroxide, Tween 20 trichloroacetic acid, triclosan, aluminum chloride and SDS. HCE-T cells were grown to 80% confluency and treated with the listed irritants at a concentration ranges of 10–100 μ M. Cell viability, Northern blot analysis and RT/PCR were performed following a 1–48 hrs incubation period. HCE-T cells were transfected with the HO-1 promoter/CAT or Luciferase constructs and CAT/Luciferase activity was measured 1–12 hrs after irritant treatment.

Results. Northern blot analysis demonstrated that levels of HO-1 mRNA transcripts increased regularly after exposure to the irritants in a concentration dependent manner. Quantitative RT/PCR confirmed the validity of the Northern analysis results and showed direct relationship to cell viability. Transient transfection of HO-1 promoter CAT/or Luciferase constructs into HCE-T demonstrated that these irritants transcriptionally activate HO-1 gene and the degree of activation correlated with the toxicity of the irritant.

Conclusions. These results are consistent with the idea that HO-1 mRNA levels may be used as an indicator of irritant toxicity and that HCE-T cells respond to stress in a fashion similar to other human cell lines. This strategy for testing will enable us to

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1. Understanding the eBook Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
 - The Rise of Digital Reading Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
 - Advantages of eBooks Over Traditional Books
2. Identifying Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
 - User-Friendly Interface
4. Exploring eBook Recommendations from Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
 - Personalized Recommendations
 - Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999 User Reviews and Ratings

- Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999 and Bestseller Lists
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 - Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999 Public Domain eBooks
 - Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999 eBook Subscription Services
 - Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999 Budget-Friendly Options
- 6. Navigating Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999 eBook Formats
 - ePub, PDF, MOBI, and More
 - Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999 Compatibility with Devices
 - Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999 Enhanced eBook Features
- 7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
 - Highlighting and Note-Taking Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
 - Interactive Elements Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
- 8. Staying Engaged with Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
- 9. Balancing eBooks and Physical Books Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
- 10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
- 11. Cultivating a Reading Routine Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
 - Setting Reading Goals Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
 - Carving Out Dedicated Reading Time
- 12. Sourcing Reliable Information of Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
 - Fact-Checking eBook Content of Annual Meeting Abstracts 1999 Asa Cba Bsa Abstracts 1999
 - Distinguishing Credible Sources

13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
14. Embracing eBook Trends
 - Integration of Multimedia Elements
 - Interactive and Gamified eBooks

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