Testing Framework for Prediction of Ocular Irritation Using the Bovine Corneal Opacity and Permeability (BCOP) Assay and Chorioallantoic Membrane Vascular Assay (CAMVA)

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ABSTRACT

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 products companies because they are rapid and economical to conduct, 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 stylers, 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 contract laboratory, a follow-up study using a second contract laboratory was conducted 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 predictions of ocular irritation potential. Our data confirmed the ocular irritation predictions made using the decision tree model for 33 of 37 test samples (89% 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 deodorants are consistently predicted not to be ocular irritants based on composition.

INTRODUCTION

The BCOP test, which uses excised bovine corneas to predict ocular irritation, is capable of differentiating a wide range of irritation responses, but has less sensitivity for discriminating irritation potential within a mild to ultra-mild range and most effectively discriminates between moderate and severe ocular irritants (Eskes et al., 2005). In addition, corneas saved from the BCOP assay may be evaluated histologically to assess the depth and degree of injury, confirming irritation potential. The CAMVA, which uses the vascular network of fertilized chicken eggs to model the conjunctiva and predict eye irritation, best discriminates between mild and moderate ocular irritants (Bagley et al., 1999; Eskes et al., 2005). These characteristics of the CAMVA effectively complement the strengths of the BCOP test.

The BCOP and CAMVA assays have been used in tandem at Kao USA Inc. for over fifteen years for product safety claims substantiation (Donahue et al., 2011). Our research using this extensive database of more than 300 tested personal care products has shown that the ocular irritation potential of new hair shampoos, ethanol-based hair stylers, 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 (Donahue et al., 2011). However, the studies comprising this database were conducted at a single contract laboratory. Therefore, a follow-up study using a second contract laboratory was conducted to demonstrate inter-laboratory reliability of the BCOP/CAMVA data-derived decision tree for prediction of ocular irritation potential. Thirty-five personal care products were assessed in the BCOP and/or CAMVA assays. The ingredient compositions of each test material were compared to the previously achieved decision tree criteria, and the experimental results of the assays were compared to the decision tree-based predictions of ocular irritation potential.

MATERIALS & METHODS

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

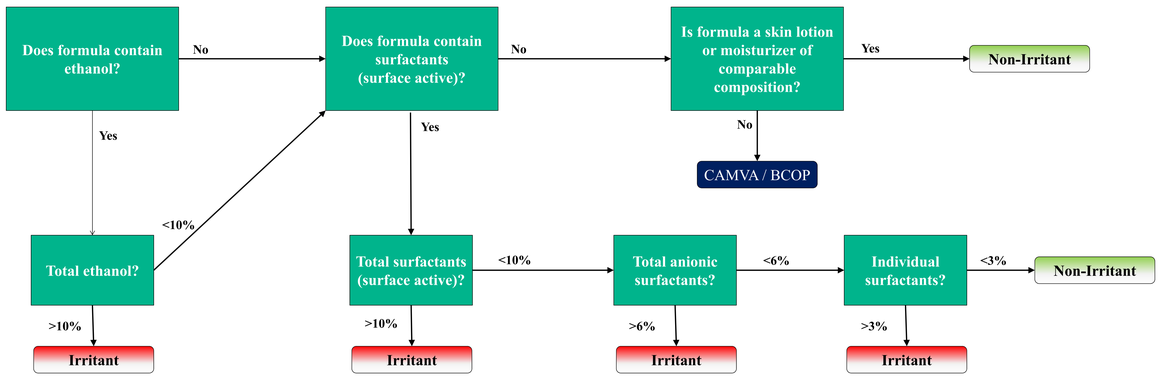
Test methods: The general BCOP methodology used in these studies was previously described by Donahue et al. (2011), and was based on the protocol developed by Gautheron et al. (1992) that was subsequently validated as OECD Test Guideline 437 (OECD, 2009). The CAMVA method used in these studies was adapted from the method described by Leighton et al. (1983). The protocol was comparable to other reported CAMVA methods (Bagley et al., 1988, 1994; Cerven and Moreno, 1992; Donahue et al., 2011).

RESULTS

Summary of test material pH and ethanol and surfactant concentrations							Summary of BCOP and CAMVA assay results				
Product category	Test material	pH (neat)[a]	Total ethanol conc. (%)	Total surfactant conc. (%)	Anionic surfactant conc. (%)	Individual high surfactant conc. (%)	BCOP irritancy grade	CAMVA irritancy grade	Overall assessment	Decision tree model prediction	Concordant with model prediction?
Hair shampoo	A (ref)	5.0	0	10-25	10-25	3-15	Non-irritant	Irritant	Irritant	Irritant	Yes
Hair conditioner	B (ref)	4.5	0	<5	0	<3	Non-irritant	Indeterminate	Non-irritant	Non-irritant	Yes
Skin lotion	C (ref)	5.0	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Astringent	10-292	4.5	15-25	<5	0	<3	Mild irritant	Non-irritant	Irritant	Irritant	Yes
Skin cleanser	10-286	6.0	0	10-25	10-25	3-15	Mild irritant	Irritant	Irritant	Irritant	Yes
Skin cleanser	10-288	5.0	0	10-25	10-25	3-15	Mild irritant	Irritant	Irritant	Irritant	Yes
Skin cleanser	10-290	5.5	0	10-25	10-25	3-15	Mild irritant	Irritant	Irritant	Irritant	Yes
Skin cleanser	11-012	5.0	0	5-10	<5	3-15	Mild irritant	Irritant	Irritant	Irritant	Yes
Skin lotion	10-028	5.0	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Skin lotion	10-031	4.0	0	<5	0	<3	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Antiperspirant/deodorant	***10-034***	***4.0***	***0***	***<5***	***0***	***3-15***	***Non-irritant***	***Non-irritant***	***Non-irritant***	***Irritant***	***No***
Antiperspirant/deodorant	10-212	5.0	0	<5	0	<3	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Antiperspirant/deodorant	10-214	4.5	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Antiperspirant/deodorant	10-233	NCC	0	<5	0	<3	Non-irritant	NA	Non-irritant	Non-irritant	Yes
Base[c]	10-231	NCC	0	<5	0	<3	Non-irritant	NA	Non-irritant	Non-irritant	Yes
Fragrance[d]	10-232	5.0	0	0	0	0	Non-irritant	NA	Non-irritant	Non-irritant	Yes
Hair conditioner	10-037	4.0	0	<5	0	<3	Non-irritant	Indeterminate	Non-irritant	Non-irritant	Yes
Hair conditioner	10-103	4.0	0	<5	0	<3	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair conditioner	10-208	4.5	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair conditioner	10-210	5.5	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair conditioner	10-228	NCC	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair conditioner	10-297	4.0	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair conditioner	10-299	4.5	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair conditioner	11-168	4.0	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair styling spray	***10-096***	***4.0***	***<5***	***0***	***0***	***0***	***Mild irritant***	***Non-irritant***	***Irritant***	***Non-irritant***	***No***
Hair styling spray	10-098	5.0	55-75	0	0	0	Moderate irritant	Non-irritant	Irritant	Irritant	Yes
Hair styling spray	10-105	4.0	<5	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair styling spray	10-116	5.0	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair styling spray	10-301	6.5	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair styling spray	***10-305***	***4.0***	***<5***	***<5***	***0***	***<3***	***Severe irritant***	***Non-irritant***	***Irritant***	***Non-irritant***	***No***
Hair styling spray	10-307	5.0	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair styling spray	***10-309***	***4.0***	***<5***	***<5***	***0***	***<3***	***Moderate irritant***	***Non-irritant***	***Irritant***	***Non-irritant***	***No***
Hair styling spray	11-160	6.0	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair styling spray	11-164	5.5	<5	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair styling spray	11-172	6.5	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair styling gel	11-170	5.0	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes
Hair mousse	10-100	5.0-5.5	0	0	0	0	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Yes

[a], pH determined during BCOP test; [b], pH determined during CAMVA; [c], test material 10-231 consisted of the base formulation for antiperspirant/deodorant 10-233 exclusive of the fragrance ingredient; [d], test material 10-232 consisted of the fragrance ingredient for antiperspirant/deodorant 10-233 (at 1% concentration in mineral oil); NA, not applicable (CAMVA not conducted for this test material); NCC, no color change (pH could not be determined because the neat test material did not cause a color change on the pH paper); NT, not tested because the neat test material was a paste.

OCULAR IRRITATION PREDICTION DECISION TREE



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 IIVS 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) confirmed the ocular irritation predictions made using the decision tree model (89% correlation rate). Test results for four materials (see table) did not follow the ocular irritancy 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.

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