Human Ocular Response to Instillation of Surfactant Solutions and Water Across 10,000 Subjects

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Summary

For the last three decades we have been dedicated to the development and use of in vitro alternatives to replace and reduce animal testing. The trans-epithelial permeability (TEP) assay has been validated internally as an alternative to the Draize rabbit eye test and has been used as part of our safety assessment program for 20 years. In parallel, we have compiled a large data set of human ocular testing with endpoints of eye stinging, bulbar conjunctive conjunctivitis, palpebral conjunctive conjunctivitis, and lacrimation. This data set includes ocular testing on 20,198 human eyes with 323,168 endpoints. This large data set allows the evaluation of variations in ocular response to formulations and to control solutions at different time points, as well as among subject populations. We observe changes in frequency of ocular conjunctivitis across the months of the year and see differences in the age dependence of ocular conjunctivitis between males and females. In addition, the neuronal eye sting response is not observed to correlate with eye conjunctivitis.

Keywords: ocular exposure, surfactant, conjunctivitis, in silico

1 Introduction

Accidental eye exposure to chemicals is a common cause of eye injury and necessitates the safety testing of many compounds. While animal alternatives testing regimes have advanced significantly, the Draize rabbit eye test developed in 1944 (Draize et al., 1944) is still widely used in many industries where validated test methods do not exist for specific ingredient classes or product forms or where international regulatory agencies do not accept in vitro data.

A modified Draize test, the low volume eye test (LVET) was developed (Griffith et al., 1980), and researchers have worked to correlate LVET results to the human ocular response (Freeberg et al., 1986; Roggeband et al., 2000) to prove its sufficiency for simulating human responses. However, the U.S. and other international regulatory agencies often still require Draize testing (ICCVAM, 2010).

The Draize test has significant limitations, however: ethical considerations limit the number of animals tested; the endpoints relate to visually observable tissue damage; and the generalizability of rabbit results to humans presents additional challenges. Furthermore, endpoints related to subject perception cannot be measured: unlike humans, rabbits do not self-report a sting response.

These limitations reinforce the desire in the field to replace animal testing with a combination of in vitro testing and in silico determinations. Much work has been done recently in developing new in vitro test methods and validating these new test methods to either Draize results or existing human ocular exposure data (Barile, 2010; McNamee et al., 2009).

The design of relevant in vitro tests or meaningful in silico models that can predict the human ocular response should be based on an in-depth understanding of the human ocular response. Recent work has capitalized on advancement of biomedical measurement techniques to observe the cellular and tissue response in order to develop a better mechanistic understanding of eye irritation (Jester et al., 2001). In vivo confocal microscopy has been used to visualize and categorize ocular irritation and the depth of injury (Jester et al., 1996, 1998).

For the past three decades Johnson and Johnson has established dedicated research programs focused on furthering the development and use of in vitro alternatives to replace and reduce animal testing across a variety of endpoints, including ocular irritation. In recent years, we have incorporated bio-informatics and knowledge management capability into these programs to further refine our safety assessment process. These systems have allowed further validation of in vitro test methods with clinical endpoints and have enabled the generation of additional mechanistic understanding of ingredient-specific responses. Ocular irritation has been a priority area for this research. Incorporating and accessing large clinical data sets into the alternative method validation process allows us to avoid limitations with Draize test data. In addition to the ethical considerations, there are objective limitations to correlating an in vitro method designed to predict a clinical response with an intermediate preclinical method that is essentially one step removed.

Here we present a very large, previously unpublished data set of human ocular exposure to a large library of relatively mild cosmetic formulations. This dataset includes 10,099 subjects, with 20,198 eyes on which four biological responses were mon-
itored over four time points, for a total of 323,168 endpoints. With this data set we can better understand the ocular response in humans across four unique responses.

This enhanced understanding of the human ocular response will assist our future development of an *in silico* model of the ingredient effects on the ocular response. Ingredient effects of surfactant solution will be modeled from this data set, and human clinical results are predicted.

### 2 Materials and methods

**Human ocular test**

Each study consisted of ten adult subjects aged 18 to 70 years. Subjects were prescreened by an ophthalmologist, and only subjects with baseline sting, lacrimation, bulbar, and palpebral irritation scores of 0 (within normal limits) were permitted to participate. The test and control (distilled water) solutions were maintained at 37-38°C before one drop of the appropriate material was instilled into the right eye followed by the other solution into the left eye. At time points of 30 sec, 15 min, and 60 min an ophthalmologist examined and graded the subjects’ eyes regarding bulbar conjunctiva, palpebral conjunctiva, and lacrimation. The following grading scales were used for bulbar conjunctiva and palpebral conjunctiva: 0 (within normal limits), 1 (mildly pink), 2 (moderately pink with some vessel dilation), 3 (intense red, dilated vessels), and for lacrimation: 0 (within normal limits), 1 (excessive wetness, no distinct tears), 2 (a few formed tears, contained in orbit), 3 (severe intense tearing). The ocular sting response was self-reported by the subject, with the scale of: 0 (within normal limits), 1 (mild, very slight), 2 (moderate), 3 (severe).

**Test articles**

All formulations tested were liquid formulations that were mostly surfactant-based cleansing systems consisting primarily of amphoteric surfactants, non-ionic surfactants, and often anionic surfactants. Additionally, nearly all formulas contained preservatives, cationic polymers, and many contained fragrance with a pH between 4.5 and 6.8. All the liquid surfactant formulas were designed to be mild (Walters et al., 2008) and were confirmed as such by *in vitro* testing: Transepithelial permeation is also known as the fluorescein dye leakage, and it assesses the aggressiveness of surfactant systems by quantifying the dye...
leakage across a monomer of epithelial cells after treatment with surfactant system (Cottin and Zanvit, 1997).

3 Results and discussion

Clinical grading and self-reported eye sting after ocular instillation of a test solution or DI water were compiled from 10,099 subjects. Scores for each parameter were averaged across the entire population to evaluate both frequency of observation and severity of the effect. As seen in Figure 1, the average scores across all endpoints measured were greater for the test solution compared to DI water. Palpebral and bulbar conjunctivitis scores were similar to each other and, on average, higher than scores for lacrimation and stinging. Conjunctivitis is very common at 30 sec for both the test and DI water ocular exposure, and it decreases with increasing time after the exposure. While lacrimation and conjunctivitis rapidly decline after 30 sec, the ocular sting response is unique in its persistence from 30 sec to 15 min. Lacrimation was observed to be infrequent for both the test eye and the control eye; it was negligible after the 30 sec time point.

Each individual subject is presented with a test solution in one eye and DI water in the other eye. Figure 2 shows the ocular responses on a per subject basis to the test article and the DI water at the 30 sec and 15 min time points; any given subject can have a response in the water eye only, test eye only, both eyes, or neither eye. For all endpoints measured, a majority of subjects exhibited no reactions in either eye (Fig. 2, blue regions). As observed in Figure 1, subjects frequently display conjunctivitis with both DI water and the test solutions, whereas lacrimation is an uncommon response to both DI water and test solutions.

For sting at both time points, lacrimation at 30 sec and conjunctivitis at 15 min, among the test results where a reaction was observed, the reaction was observed most commonly in the test eye alone. However, for both conjunctivitis endpoints at 30 sec, reactions in both the test eye and control eye were more common than reactions in the test eye alone, suggesting that the response is a general response to anything being placed into the eye. It is interesting to note that for both bulbar and palpebral conjunctivitis, the number of test eye only reactions is greater at 15 min than it is at 30 sec, even though the total number of test eye reactions is greater at 30 sec.

The DI water control eye frequently exhibited conjunctivitis at 30 sec, and this was further investigated with respect to the seasonality of the ocular exposure. The seasonality of eye redness is well studied, and it is caused primarily by seasonal allergies with a peak in June and July (Wolffsohn et al., 2011; Singh et al., 2010). Figure 3 shows the seasonality of the conjunctivitis response where the abscissa marks the months, with the width of each bar proportional to the number of subjects tested in that month. While the conjunctivitis score range is 0, 1, 2, 3, nearly all responses are either 0 or 1, so each bar consists of a red and blue bar, where the relative length of each bar is respectively proportional to the fraction of 0 (within normal limits) and 1 (mildly pink).

A two-tailed t-test assuming equal variance was performed on the responses in each month compared to the overall population.

![Fig. 2: Within subject global correlation between water and test](image-url)

The number of subjects reporting reactions in their water eye, test eye, both eyes, or neither eye at time points 30 sec and 15 min for Bulbar Conjunctivitis (Bulb), Palpebral Conjunctivitis (Palp), Eye Sting (Sting), and Lacrimation (Lacr).
As can be seen in Figure 3A, in the test eye, the months displaying a significantly ($\alpha = 0.05$) higher fraction of conjunctivitis responses are April ($p < 0.05$), June ($p < 0.001$), and October ($p < 0.001$), while significantly lower responses are seen in May ($p < 0.001$) and July ($p < 0.05$), exhibiting lower response fractions. In the DI water eye, Figure 3B, the months displaying significantly higher conjunctivitis response compared to the total subject set are October ($p < 0.01$), November ($p < 0.05$), and December ($p < 0.01$), while significantly lower conjunctivitis responses are observed in May ($p < 0.001$). A clear seasonality trend is not observed, and certainly not one that corresponds to allergy season. Perhaps in the water eye slightly increased conjunctivitis is observed during the winter months (October to February). The other endpoints were evaluated for seasonal effects, but similarly inconclusive results were found. Subjects are prescreened, and subjects suffering conjunctivitis from allergies, or any other cause, would not be selected for study.

To evaluate potential effects due to population variability over test panels, the effects of age and gender on the four endpoints (bulbar and palpebral conjunctivitis, eye sting, and lacrimation) are shown in Figure 4 at the 30 sec and 15 min time points. In Figure 4, the width of the columns represents the relative number of subjects within that age/gender grouping. With this large data set, even the smaller columns represent 100-300 subjects and the larger columns represent ~2000 subjects.

The fraction of subjects that exhibit ocular sting is not observed to be a function of age or gender; there is no age or gender effect observed for the sting at either 30 sec or 15 min. Lacrimation at 30 sec in both females and males tends to decrease with age. Lacrimation at 15 min is infrequent for all populations.

The bulbar and palpebral conjunctivitis responses at both 30 sec and 15 min time points have similar trends between the demographic groups. For females, both bulbar and palpebral conjunctivitis response at 30 sec does not display a strong effect of age; at 15 min, however, the conjunctivitis response increases with female age. A different response is observed among men. Interestingly, both the bulbar and palpebral conjunctivitis response at 30 sec does display an age effect for men, with men age 25-45 displaying significantly more conjunctivitis. In contrast to women, an age effect is not observed among men. Across most age groups and time points, men generally exhibit more conjunctivitis than women.

4 Conclusions

While surfactant interaction with skin is generally well understood (Saad et al., 2011), the interaction with the eye has not been as well studied, due to various limitations. Many factors complicate the development of an in silico model of the human ocular response to chemical exposure. A strong understanding of the human response with respect to a variety of endpoints is necessary if we are eventually to replace the Draize test and human ocular testing. Perhaps not surprisingly, the human ocular response to exposure is complicated, and it is found to be a function of time after exposure, gender, and age, with interac-

![Fig. 3: Seasonality of human ocular response](image)

The seasonal effect on bulbar eye conjunctivitis was investigated by showing the subject data distribution (of 1 sec and 0 sec) for conjunctivitis at 30 sec for the test eye.


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