Correlation of Two In-Vitro EpiOcular™ Test Methods and Consumer Eye Irritation Data for Cleaning Products

Kathryn E. Page1, Lori A. Stradley2, Patrick D. Elias3, Nathan Wilf4, Greg Mum2, Elizabeth Sly5, Sarah Nadowski2, Jacob Sauran2, Keith Mainquist1

1The Clorox Company, Pleasanton, CA; 2Institute for In Vitro Sciences, Inc., Gaithersburg, MD.

ABSTRACT

The Clorox Company has used the EPA EpiOcular™ assay (EO) to predict ocular irritant potential of cleaning products without the use of animals. The US EPA – Office of Pesticide Programs has accepted EO data in replacement of several tests for ocular irritancy of cleaning products with antimicrobial claims. Another assay utilizing EpiOcular™ tissue, the Eye Irritation Test (EIT), was recently accepted by the OECD  (TG 492) for classifying materials that do not require ocular irritation labeling under GHS. EO utilizes multiple exposure times to calculate the test material exposure time to cause 50% viability (ET50). In the EO, test materials were exposed to test materials for up to 4 hours, followed by rinsing, and exposure to keratinocytes, which models the effect of a test article to the corneal epithelium. Both EIT and EO give similar rankings in level of irritation. For EO, both ET50 values and estimated viability values after 30 minutes of exposure to test materials (same exposure time as EIT) were compared to ET50 results. Correlation of the EO and EIT MV30 scores resulted in an r2 value of 0.91, adding strength to the observed correlation of the methods. The collected human eye irritation data further supported this case, aligning with predicted irritation of the tested materials.

METHODS

In Vitro Ocular Irritation

Two protocols for ocular irritation were evaluated using EpiOcular™ tissues obtained from MedTox Corporation. The EpiOcular™ tissues are 3-dimensional tissue constructs grown from human keratocytes, which models the effect of a test article to the corneal epithelium. In general, the procedures used to conduct the 2 protocols are essentially the same as outlined below:

- Tissue Pre-incubation
- Tissue Treatment
- Tissue Rinsing
- Post-Soak

The main differences between the 2 protocols are as follows:

1. In addition to the 1 hour pre-incubation in assay medium, the EIT protocol also requires an additional 24-hour pre-incubation.
2. For the EIT protocol, 100 µL of each test article were dosed on duplicate tissue, for four exposure times. For the EO protocol, the tissues were pre-treated with 20 µL of DPBS for 30 minutes, followed by dosing on duplicate tissue, with 30 µL of each test article for 30 minutes.
3. EO tissues were rinsed using DPBS (Dulbecco's Phosphate Buffered Saline) from a spray bottle, while EIT tissues were rinsed in sterile cups containing 1×DPBS.
4. Following the rinsing and post-soak procedures the EIT tissues were placed in assay medium for a post-incubation period of 2 hours, whereas the EO tissues were transferred to MTT.
5. Percent viability was calculated relative to the control. In addition to percent viability, an ET50 value (estimated time to reduce viability to 50%) was calculated for the EO assay.

Correlation Analysis

The EO ET50 was converted to a MV30 score; similar to that of EIT. Mean viability values at 15 (MV15) and 45 (MV45) minutes were determined from raw EO data. These MV15 and MV45 values were then plotted against time, and an equation was generated to represent the connecting line; y = viability, and x = time. The value of y (viability) was then determined for x = 30 minutes.

Human Experience

Adverse events data (involving the eye) were collected for products of similar formulation to those tested. A “human experience” score was generated using the percent of moderate cases (% Mo), and an estimated MV30 value for EO (estimated % viability after 30 minutes of exposure) using a simpler model; indicating a significant relationship between the results from the two assays.

SUMMARY / CONCLUSION

- Using a predicted viability at 30 minutes of exposure in the EO protocol, there is a strong linear correlation between the results using EO and EIT.
- The correlation has a 95% confidence interval of ±13.4% due to the small amount of samples tested; increasing sample number may help narrow the confidence interval.
- With improved predictability, the model can potentially be used as part of a product development testing strategy to predict the score of one test from the results of another (e.g., using EPA results to predict GHS score).
- Continued testing may lead to reduced data duplication by further correlation between the EO (EPA) and GHS protocols, which would result in cutting time and cost for safety testing. This could be supported by future alignment of EPA and GHS classifications (if successful).
- Both in vitro eye irritation methods correlate with consumer human data from similar formulations; however, EIT is stronger. This was demonstrated by the decrease in the human experience score accompanied by an overall increase in viability (EIT) and ET50 values (EO).

Figure 1: In Vitro Eye Irritation - EPA EpiOcular (EO)

Figure 2: In Vitro Eye Irritation - OECD EpiOcular (EIT)

Figure 3: In Vitro Eye Irritation - EO/EIT Correlation

Figure 4: In vitro Eye Irritation & Human Experience Correlation

Visit Our Websites

In Vitro Eye Irritation Results for 30 Cleaning Product Formulations using the EPA EpiOcular™ (EO) and OECD EpiOcular (EIT) methods. The EIT and EO methods appear to give similar rankings in level of irritation. For EO, both ET50 values and estimated viability values after 30 minutes of exposure to test materials (same exposure time as EIT) were compared to ET50 results. Correlation of the EO and EIT MV30 scores resulted in an r2 value of 0.91, adding strength to the observed correlation of the methods. The collected human eye irritation data further supported this case, aligning with predicted irritation of the tested materials.