

Categorical evaluation of the ocular irritancy of cosmetic and consumer products by human ocular instillation procedures

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Synopsis

The assessment of ocular irritation potential is an important part of safety testing for cosmetic and consumer products. The purpose of this investigation was to examine ocular irritancy levels elicited in humans by various categories of a specific class of cosmetic and consumer products that have a potential to enter the eye inadvertently during use. Test materials assessed belonged to one of seven categories, which included liquid makeup, shampoo, baby wash, mascara, eye makeup remover, powder eye shadow, and facial cleanser. These test materials were evaluated by human ocular instillation, followed by examinations, for which subjective perceptions of irritation were recorded, and component areas of ocular tissues were individually examined for inflammation and for the area and density of fluorescein staining patterns at 30 seconds and at 5, 15, 60, and 120 minutes post-instillation. Subjective and objective ocular irritation scores of 410 eyes were analyzed by product classification. Average score levels were determined for subjective responses, inflammation, and fluorescein staining patterns. This investigation determined that irritation levels of the evaluated test materials varied markedly with respect to product category, type of ocular irritation, and ocular tissue, demonstrating that these factors are important considerations for the prediction of the ocular irritancy of a test material.

INTRODUCTION

Ocular irritation testing represents an important step in the safety evaluation of cosmetic and consumer products. Different types of cosmetic and consumer products may irritate ocular tissues at different levels, with various irritancy patterns. A number of studies have been made in which the ocular irritative effects of various compositions of cosmetic products *in vitro* and *in vivo* were compared (1,2). There, however, is a relative lack of studies in which human responses have been carefully compared among cosmetic or

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consumer products under comparable, controlled conditions. The purpose of this investigation was to examine ocular irritancy levels elicited in human subjects by various categories of a specific class of cosmetic and consumer products that have a potential to enter the eye inadvertently during use.

MATERIALS AND METHODS

SUBJECTS AND MATERIALS

The results from 205 human subjects (410 eyes) with normal, non-contact-lens-wearing eyes, ranging in age from 19 to 69 years, were reviewed from 12 ocular instillation studies. All subjects were instructed not to wear any eye makeup for their pre-test qualifying ophthalmic examination. Prior to enrollment, all inclusion/exclusion criteria were verified, an informed consent form was obtained, and a medical history was taken. The test materials assessed, which were provided by multiple clients, belonged to one of seven categories (Table I), which included liquid makeup (9.76%), shampoo (39.02%), baby wash (19.51%), eye makeup remover (14.63%), mascara (2.44%), powder eye shadow (9.76%), and facial cleanser (4.88%).

PROCEDURE OF ACUTE INSTILLATION

Each subject was reclined in an automated ophthalmic chair (Isell/Diversatronics, Inc.) at a 60° angle. A 75–100- μ l or 30-mg dose of each test material was instilled into the inferior cul de sac, while the lower eyelid was retracted downward. Each test material was instilled in the right or left eye, in rapid sequence. Any excess tearing resulting from the instillation was gently blotted, with a separate tissue used for each eye. Ophthalmic examinations were performed at 30 seconds, 5 minutes, 15 minutes, 60 minutes, 120 minutes, and 24 hours post-instillation. During the course of the study, subjects remained in a controlled environment to preclude exposure to any external factors that could influence the evaluation of the test material (i.e., rubbing of eyes, smoke, bright lights, etc.).

OPHTHALMIC EXAMINATION

The ophthalmic evaluation included assessments of subjective reports of ocular symptoms, objective ophthalmic irritation, and ocular surface fluorescein staining. Subjective

Table I
Distribution of Product Types

Products	Preparation	Number of eyes	%
Liquid makeup	Neat	40	9.76
Shampoo	10%	160	39.02
Baby wash	Neat	80	19.51
Eye makeup remover	Neat	60	14.63
Mascara	Neat	10	2.44
Powder eye shadow	Neat	40	9.76
Facial cleanser	Neat	20	4.88
Total		410	100.00

irritation was determined by ascertaining from the subject any experiences of ophthalmic irritation (i.e., stinging, burning, itching, dryness, and/or 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, were examined for evidence of redness, scaling, swelling, and/or excessive secretions of the meibomian gland orifices. The palpebral and bulbar conjunctivae were examined and scored for redness, inflammation, and follicular and/or papillary reactions. The cornea was examined for evidence of any 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 Z 2982A slit lamp biomicroscope, and the tear film break-up time was assessed.

DATA ANALYSES

The grading scale, designed by Bruce E. Kanengiser (3), for all parameters was 0–4 (0 = normal, 1 = trace, 2 = mild, 3 = moderate, and 4 = severe), with the exception of fluorescein ophthalmic staining, in which a 14-point scale was employed to evaluate the integrity of the palpebral and bulbar conjunctivae, corneal epithelium, and caruncle (Table II). Additionally, tear film break-up time was assessed by determining the time that elapsed before the first hole (dry spot) appeared in the corneal fluorescein layer (≥ 10 seconds is normal). Microsoft Excel (2000) and SigmaStat/SigmaPlot Version 5 were used to compile and statistically analyze the data. Student's *t*-test and ANOVA and correlation (*r*-value) tests were performed. Statistical significance was declared for all

Table II
Palpebral and Bulbar Conjunctival, Caruncular, and Corneal Fluorescein Ophthalmic 14-Point Area Staining Scale (3)

Ocular irritancy	Level	Description (% staining in quadrant)
No ocular irritancy	0	No staining
Mild ocular irritancy range	1	>0 and $\leq 10\%$
	2	>10% and $\leq 20\%$
	3	>20% and $\geq 30\%$
Moderate ocular irritancy range	4	>30 and $\leq 40\%$
	5	>40% and $\leq 50\%$
	6	>50% and $\geq 60\%$
Severe ocular irritancy range	7	>60% and $\leq 70\%$
	8	>70% and $\leq 80\%$
	9	>80% and $\geq 90\%$
	10	>90% and $\geq 100\%$
	11	Mild superficial tissue abrasion
	12	Moderate superficial tissue abrasion
	13	Severe superficial tissue abrasion

Density grading scale: 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.

p-values less than or equal to 0.05 at the 95% confidence level. The intensity values were given as means \pm SEM.

RESULTS AND DISCUSSION

Ocular tissues consisting of palpebral conjunctiva, bulbar conjunctiva, cornea, and caruncle respond to cosmetic exposure with different severity, pattern, and/or onset of symptoms, with respect to inflammation, abnormalities, and/or observed tissue abrasion. Figure 1 depicts ocular irritation induced by instillation of a cosmetic or consumer product. Moderate inflammation of the conjunctivae at a level 3 (Figure 1A); punctate fluorescein staining patterns of the palpebral conjunctivae at an area level 3 with a density level 2 (Figure 1B); superficial punctate keratopathy at an area level 3 with a density level 3 (Figure 1C); and punctate staining of the caruncle at an area level 4 with a density level 3 (Figure 1D) were observed.

INCIDENCE OF UNEXPECTED ADVERSE EVENTS IN HUMAN OCULAR INSTILLATION TESTS

Studies on humans proceed only after a potentially suitable formulation has been identified as a result of irritation tests in animals and *in vitro* (4), minimizing the potential of unexpected adverse events during *in vivo* studies. The frequency of occurrence of adverse events was minimal in human ocular instillation studies. Of 205 human subjects who participated in ocular instillation studies from 1998 to 2003, only one subject

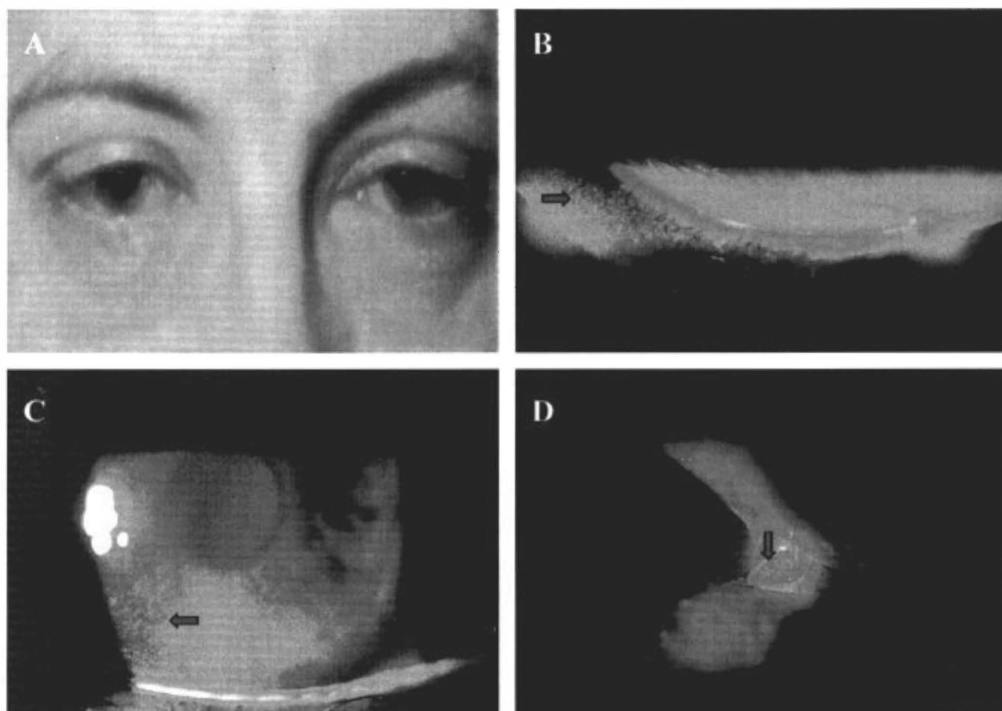


Figure 1. Representative illustrations of ocular irritation induced by a cosmetic product.

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

Average maximum score levels of subjective irritation (Figure 2), including stinging, burning, itching, dryness, and/or foreign body sensation, demonstrated that mascara and powder eye shadow exhibited mild to moderate irritation (2.8 ± 0.102 and 2.45 ± 0.182 , respectively), which was statistically higher ($p < 0.05$) than for the remaining product types, except for baby wash and eye makeup remover as compared to powder eye shadow. Liquid makeup with an average maximum score level of 0.5 ± 0.088 elicited significantly lower levels of reported subjective irritation than all other products ($p < 0.05$).

OBJECTIVE IRRITATION (SLIT LAMP BIOMICROSCOPE EXAMINATION)

Objective ophthalmic evaluation consisted of examination of lacrimation, eyelid inflammation, palpebral and bulbar conjunctival inflammation, and corneal abnormalities. No lacrimation, eyelid inflammation, or corneal abnormalities were observed for all product types. The average maximum score levels of combined palpebral and bulbar conjunctival inflammation scores (maximum combined score of 8) were significantly higher ($p < 0.05$) for shampoo and baby wash, with scores of 4.075 ± 0.126 and 4.1 ± 0.16 , respectively, than for liquid makeup, eye makeup remover, mascara, powder eye shadow, and facial

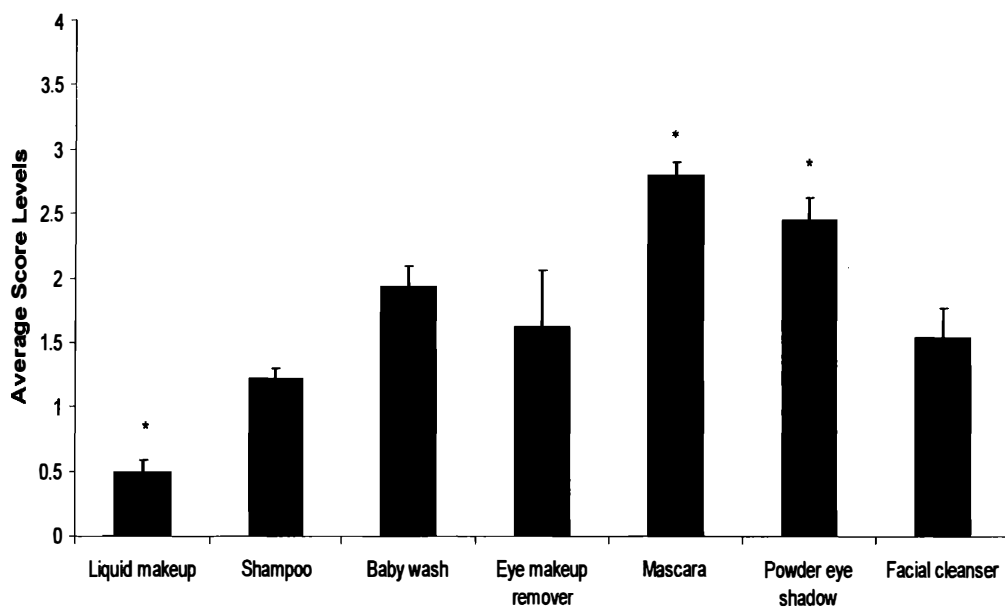


Figure 2. Average maximum levels of subjective irritation.

cleanser, with scores of 3.025 ± 0.12 , 2.783 ± 0.141 , 1.8 ± 0.25 , 3.4 ± 0.11 , and 2.34 ± 0.25 , respectively (Figure 3).

DISTRIBUTION OF FLUORESC EIN STAINING

The maximum area and density scores at each examining timepoint for the fluorescein staining patterns of each ocular tissue were multiplied and totaled for all examination intervals. Scores from each evaluated tissue were weighted, based on a modification of the Draize method (5,6) for assessing ocular irritancy potential, and totaled to determine an overall fluorescein staining score for all eyes by the following equation: Total weighted score = (palpebral + bulbar) \times 2 + cornea \times 5 + caruncle \times 1. The distributions of the average weighted score (Figure 4) for liquid makeup, shampoo, baby wash, eye makeup remover, mascara, powder eye shadow and facial cleanser were 11.4 ± 1.34 , 31.03 ± 1.52 , 39.19 ± 2.70 , 9.6 ± 0.91 , 39.92 ± 3.45 and 9.55 ± 1.98 , respectively. The scores for shampoo, baby wash, and powder eye shadow were statistically significantly higher than those of the others ($p < 0.05$).

POST-INSTILLATION EVALUATION INTERVALS

The Draize method and the modified Draize methods, such as the FHSA method, the OECD method, and the FIFRA/TSCA method (7,8), established the stipulated intervals for observing irritation, which were limited to 1, 24, 48, and 72 hours after administration of the test material in animals. Human eyes are highly sensitive to cosmetic products and respond frequently and quickly to test material exposure. Therefore ophthalmic evaluations were performed at the following time intervals: 30 seconds, 5 minutes, 15 minutes, 60 minutes, 120 minutes, and 24 hours post-instillation. Sub-

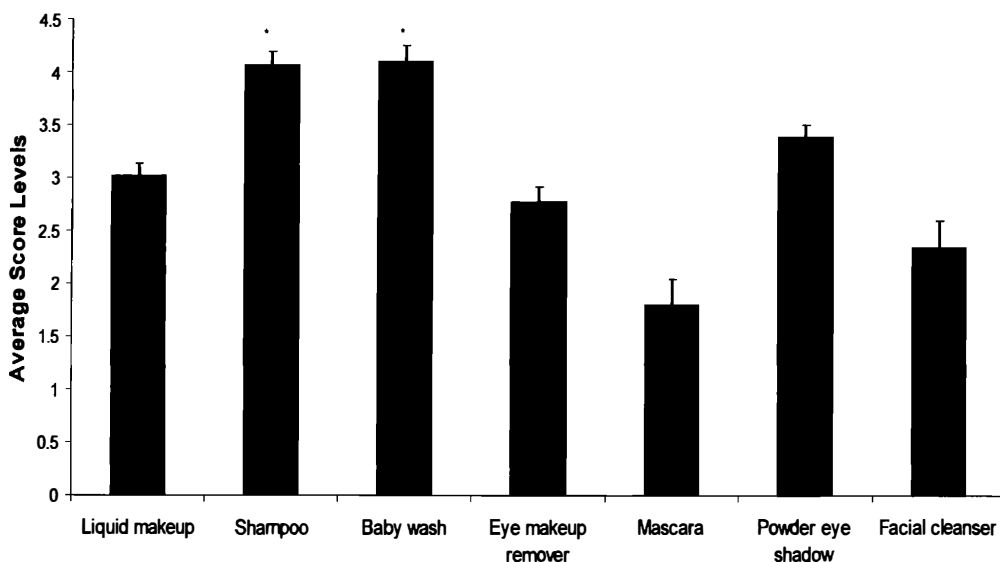


Figure 3. Average maximum levels of objective irritation.

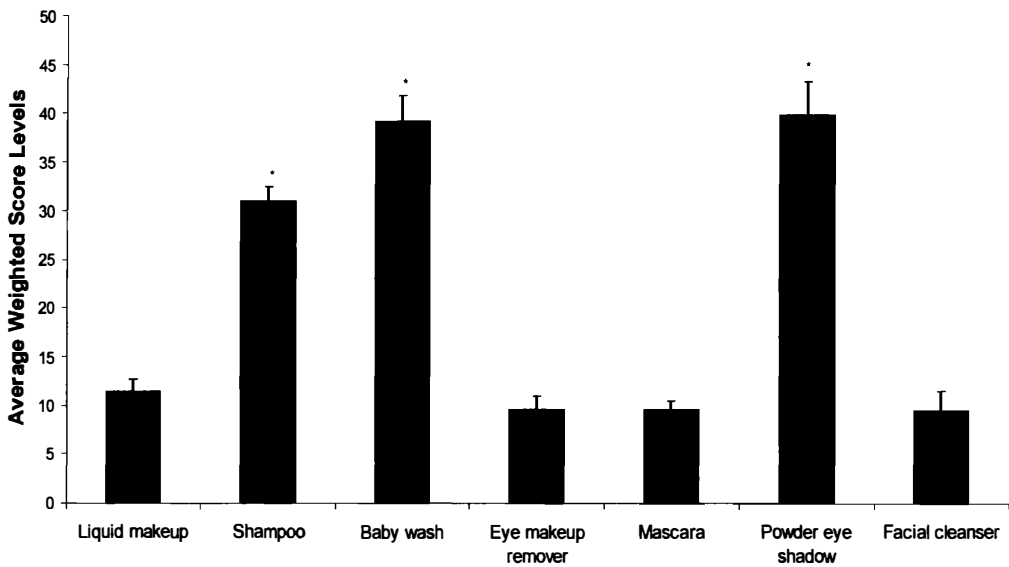


Figure 4. The distribution of weighted scores of area and density of fluorescein staining patterns.

jective reports of irritation and objective ophthalmic scores, as well as fluorescein staining patterns, were observed during the post-instillation examinations. The average subjective irritation scores for all product types peaked at 30 seconds post-instillation and decreased markedly at the 5 minute examination, with the exception of powder eye shadow, which decreased at 15 minutes post-instillation (Figure 5A). Objective ophthalmic irritation was observed in various patterns (Figure 5B). Objective irritations decreased at the 5 minute examination for liquid makeup, mascara, eye makeup remover, and powder eye shadow, and at the 60 minute examination for shampoo and baby wash. However, objective irritation for facial cleanser persisted at 120 minutes following ocular instillation. The average weighted scores (Figure 5C) of fluorescein ophthalmic staining of ocular tissues revealed that superficial punctate staining peaked at 15 minutes post-instillation for shampoo, baby wash, eye makeup remover, mascara, and powder eye shadow, and at 60 minutes post-instillation for liquid makeup and facial cleanser. Most ocular irritation resolved after 24 hours. The statistical analysis of weighted scores for fluorescein staining at post-instillation examination intervals demonstrated a very good correlation ($r > 0.95$) between facial cleanser, shampoo, or powder eye shadow and baby wash, and a fair correlation ($r < 0.75$) between powder eye shadow or facial cleanser and mascara (Table III).

CONCLUSIONS

Based on the comparison analysis of the limited data in each product category, this study has demonstrated that subjective irritation reports, objective ocular irritation, and fluorescein staining patterns attenuated rapidly during the two-hour study period for all categories except facial cleanser. Mascara and powder eye shadow elicited more subjective ocular discomfort; shampoo and baby wash exhibited higher objective ocular irritation scores; and shampoo, baby wash, and powder eye shadow elicited greater ocular tissue

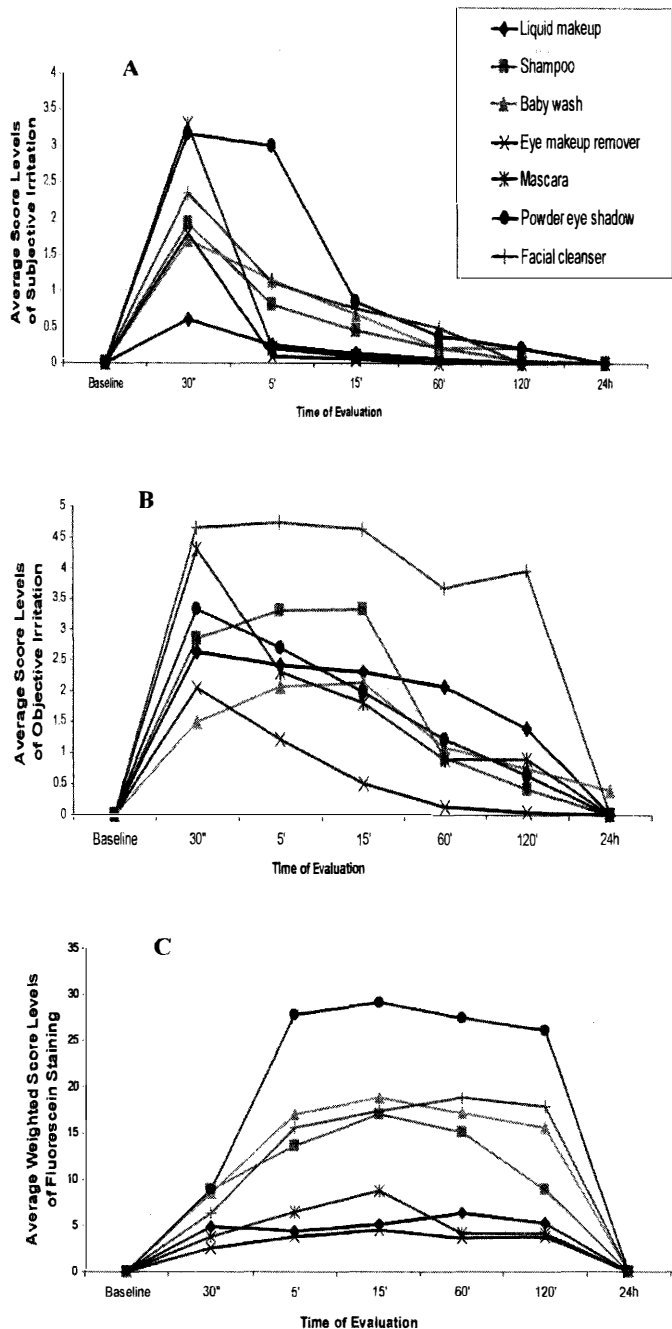


Figure 5. Average score levels of ocular irritation at post-instillation evaluation intervals.

staining than the remaining product types, in which mascara exhibited the lowest correlation between subjective response and objective irritation. Epithelial defects of the ocular surface were observed at later post-instillation evaluations, compared to the subjective responses and objective irritation, in which facial cleanser, shampoo, baby

Table III
Correlation Coefficient (r value) of Fluorescein Staining Between Categories

	Liquid makeup	Shampoo	Baby wash	Eye makeup remover	Mascara	Powder eye shadow	Facial cleanser
Liquid makeup	1.00						
Shampoo	0.92	1.00					
Baby wash	0.91	0.95	1.00				
Eye makeup remover	0.94	0.88	0.82	1.00			
Mascara	0.85	0.88	0.77	0.97	1.00		
Powder eye shadow	0.88	0.92	0.99	0.77	0.71	1.00	
Facial cleanser	0.92	0.95	0.98	0.79	0.71	0.98	1.00

wash, and powder eye shadow elicited the most similar fluorescein staining patterns at post-instillation intervals.

Human ocular instillation is an effective and safe *in vivo* methodology for the assessment of cosmetic irritancy. Ocular irritation elicited by the evaluated test materials varied markedly by product category, with respect to the type (subjective reports, inflammation, and fluorescein staining), duration, and ocular tissue involvement, demonstrating that these factors are important considerations for the prediction of the ocular irritancy of a test material.

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