Evaluating the Safety and Cytotoxic Effects of Make-up Removers in a Human Corneal Epithelial Cell Live/Dead Cell Model

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Executive Summary

This academic poster presents evidence that preservative-free makeup removers demonstrate superior safety profiles compared to those containing common preservatives like benzalkonium chloride (BAK), polyhexamethylene biguanide (PHMB), and alexidine hydrochloride (Table 1), when tested on human corneal epithelial cells. The findings have important implications for patients with dry eye disease and compromised ocular surfaces.

Background:

- Patients with dry eye and compromised ocular surface often use a variety of cosmetic products that can impact the ocular surface and periocular skin areas.[1-4]
- Cosmetic preservatives at approved concentrations can induce cellular atrophy and death within hours of exposure[5]
- These products can remain on the skin around the eye for hours throughout the day and are often used chronically throughout a person's lifetime.
- Preservatives such as those belonging to the biguanide class and benzalkonium chloride are often avoided in artificial tears and in glaucoma products.[6-8]
- Preservatives in cosmetics are rarely considered a problem for eyes, as they are not expected to come into contact with the ocular surface, though studies indicate they can.[9,10]

TABLE 1: Safety Concerns of Common Cosmetic Preservatives

	Benzalkonium Chloride (BAK)[11]	Polyhexamethylene Biguanide (PHMB)[12]	Alexidine Hydrochloride[13]
DOSAGE	• Safe for use as a cosmetic ingredient at concentrations up to 0.1% as a free active ingredient	• EU assessment (2015): Not safe at 0.2% • Revised Assessment (2017): Safe up to 0.2% with restrictions	• Not regulated in the EU for use in cosmetics
SAFETY CONCERNS	• Toxic to meibomian gland, corneal and conjunctival epithelial cells12 • Destabilizes tear film lipid layer • Increased evaporation & dry eye disease • Dose-dependent toxicity: cell death at ≥0.25 mg/ml within 24 h • Clinical manifestations: irritation, burning, stinging, unstable tear film, meibomian gland dysfunction	• Significant corneal toxicity including epithelial erosion and neovascularization	• Causes serious eye irritation (H319 classification) • Causes skin irritation (H315 classification)
RESTRICTIONS / CONSTRAINTS	• Max 0.1% in cosmetics	• Max 0.1% in cosmetics • Not recommended for sprays (inhalation risk) • Requires quantitative risk assessment	• Requires protective handling during manufacturing • Limited long-term cosmetic safety data

Aim:

The purpose of this study was to evaluate the impact of commercial eye makeup removers with and without preservatives using a live/dead cell assay in vitro model.

Study Design and Methodology

This study assessed the toxicity of four commercial makeup remover formulations on immortalized HCECs using live/dead staining and alamarBlue™ metabolic assay.

TESTED FORMULATIONS

- Optase Life Sensitive Eye Makeup Remover (Preservative Free)*
- LUMIFY Eye Illuminations 3-in-1 Micellar Water Cleansing Water & Eye Makeup Remover (Alexidine Hydrochloride)
- Garnier Micellar Water Sensitive Skin (Polyhexamethylene biguanide (PHMB))
- Neutrogena Oil Free Eye Make Up Remover (Benzalkonium chloride (BAK))

EXPERIMENTAL CONDITIONS

- HCECs were treated with 30 µL of each sample for 10 minutes at 37 °C with 5% CO_2.
- Cell viability was assessed using fluorescence measurement at 540/590 nm (alamarBlue™ assay) and by fluorescence microscopy (LIVE/DEAD staining).

Results

The study revealed a clear hierarchy of cytotoxicity among the tested preservatives (Figure 1 and 2):

- Highest toxicity: Benzalkonium chloride (BAK) and PHMB caused complete the highest cell death in the live/dead assay, consistent with previous research showing BAK's severe impact on corneal epithelial cells at concentrations as low as 0.005%.
- Moderate toxicity: Alexidine hydrochloride showed intermediate effects, caused significant cell viability reduction ($p < 0.001$) but allowed some cell survival.
- No significant toxicity: The preservative-free formulation demonstrated safety equivalent to saline and hyaluronic acid controls ($p > 0.05$).

Except for OLMUR (pH 7.2), all preservative-containing MUR formulations showed cytotoxicity in HCECs

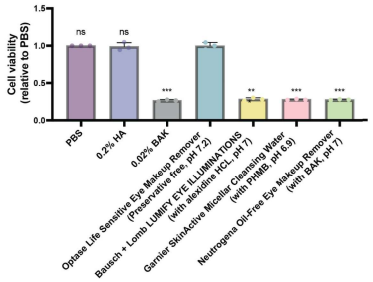

FIGURE 1. Cell viability of HCECs following treatment. Human corneal epithelial cells (HCECs) were treated for 10 minutes with one of the following: 30 µL of PBS, 0.2% hyaluronic acid (HA) in PBS, 0.02% benzalkonium chloride (BAK) in PBS, Optase Life Sensitive Eye Makeup Remover (OLMUR, pH 7.2), MUR with alexidine hydrochloride (alexidine HCl, pH 7.0), MUR with polyhexamethylene biguanide (PHMB, pH 6.9), or MUR with BAK (pH 7.0). n = 3. Data are normalized to the PBS control and presented as mean ± SD. ns = not significant ($p > 0.05$); ***$p < 0.001$ vs. neutralized OLMUR (one-way ANOVA).

Optase Life Sensitive Eye Makeup Remover at pH 7.2 is relatively non-cytotoxic and safe to corneal epithelial cells

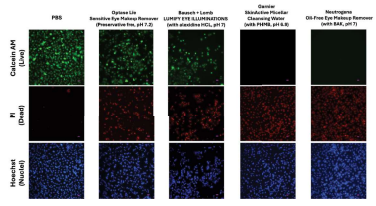

FIGURE 2. Live/Dead staining of HCECs post treatment: Representative fluorescence images of HCECs stained with a Live/Dead assay to assess cell viability. Live cells appear green (Calcein-AM), while dead cells appear red (PI). Cells were treated with 30 uL of PBS or makeup removers for (A-B) 10 minutes. Scale bar = 40um. n=3.

Key Takeaway:

FINDING:

- Preservative-free Optase Life Sensitive Eye Makeup Remover maintained corneal cell viability, while preservative-containing Makeup Removers (Alexidine, PHMB, BAK) caused significant cytotoxicity.
- alamarBlue™ assay confirmed reduced metabolic activity; results are consistent with live/dead imaging.
- Preservative-containing Makeup Removers may worsen ocular surface damage in dry eye patients.

SIGNIFICANCE AND FUTURE PERSPECTIVE:

- Eye care professionals should advise careful selection of makeup removers, especially for patients with dry eye, contact lens wear, ocular surface disorders, or preservative sensitivity.
- Clinical validation in dry eye patients, repeated exposure safety studies, evaluation across durations and concentrations, and assessment of tear film interactions.

Conclusion

This *in vitro* data suggests that Makeup Removers with commonly used preservatives can cause cytotoxicity whilst the Optase Life Sensitive Eye Makeup Remover with no preservatives demonstrated no significant cytotoxicity. The results underscore the importance of carefully selecting Makeup Removers, particularly for patients with chronic or moderate-to-severe dry eye disease.

These findings warrant further investigation of Makeup Removers in future clinical studies for their effect in dry eye patients.

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*The OLMUR was an ionized water formula that was neutralised to a pH of 7.2 to reflect its solution chemistry mechanism of action when applied to the eye.[14]