General Behaviors of Surfactants in the Bovine Corneal Opacity and Permeability (BCOP) Assay

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Introduction

Surfactants, or surface-active agents, are compounds used to decrease surface tension of a liquid, with wide ranging applications from household and personal care products to industrial and agrochemical use. Surfactants exhibit a wide range of responses based on concentration, consumer or industrial use, chemical structure, charge, etc. Surfactants can be classified based on the charge found on the head group of the molecule, and are generally divided into positively charged cationic, negatively charged anionic, and uncharged nonionic groups.

Here we present the observed responses of surfactants for each of these charge groups in the Bovine Corneal Opacity and Permeability (BCOP) assay with respective histopathological evaluations to provide context and insight into addressing ocular irritation with *in vitro* and *ex vivo* methodologies. Using methods established in OECD TG 437, as well as the Guidelines for Histopathological Evaluation of the BCOP Assay (2016), we present a quantitative and mechanistic understanding of the ocular irritation caused by each surfactant group. The BCOP assay utilizes a full thickness corneal model to evaluate ocular irritation which allows for preservation of treated corneas for further histopathological evaluation of the epithelial, stromal, and endothelial layers of the cornea.

Materials & Methods

The BCOP assay was performed as outlined in TG 437, and as presented in **Figure 1** below. Three corneas were used per treatment group, including the negative control (deionized water) and positive control (ethanol). The change in opacity was determined by subtracting the final opacity value (*i.e.*, opacity value after treatment followed by 2-hour post-exposure incubation) from the initial opacity value (*i.e.*, baseline opacity prior to treatment). The permeability changes were quantified at an optical density (OD) of 490 nm. Both the opacity and permeability scores were adjusted by the changes in the opacity and permeability for the negative control (NC) treated corneas. The *In Vitro* Irritation Score (IVIS) for each treatment group was calculated by adding the mean opacity score to 15X the mean permeability score (**Figures 2-13**). The histopathology was performed and evaluated under the guidance of the Histopathology Guidelines (2016) (**Figures 14-17**).

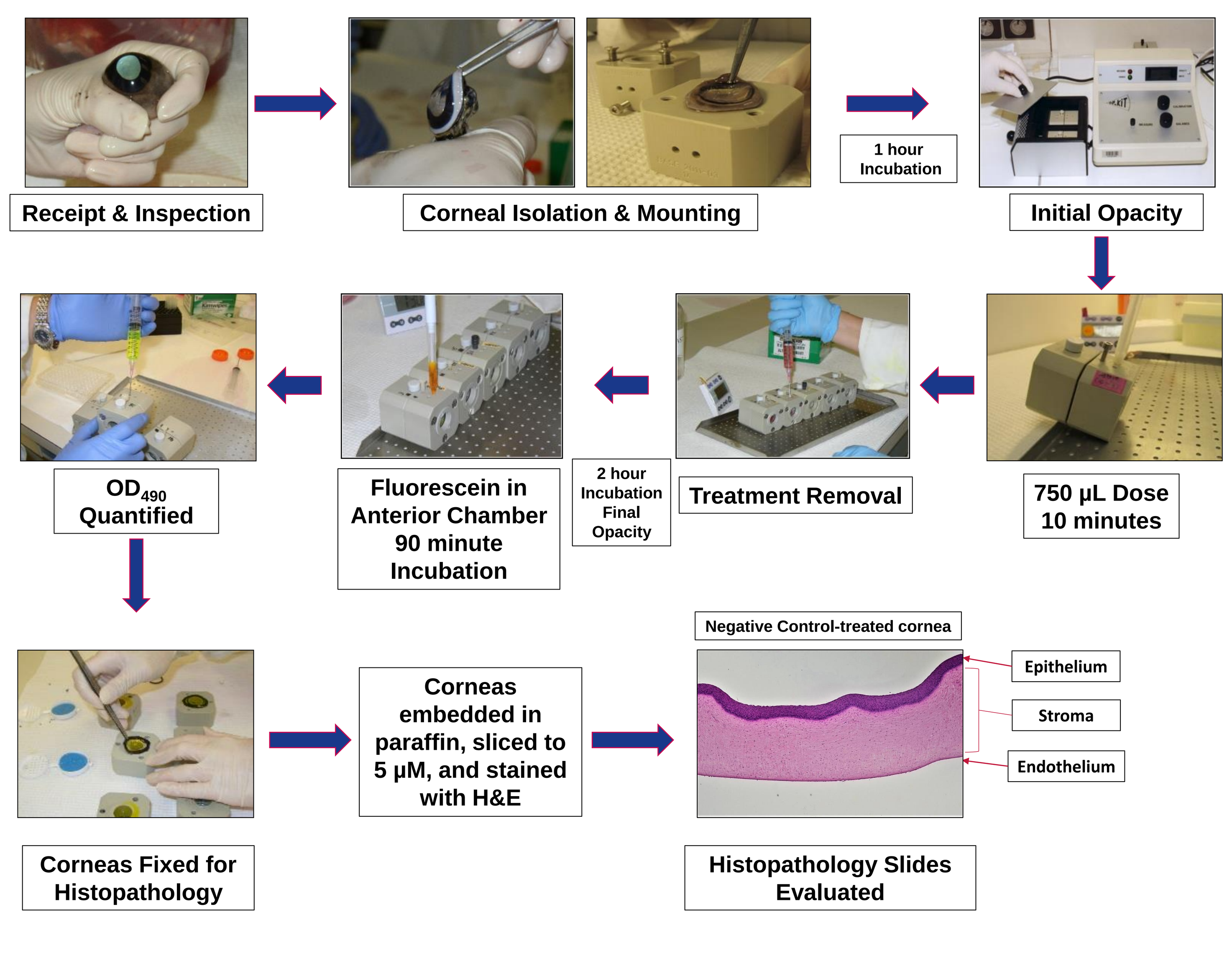


Figure 1. Critical Steps of BCOP Assay

Surfactant Type	General Data Observations in BCOP	General Histopathology Observations
Cationic (positive charge)	Higher opacity values and lower permeability values (*e.g.*, IVIS is weighted most by opacity)	Coagulation of epithelial cell proteins noted, supporting the higher opacity values and lower permeability values due to limited flow of fluorescein through the cornea
Anionic (negative charge)	Lower opacity values and higher permeability values (*e.g.*, IVIS is weighted most by permeability)	Epithelial cell layer erosion and/or detachment, supporting the lower opacity values (*e.g.*, less layers for light to pass through), and leading to higher permeability values due to compromise of corneal barrier properties
Non-ionic (no charge)	Most tested non-ionics had low ocular irritation potential. Triton™-X-100 and IGEPAL CA-630 were more irritating, and showed higher opacity and permeability scores	In general, non-ionics with minimal, if any, irritation, appeared similar to the negative control-treated corneas. However, more irritating non-ionics (*e.g.*, Triton™-X-100 and IGEPAL CA-630) showed epithelial layer erosion or detachment (*e.g.*, lower opacity changes) and compromise of barrier properties (*e.g.*, higher permeability changes)

Table 1. General Observations by Surfactant Type

Results

= Average Permeability Value (OD_{490} x 15) = Average Change in Opacity

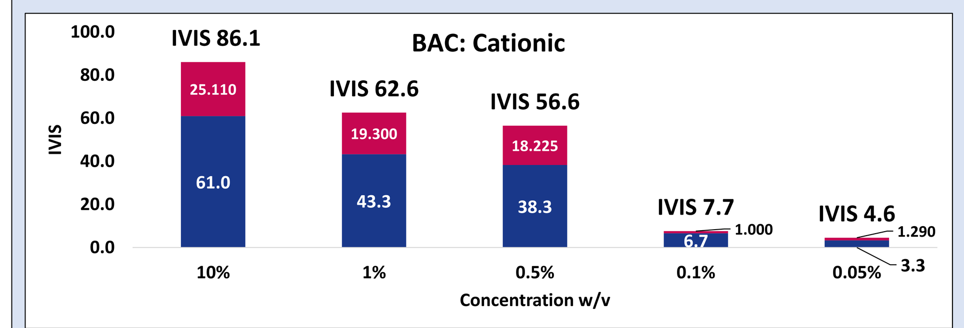


Figure 2. Weighted IVIS of Benzalkonium Chloride (BAC) tested at 10%, 1%, 0.5%, 0.1%, and 0.05%

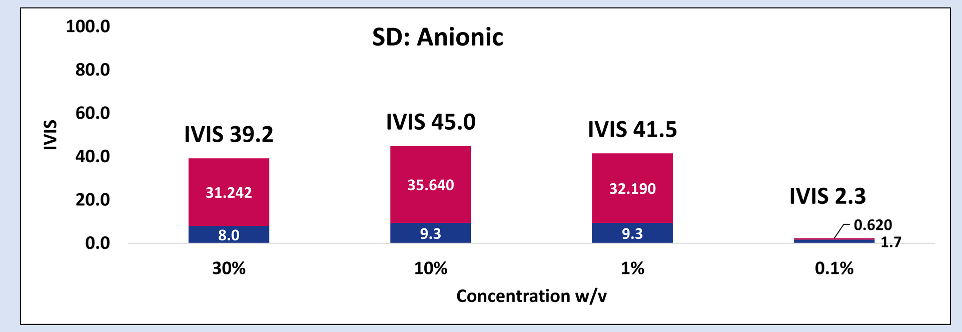


Figure 3. Weighted IVIS of Sodium Deoxycholate (SD) tested at 30%, 10%, 1%, and 0.1%

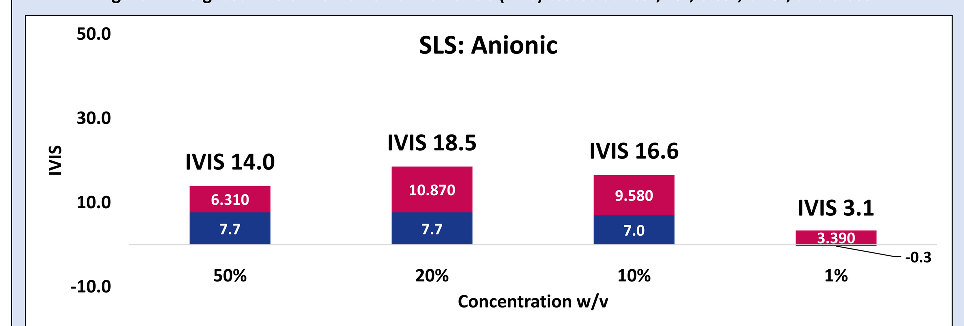


Figure 4. Weighted IVIS of Sodium Laureth Sulfate (SLS) tested at 50%, 20%, 10% and 1%

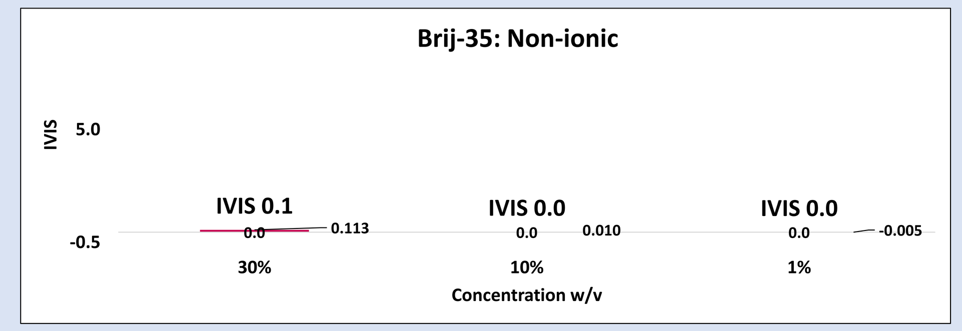


Figure 5. Weighted IVIS of Brij-35 tested at 30%, 10%, and 1%

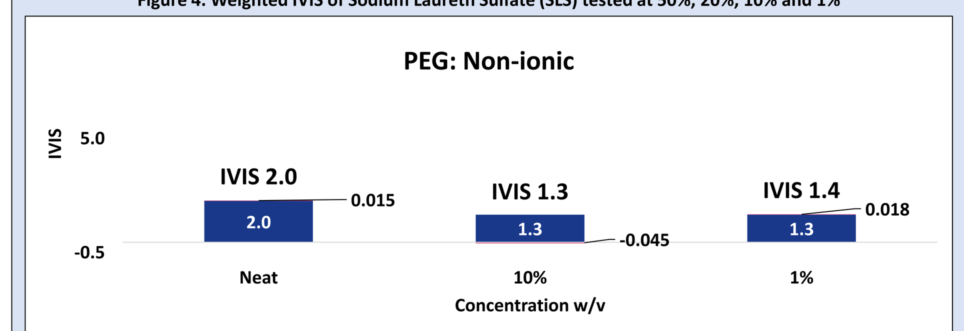


Figure 6. Weighted IVIS of Polyethylene Glycol (PEG) tested Neat, 10%, and 1%

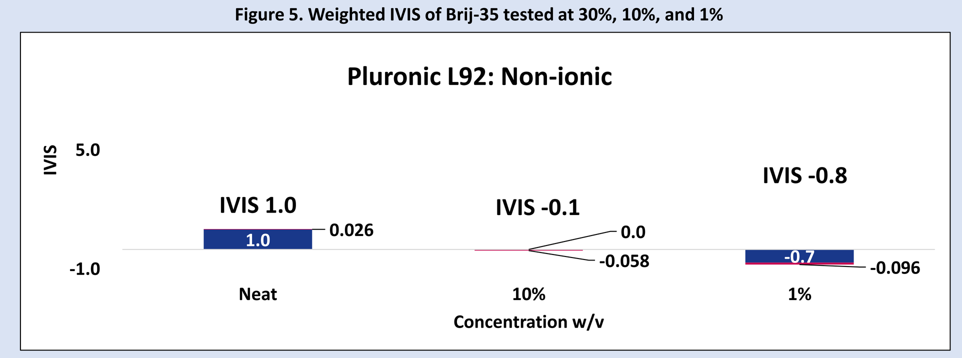


Figure 7. Weighted IVIS of Pluronic L92 tested Neat, 10%, and 1%

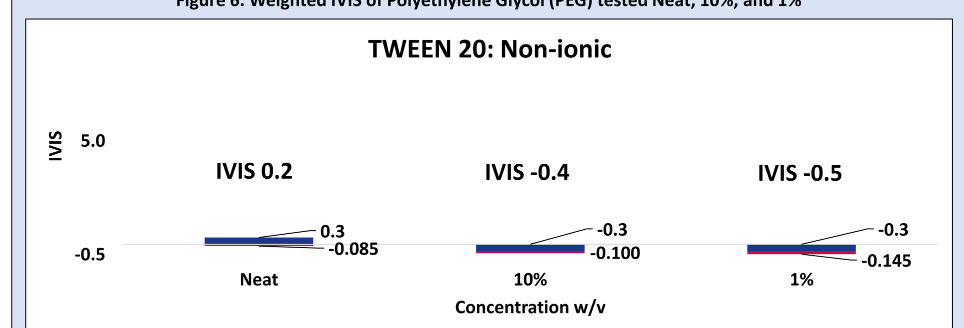


Figure 8. Weighted IVIS of TWEEN 20 tested Neat, 10%, and 1%

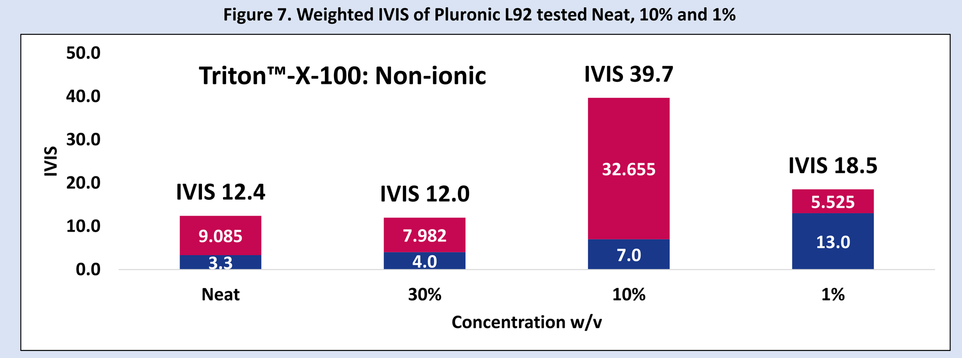


Figure 9. Weighted IVIS of Triton™-X-100 tested Neat, 30%, 10%, and 1%

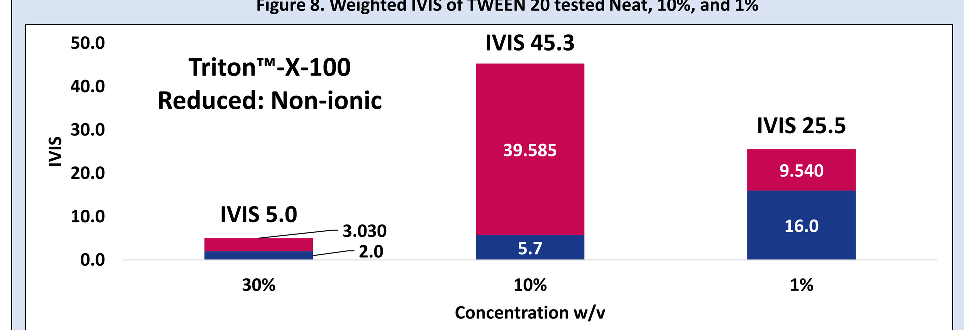


Figure 10. Weighted IVIS of Triton Reduced tested at 30%, 10% and 1%

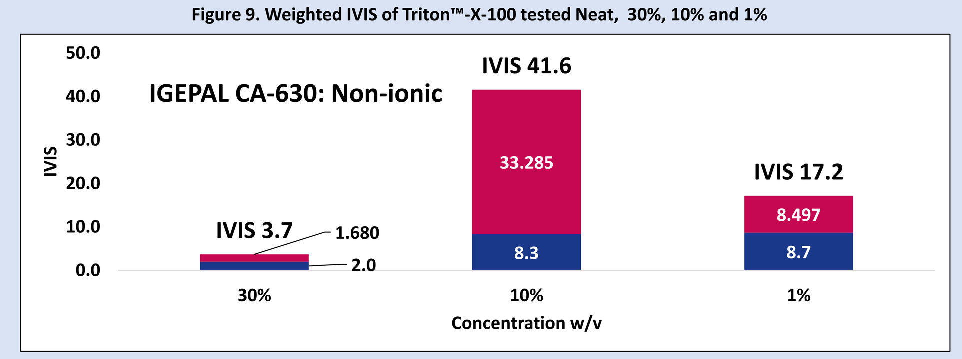
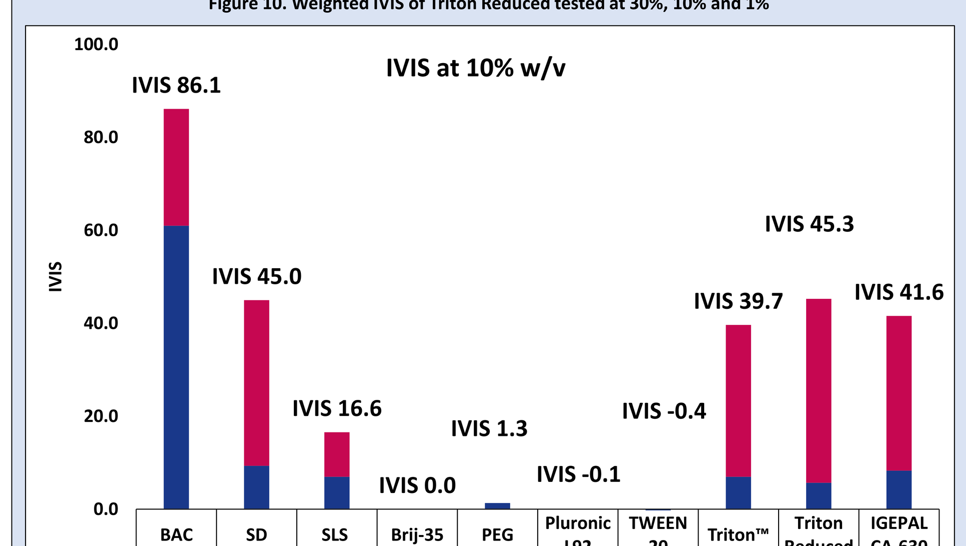
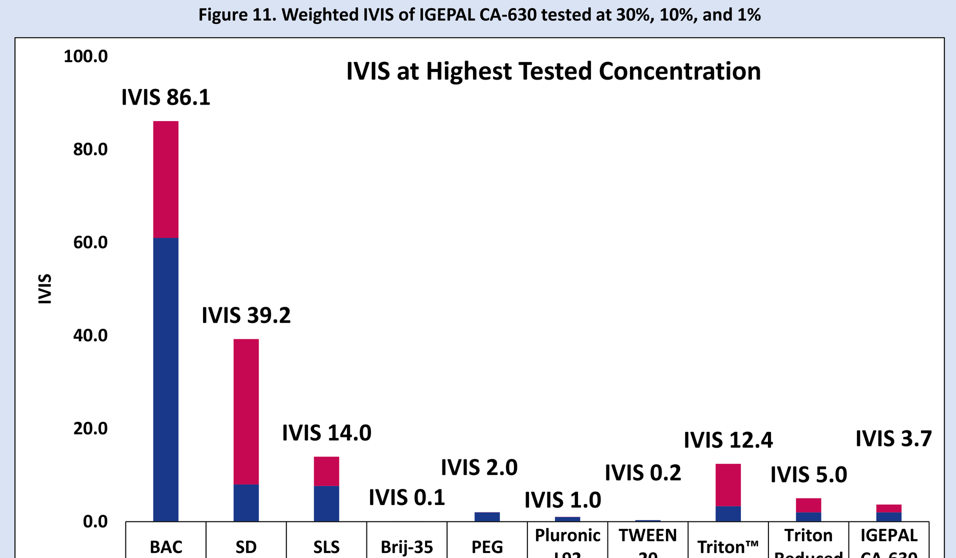


Figure 11. Weighted IVIS of IGEPAL CA-630 tested at 30%, 10% and 1%



	BAC	SD	SLS	Brij-35	PEG	Pluronic L92	TWEEN 20	Triton™	Triton Reduced	IGEPAL CA-630
OD490	25.110	35.640	9.580	0.010	-0.045	-0.058	-0.100	32.655	39.585	33.285
Opacity	61.0	9.3	7.0	0.0	1.3	0.0	-0.3	7.0	5.7	8.3
IVIS	86.1	45.0	16.6	0.0	1.3	-0.1	-0.4	39.7	45.3	41.6

Figure 12. Weighted IVIS of All Surfactants Tested at 10% w/v



	BAC	SD	SLS	Brij-35	PEG	Pluronic L92	TWEEN 20	Triton™	Triton Reduced	IGEPAL CA-630
OD490	25.110	31.242	6.310	0.113	0.015	0.026	-0.085	9.085	3.030	1.680
Opacity	61.0	8.0	7.7	0.0	2.0	1.0	0.3	3.3	2.0	2.0
IVIS	86.1	39.2	14.0	0.1	2.0	1.0	0.2	12.4	5.0	3.7

Figure 13. Weighted IVIS of All Surfactants at Highest Tested Concentration

Conclusions & Future Directions

This data supports the central tenet that surfactants exhibit a range of responses, and illustrates how different surfactant types have different molecular initiating events resulting in different modes of irritation in the eye. As expected, the IVIS was driven by opacity in cationic surfactants and permeability in anionic surfactants. Non-ionic surfactants generally were least irritating, with some exceptions. In some cases, such as Triton™-X-100, a more conservative prediction was obtained when tested at 10%, rather than higher tested concentrations, as shown in **Figures 12-13**. Histopathology analysis provides additional data and may elucidate damage not picked up in the assay alone. Similarly, opacity and permeability changes should be independently reviewed and considered in addition to IVIS scores. Future investigations on the structural differences between mild non-ionic surfactants like PEG and irritating non-ionic surfactants like Triton™-X-100 may provide additional insights. Our findings provide a reference for industry or research in formulation development, highlight structural or chemical-based mechanisms for ocular irritation, and demonstrate surfactant behavior.

Histopathology Results

Figure 14. Representative image of Negative Control-treated cornea with intact cellular layers.

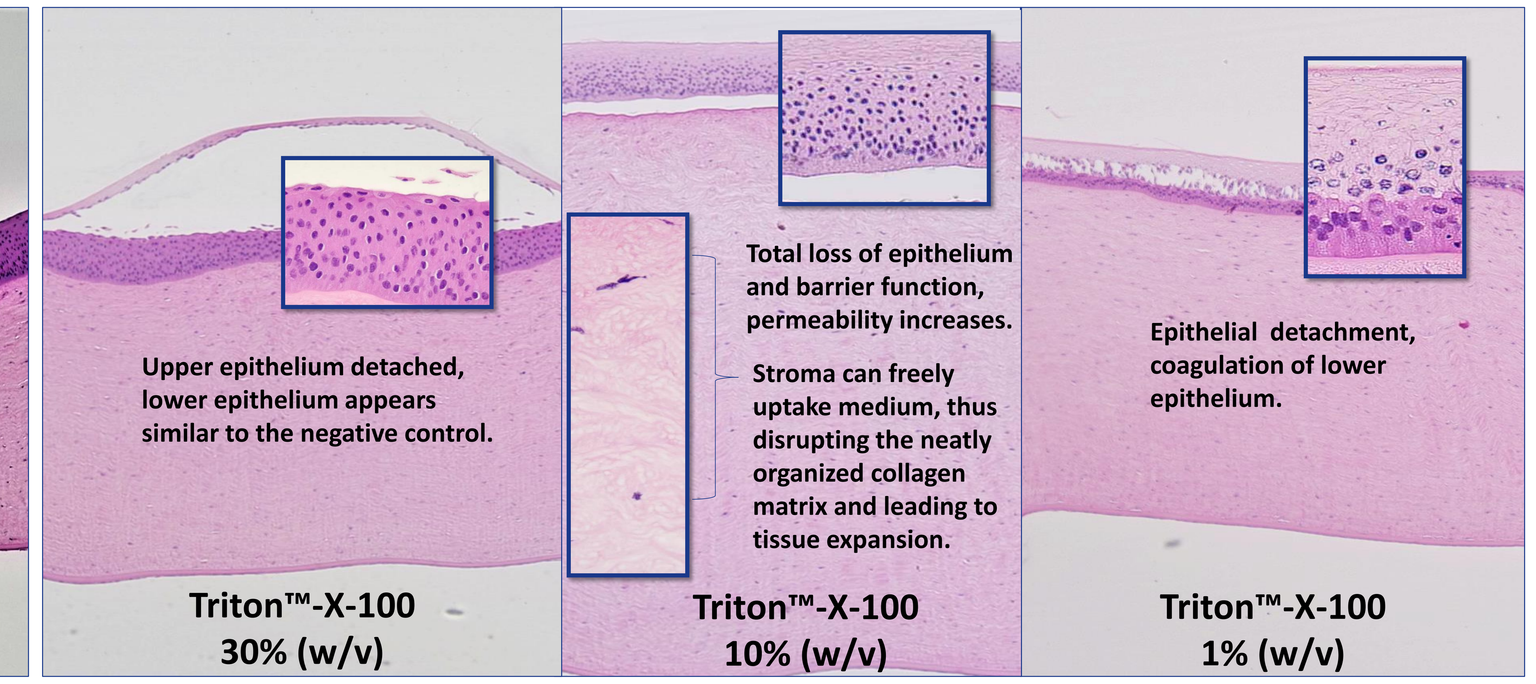


Figure 15. Representative images of Triton™-X-100, a non-ionic surfactant, at the 30%, 10%, and 1% concentrations. These responses indicate a maximum ocular irritation at 10% (w/v), potentially due to optimal micelle formation.

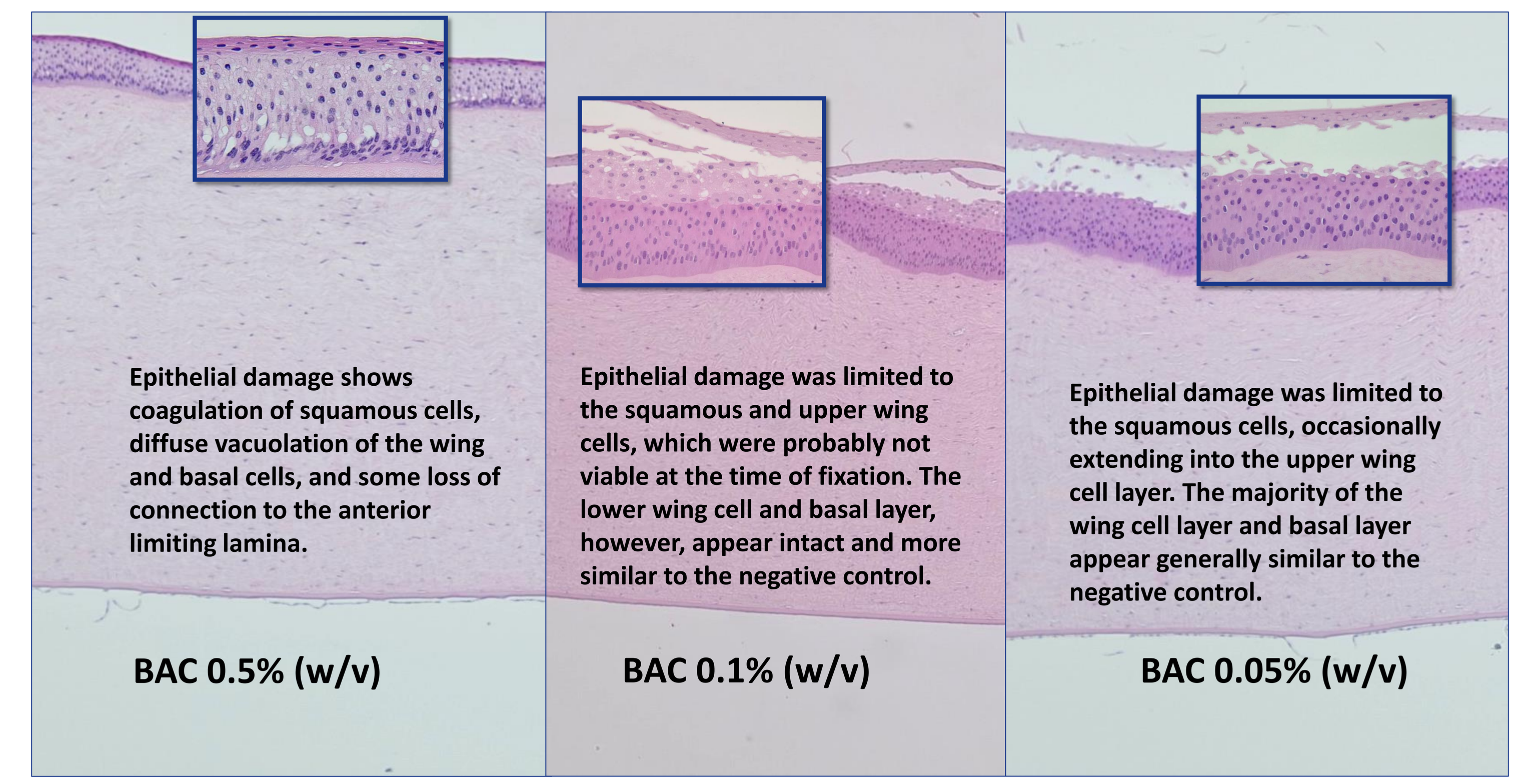


Figure 16. Representative images of Benzalkonium Chloride (BAC), a cationic surfactant, at the 0.5%, 0.1%, and 0.05% concentrations. These concentrations were chosen as a model for BAC concentrations used in final formulations of personal care products.

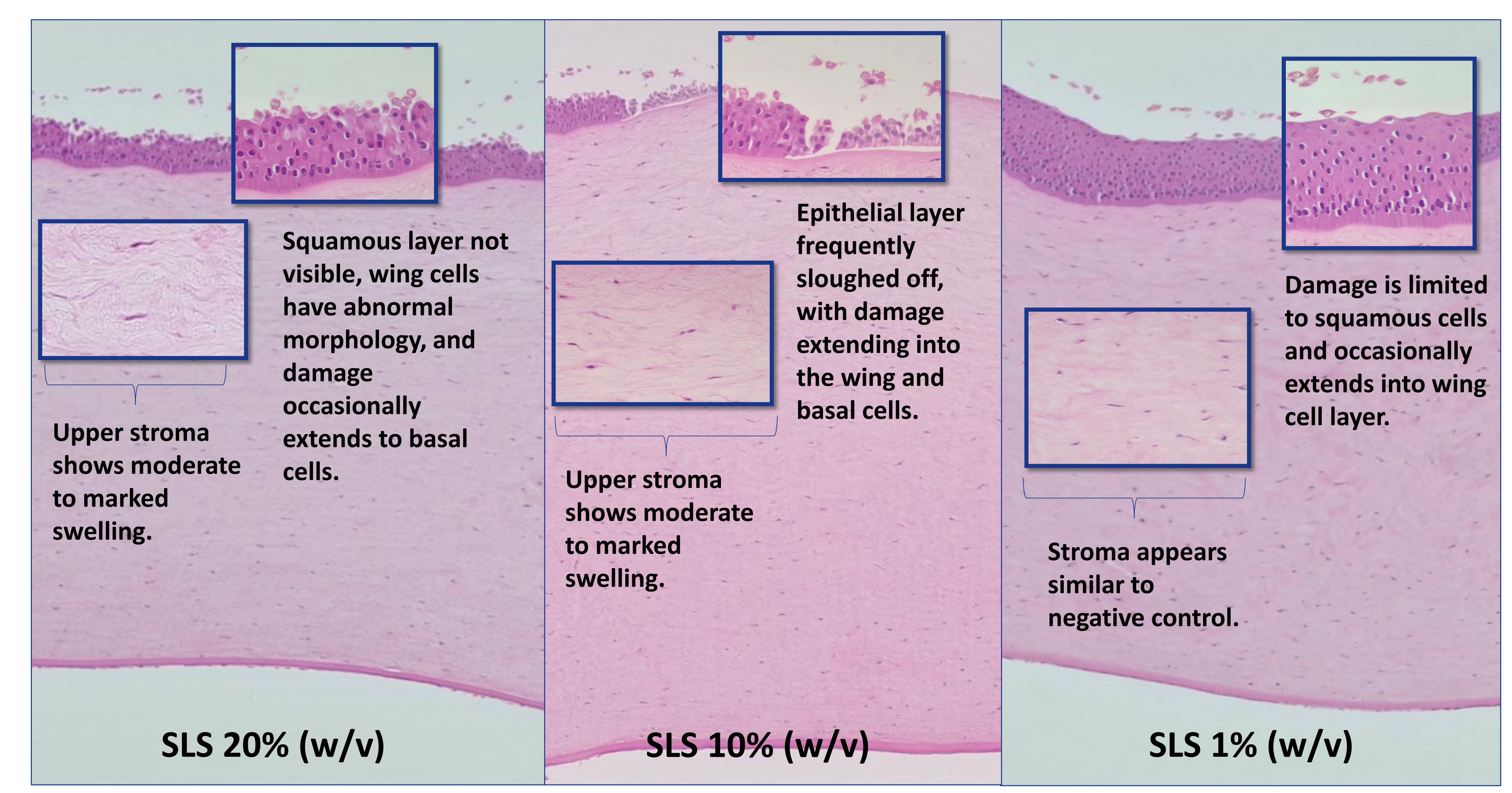


Figure 17. Representative images of Sodium Laureth Sulfate (SLS), an anionic surfactant, at the 20%, 10% (highest IVIS), and 1% concentrations.

References

OECD (2020), *Test No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264203846-en.

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Acknowledgements

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