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

Kathryn E. Page[1], Lori A. Strazdas[1], Patrick D. Elias[1], Nathan Wilt[2], Greg Mun[2], Elizabeth Sly[2], Norah Sadowski[2], Jacob Saudan[1], Keith Mainquist[1]

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

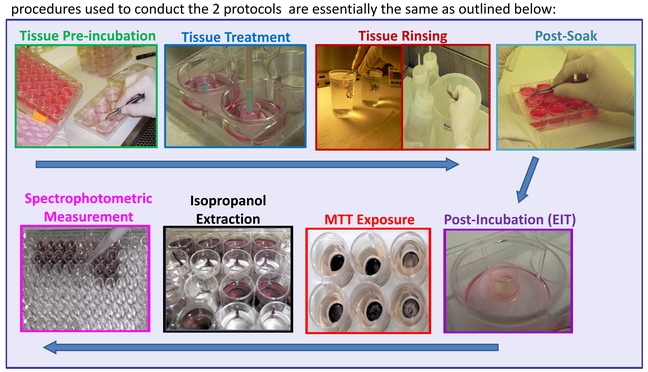
ABSTRACT

The Clorox Company has used the EPA EpiOcular™ assay (EO) to predict ocular irritancy potential of cleaning products without the use of animals. The US EPA - Office of Pesticide Programs has accepted EO data in replacement of animal tests for ocular irritancy of cleaning products with antimicrobial claims. Another assay utilizing EpiOcular™ tissue, the Eye Irritation Test (EIT), was recently accepted by 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 (ET_{50}). EIT also evaluates ocular irritation, using mean relative viability (MV_{30}) after single exposure time (30 minutes) and post exposure incubation. Ten reference materials with known irritancy levels were tested in EO and EIT. In the EIT, 5 materials resulted in MV_{30} of >60% with GHS "No Category" classification. The remaining 5 test materials with MV_{30} <60% were classified as ocular irritants. In the EO, two test materials were predicted to be EPA Category IV (ET_{50} > 70min), 5 were predicted to be Category III (70 min > ET_{50} >4 min), and 3 were predicted to be Category I (ET_{50} < 4 min). The results of both EIT and EO assays gave similar rankings in level of irritancy. For EO, both ET_{50} values and estimated viability values after 30 minute of exposure to test materials (same exposure time as EIT) were compared to EIT results. Correlation of the EO and EIT MV_{30} scores resulted in an r^2 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 MatTek® Corporation. The EpiOcular™ tissues are 3-dimensional tissue constructs grown from human keratinocytes, 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:



OECD Guidelines for the Testing of Chemicals. Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage (OECD TG 492), adopted 28 July 2015.
Office of Pesticide Programs, U.S. Environmental Protection Agency (2 March 2015) Use of an Alternate Testing Framework for Classification of Eye Irritation Potential of EPA Pesticide Products. http://www.epa.gov/sites/production/files/2015-05/documents/eye_policy2015update.pdf

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 16-24 hour pre-incubation.
2. For the EO protocol, 100µL of each test article were dosed on duplicate tissue, for four exposure times. For the EIT 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 ~100mL of 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 negative control. In addition to percent viability, an ET_{50} value (estimated time to reduce viability to 50%) was calculated for the EO assay.

Correlation Analysis

The EO ET_{50} 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; result = MV30. The relationship between the two assays was evaluated using linear regression techniques in Minitab 16 Statistical Software, Matlab, and Microsoft Excel. The 95% confidence interval for the data was found using Minitab 16.

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 correcting for the number of cases (total number of cases with effect divided by those with no effect):

Human Experience Score = %Mo x (E / NE)

Visit Our Websites

THE CLOROX COMPANY

www.TheCloroxCompany.com

IIVS Institute for In Vitro Sciences — Advancing Science & Animal Welfare Together

www.IIVS.org

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

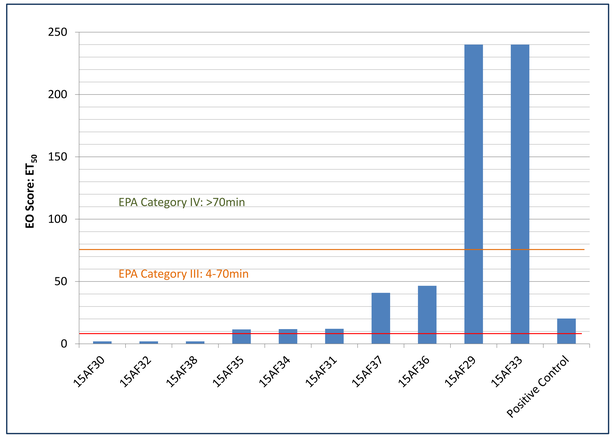


Figure 1 shows *in vitro* eye irritation results for 10 cleaning product formulations using the EPA-approved 3D-tissue EpiOcular™ (EO) method. The EO utilizes multiple exposure times to calculate the test material exposure time to reduce viability to 50% (ET_{50}). 2 test materials were predicted to be EPA Category IV (ET_{50} > 70min), 5 were predicted to be Category III (70 min > ET_{50} >4 min), and 3 were predicted to be Category I (ET_{50} < 4 min; below red line on graph).

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

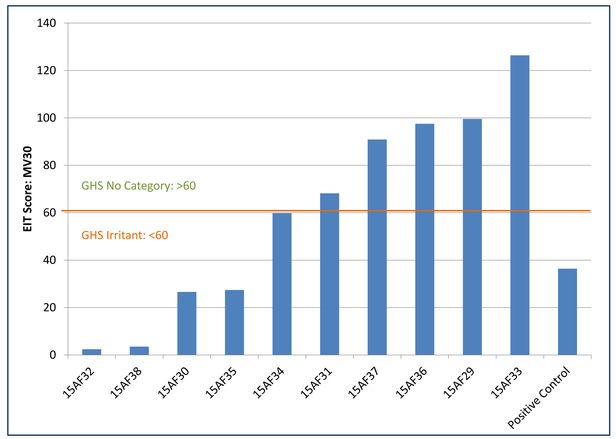


Figure 2 shows *in vitro* eye irritation results for 10 cleaning product formulations using the ECVAM-validated 3D-tissue EpiOcular™ Eye Irritation Test (EIT) method (OECD 492). The EIT evaluates ocular irritation, using mean relative viability (MV_{30}) after single exposure time (30 minutes) and post exposure incubation. Five materials resulted in MV_{30} of >60% and were predicted to not require labelling or classification for ocular irritation (GHS "No Category" classification); the remainder were predicted to be GHS ocular irritants (MV_{30} <60%)

Figure 3: *In vitro* Eye Irritation – EO/EIT Correlation

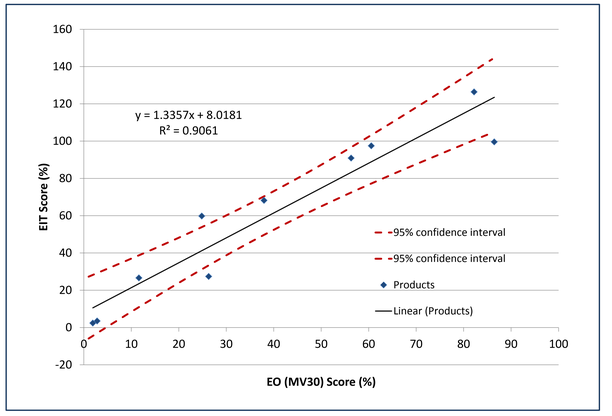


Figure 3 shows the correlation between the two *in vitro* ocular irritation methods (% viability for EIT, and an estimated MV30 value for EO (estimated % viability after 30 minutes of exposure)) using Excel. The graph shows a linear fit of $1.3357 * (EO) + 8.0181 = EIT$, with an R^2 coefficient of 0.9061. Alternate analysis in MatLab using a power law fit of $14.77 * (EO)^{0.4976} - 17.5 = EIT$, provides an R^2 coefficient of 0.9358. Both R^2 values represent strong correlations, with the linear fit using a simpler model; indicating a significant relationship between the results from the two *in vitro* tests, and highlighting the linear fit as the preferable model. The 95% confidence interval for this data is ±13.4 %. This represents a spread of about 22% of the total range of data, providing a weak/unstable predictability; due to the low number of data points. The predictive quality of the model may be improved with the testing of more products in both assays. The main caveat in this comparison is that although the ET_{50} score of the EO can be converted to an MV30 score (using the raw data), the MV30 score of the EIT cannot be converted into an ET_{50} (due to lack of data points required to generate the curve).

Figure 4: *In vitro* Eye Irritation & Human Experience Correlation

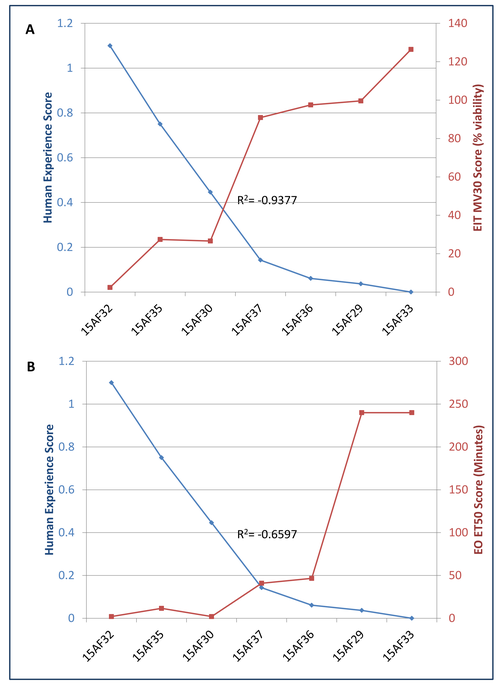


Figure 4 shows interaction of Human Experience data for eye irritation (for products with similar formulations) and 7 of the *in vitro* EIT (A), and EO (B) tested formulations. The data in figure 4A shows correlation between human eye irritation, and the *in vitro* results from the EIT test method (R^2= -0.9377). The sample 15AF30 has an oxidative chemistry, and showed blistering of the tissues; when removed the strength of the correlation is increased (R^2= 0.9812). The EPA EO test method also correlates with the human experience data, although not as strongly (R^2 = 0.6597). Removing the 15AF30 sample from the data set also increases the EO vs human experience data correlation strength (R^2 = 0.6711).

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 EIT (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 human experience 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 ET_{50} values (EO).