In vitro ocular irritation assays, such as the Chorioallantoic Membrane Vascular Assay (CAMVA) and Bovine Corneal Opacity and Permeability (BCOP) test, are routinely used by personal care product companies because they are rapid and economical to conduct, but do not require the use of live animals, and provide reliable predictive data. Previous research using an extensive CAMVA and BCOP database at Kao USA Inc. has shown that ocular irritation potential for new hair shampoos, ethanol-based hair styles, skin cleansers, and skin lotions can be reliably predicted using a decision tree that systematically compares the ingredient composition, particularly ethanol and surfactant content, of the new formulation to previously tested formulations. Because the studies comprising this original database were conducted at a single testing laboratory, a follow-up study was conducted using a second contract laboratory to conduct in vivo testing using human subjects to demonstrate inter-laboratory reliability of the CAMVA/BCOP data-derived decision tree for prediction of ocular irritation potential. Thirty-five personal care products were tested using the CAMVA and/or BCOP assays. The ethanol and surfactant content of each test material was evaluated, and the results of the assays were compared to the decision tree-based prediction of ocular irritation potential. Our data confirmed the ocular irritation potential made using the decision tree model for 35 of 37 test samples (95% correlation rate) and verified the inter-laboratory reliability of the CAMVA and BCOP assays when conducted using appropriate controls. Our results also strengthened the ocular irritation decision tree model by confirming that odorants are consistently predicted not to be ocular irritants based on composition.

## MATERIALS & METHODS

Testing laboratory qualification: In order to strengthen the ocular irritation prediction model by demonstrating inter-laboratory reliability of the core assays (CAMVA and BCOP) that support the decision tree, a qualification of the candidate laboratory, Institute for Vitro Sciences (IVS, Gaithersburg, Maryland) was conducted and included a full audit of the facility.

Test methods: The general BCOP methodology used in these studies was previously described by Donahue et al. (2011), and was based on the protocol developed by Gaborek et al. (1992). This was subsequently validated as OECD Test Guideline 417 (OECD, 2008). The CAMVA method used in these studies was adapted from the method described by Leighton et al. (1983). The protocol was comparable to other reported CAMVA methods (Bagley et al., 1998, 2003; Cerven and Moreo, 2011; Donahue et al., 2011).

## CONCLUSIONS

The successful concordance of experimental results obtained in this study with the ocular irritation predictions from the decision tree model confirmed the inter-laboratory reliability of the CAMVA and BCOP assay results at IVS and the experimental results for over 300 products evaluated at another contract laboratory over the past 15 years (Donahue et al., 2011).

In addition, the experimental BCOP/CAMVA results for 33 of 37 test materials (including the reference materials) confirm that the ocular irritation predictions made using the decision tree model (89% correlation rate). Test results for four materials (see table) did not follow the irritation classification predicted by the decision tree classification model. Further investigation into high volatile organic chemical containing formulations is needed.

Overall, the decision tree prediction model developed to assess eye irritation potential based on chemical composition of formulations has been strengthened in key areas. Our iterative approach to development of this decision tree classification model is an example of efficient improvement of toxicological assessment of eye irritation potential.