



**Introduction**

Ocular irritation testing represents an important step in the safety evaluation of cosmetic products. Increasing concern regarding the ethics of animal testing have prompted the development and use of numerous *in vitro* systems to approximate the ocular irritation potential of cosmetics designed for human application. While these systems are capable of demonstrating potential tissue damage at a cellular level, human subjective responses to ocular cosmetic exposure, which may be more or less sensitive to particular irritants, cannot be accurately predicted from *in vitro* results. For the same reasons that the rabbit eye has been disputed as a model for the human eye, i.e. differences in corneal ultrastructure and in the sensitivity to eye irritants, cell lines cannot effectively simulate the response of ocular tissues composed of a range of cell types. Similarly, *ex vivo* models, which lack the tear film, do not provide a representative model of the intact eye. The ideal system in which to assess the ocular safety of cosmetics is the human eye, which is frequently disregarded due to issues of safety and compliance of the human subject. Since 1993, we have utilized ocular instillation of test cosmetics into the human eye as a method to assess irritancy. Our studies have demonstrated that this methodology is safe and that obstacles related to subject compliance are minimized by the design of the test. Human ocular instillation provides a means of distinguishing effects of test materials on individual ocular tissues. Using this testing methodology in conjunction with our grading system, which is a more definitive irritation scoring system than the standard Draize scale, we are capable of categorizing test material irritation levels. The Kanengiser grading system records human subjective responses in addition to objective ocular irritation, which is scored in terms of area and density of tissue loss. As a result of the comprehensive nature of the Kanengiser grading method, we have demonstrated that differences in sensitivity to cosmetics exist among the ocular tissues. The thirteen-point scoring scale, assessing the area of tissue loss in ocular tissues (cornea, palpebral and bulbar conjunctivae, and caruncle), is capable of describing a range of tissue damage, encompassing minor lesions as well as severe tissue abrasion. This scoring system yields greater sensitivity and specificity than the Draize scale. Human subjective irritation scores demonstrate greater correlation with scores for corneal irritation assessed by the Kanengiser method than by the Draize method. While we have considered the cornea a predictive tissue with respect to ocular safety because of its importance to visual acuity, the correlation of our objective corneal irritation scores with subjective reports of irritation provides further evidence of the significance of this tissue in predicting ocular irritancy.

**Purpose**

The objective of this study was to introduce a method of assessing ocular irritation in human subjects and to illustrate the significance of a new expanded grading system, designed by Kanengiser, for the precise evaluation of ocular lesions and quantitative assessment of ocular surface responses to cosmetic products (i.e. shampoos, soaps, sunscreens, eye area, and facial cosmetics).

**Methods**

A total of 19 subjects, consisting of 12 males and 7 females, ranging in age from 31 to 69 years old, were impaneled. A designated quantity of test material was administered to each eye. Ocular examinations were performed at scheduled intervals following product instillation.

**Procedure**

**Acute Instillation**  
 -Subjects were reclined in ophthalmological chair at a 60° angle.  
 -0.1 ml of test material was instilled in the inferior cul de sac while the lower eyelid was retracted downward.  
 -Ophthalmic examinations were performed at 30 seconds, 5 minutes, 15 minutes, 60 minutes, and 120 minutes post-instillation.

The ophthalmic evaluation included assessments of subjective reports of ocular symptoms, objective ophthalmic irritation, and ocular surface fluorescein staining. Subjective irritation was determined by ascertaining from the subject any experiences of ophthalmic irritation (i.e. stinging, burning, itching, dryness, and foreign body sensation) at the time of the specified examination. Subjects were examined for evidence of excessive lacrimation. Each subject's upper and lower eyelids, specifically the lid margins and meibomian gland orifices, were examined for evidence of redness, scaling, swelling, and/or excessive meibomian secretions. The palpebral and bulbar conjunctivae were examined and scored for redness, inflammation, follicular, and/or papillary reactions. The cornea was examined for evidence of any inflammation, neovascularization, edema, infiltrates, opacities, and/or epithelial defects. To assess fluorescein staining patterns, a Fluorets sterile ophthalmic strip [fluorescein sodium BP - Smith & Nephew] was inserted into the inferior cul de sac of each eye, after a small amount of Dacriose sterile irrigating solution had been dropped onto the Fluorets strip. The integrity of the palpebral and bulbar conjunctivae, corneal epithelium, and caruncle were then evaluated with a Haag-Streit Bern Model No. Z 2982A Slit Lamp Biomicroscope, and tear film break-up time was assessed. All data were analyzed with Student's t-test, ANOVA or correlation (r-value).

**Scoring scale**

**Subjective Ophthalmic Scoring Scale**

**Subjective Irritation** (stinging, burning, itching, dryness, and/or foreign body sensation)  
 0= None 1=Slight 2=Mild 3=Moderate 4=Severe

**Objective Ophthalmic Scoring Scale (Slit Lamp Biomicroscope Examination)**

**Lacrimation** **Tear Film Break-up Time**  
 0 = Normal tear production (no excess wetness) ≥10 = normal  
 1 = Trace increase in wetness  
 2 = Mild increase in wetness (no distinct formed tears)  
 3 = A few formed tears (contained within the cul de sac and on surface of globe)  
 4 = Intense tearing (leaving cul de sac and globe, wetting lids and face)

**Eyelid Irritation** (redness, scaling, swelling, and/or meibomian secretions)  
 0 = No evidence of inflammation  
 1 = Trace inflammation  
 2 = Mild inflammation  
 3 = Moderate inflammation  
 4 = Severe inflammation

**Palpebral Conjunctival Irritation**  
 0 = No evidence of inflammation  
 1 = Trace redness (very mild inflammation)  
 2 = Mild redness (mild inflammation)  
 3 = Moderate redness (moderate inflammation)  
 4 = Marked, intense redness (severe inflammation)

**Bulbar Conjunctival Irritation**  
 0 = No evidence of inflammation  
 1 = Trace redness (very mild inflammation)  
 2 = Mild redness (mild inflammation)  
 3 = Moderate redness, some dilation of blood vessels (moderate inflammation)  
 4 = Marked, intense redness several dilated blood vessels (severe inflammation)

**Corneal Abnormalities** (opacities, edema, infiltrates, vascularization, and/or epithelial defects)  
 0 = Normal, no abnormality  
 1 = Trace, very mild abnormality  
 2 = Mild abnormality  
 3 = Moderate abnormality  
 4 = Severe abnormality

**Palpebral and Bulbar Conjunctival, Caruncular, and Corneal Fluorescein Ophthalmic Staining Scale**

**AREA**

**No Ocular Irritancy**  
 0 = No staining

**Mild Ocular Irritancy Range**  
 1 = >0 and ≤ 10%  
 2 = >10% and ≤ 20%  
 3 = >20% and ≤ 30%

**Moderate Ocular Irritancy Range**  
 4 = >30% and ≤ 40%  
 5 = >40% and ≤ 50%  
 6 = >50% and ≤ 60%

**Severe Ocular Irritancy Range**  
 7 = >60% and ≤ 70%  
 8 = >70% and ≤ 80%  
 9 = >80% and ≤ 90%  
 10 = >90% and ≤ 100%  
 11 = Mild superficial tissue abrasion  
 12 = Moderate superficial tissue abrasion  
 13 = Severe deeper tissue abrasion

**DENSITY**

1 = Occasional, scattered punctate staining  
 2 = More uniform pattern of diffusely scattered punctate staining  
 3 = Dense foci of punctate staining within the areas of diffuse punctate staining  
 4 = General pattern of dense punctate staining

**Results**

**Incidence of Adverse Events in Human Ocular Instillation Tests**

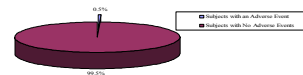


Figure 1: Incidence of Adverse Events in Human Ocular Instillation Tests

The frequency of occurrence of adverse events is minimal in the human ocular instillation studies that we have performed. Of 196 human subjects who participated in ocular instillation studies since 1998, only 1 subject experienced an adverse event. This event, which occurred prior to test material instillation, was unrelated to the test material or to the study procedures. Other evidence of the safety of this methodology is provided by the repeated willingness of subjects to enroll in these studies as well as the resolution of all observed ocular irritation during the course of the studies.

**Subjective Irritation**

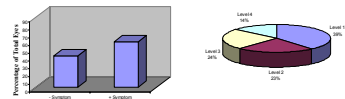


Figure 2: Subjective Irritation

Approximately 59% of the total 228 individual eye evaluations, performed post-instillation, demonstrated reports of subjective irritation, including stinging, burning, itching, dryness, and/or foreign body sensation. The distribution of subjective irritation levels was as follows: 39% reported level 1, 23% reported level 2, 24% reported level 3, and 14% reported level 4.

**Objective Ophthalmic Scores (Slit Lamp Microscope Examination)**

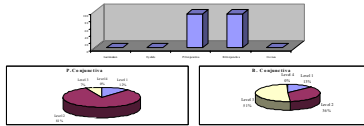


Figure 3: Objective Ophthalmic Scores (Slit Lamp Microscope Examination)

Approximately 94% of the total 228 individual eye evaluations revealed both palpebral and bulbar conjunctival irritation. No lacrimation, eyelid or corneal irritation was observed. The levels of palpebral conjunctival irritation were 12%, 81%, 7%, and 0% at levels 1, 2, 3, and 4, respectively. The levels of bulbar conjunctival irritation were 13%, 36%, 51%, and 0% at levels 1, 2, 3, and 4, respectively.

**Distribution of Fluorescein Staining**

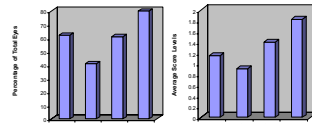


Figure 4: Distributions of Fluorescein Staining

The percentages of fluorescein staining, with both area and density scores, in the total 228 individual eye evaluations were 62%, 41%, 61%, and 80% on palpebral and bulbar conjunctiva, cornea, and caruncle, respectively. The distributions of the average score levels for palpebral and bulbar conjunctivae, cornea, and caruncle staining were 1.15, 0.91, 1.4, and 1.83, respectively. We have observed higher fluorescein staining of the caruncle than that of other ocular tissues. This is due to the effect of gravity, the tear fluid dynamics of the blinking eye, and the flow of tears through the nasolacrimal system, causing the instilled test material to be directed infranasally.

**Area Levels of Fluorescein Staining (Kanengiser vs. Draize)**

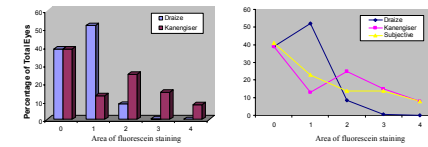


Figure 5: Area Levels of Fluorescein Staining on Cornea (Kanengiser vs. Draize)

The area of corneal fluorescein staining was judged on a 0 to +4 scale using the same terminology as for corneal cloudiness (Draize). Corneal fluorescein staining was concurrently assessed by Kanengiser's scoring scale. The percentages of fluorescein staining observed in the total 228 eye evaluations exhibited 39%, 52%, 8.5%, 0.5%, and 0% at level 0, 1, 2, 3, and 4 with Draize's scoring scales comparing to 39%, 13%, 25%, 15%, and 8% at level 0, 1, 2, 3, and 4 with Kanengiser's scoring scales. The statistical analysis of correlation between subjective irritation levels and area levels of corneal staining demonstrated better correlation for Kanengiser's scoring system (r = 0.82) than for Draize's scoring system (r = 0.74).

**Objective Ophthalmic Scores (Slit Lamp Biomicroscope Examination) and Fluorescein Staining Observed during Ocular Irritation**

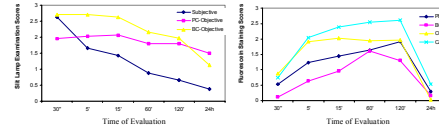


Figure 6: Objective Ophthalmic Scores (Slit Lamp Biomicroscope Examination) and Fluorescein Staining Observed during Ocular Irritation

Average subjective irritation scores decreased from level 2.63 at 30 seconds to level 1.66 at 5 minutes and to level 0.87 at 60 minutes with significance (p<0.05). Objective ophthalmic irritation was observed at score levels of approximately level 2 during the post-instillation time course. Fluorescein ophthalmic staining on ocular surfaces revealed that superficial punctate staining was increased to peak at 120 minutes on the palpebral conjunctiva and caruncle, at 60 minutes on the bulbar conjunctiva, and at 15 minutes on the cornea following ocular instillation. The significant changes (p<0.01) on all ocular surface tissues started at 5 minutes after ocular instillation. Most ocular irritation resolved after 24 hours, with the palpebral and bulbar conjunctival irritation observed at level 1.5 and 1.13, respectively. All data was analyzed by one-way analysis of variance followed by Tukey's test.

**Summary**

- Human ocular instillation is an effective and safe *in vivo* methodology for the assessment of cosmetic irritancy.
- Kanengiser's grading system assesses subjective human responses in addition to objective irritation and ocular surface tissue staining, which is scored on a scale with 13 area and 4 density classifications.
- Ocular tissues consisting of palpebral conjunctiva, bulbar conjunctiva, cornea, and caruncle respond to cosmetic exposure with different levels of irritation and tissue abrasion.
- When scoring methodologies are compared in assessing corneal irritation, Kanengiser's scoring system has a higher level of correlation with subjective irritation responses than Draize's scoring system.
- The cornea is a predictive tissue in the evaluation of ocular irritancy because of its importance to visual acuity. The relevance of this tissue in assessing test material safety is supported by the correlation of the objective and subjective scores of our grading system.
- Caruncular fluorescein staining scores during ocular instillation are higher than those of other tissues due to gravity, tear fluid dynamics of the blinking eye, and the flow of tears directing the instilled test material infranasally.

**Conclusion**

Human ocular instillation represents a reliable, predictable and reproducible ocular irritant testing methodology to assess the safety of many substances. Kanengiser's ocular grading system is an efficient assay method for determining the ocular irritant potential of cosmetic products in human eyes.