

ANNEX II

PROFICIENCY STANDARDS FOR THE ICE AND BCOP TEST METHODS

Prior to routine use of a test method that adheres to this Test Guideline (TG), laboratories may wish to demonstrate technical proficiency by correctly identifying the ocular corrosivity classification of the 10 substances recommended in table 1. These substances were selected to represent the range of responses for local eye irritation/corrosion, which is based on results in the *in vivo* rabbit eye test (TG 405) (*i.e.*, Categories 1, 2A, 2B, or Not Labeled according to the UN GHS). However, considering the validated usefulness of these assays (*i.e.*, to identify ocular corrosives/severe irritants only), there are only two test outcomes for classification purposes (corrosive/severe irritant or noncorrosive/nonsevere irritant) to demonstrate proficiency. Other selection criteria were that substances are commercially available, there are high quality *in vivo* reference data available, and there are high quality data from the two *in vitro* methods for which Test Guidelines are being developed. For this reason, irritating substances were selected from the US-ICCVAM recommended list of 122 reference substances for the validation of *in vitro* ocular toxicity test methods (see Appendix H, ICCVAM 2007¹). Reference data are available in the ICCVAM Background Review Documents for BCOP and ICE (ICCVAM 2006^{2,3}).

¹ ICCVAM (2007). Test Method Evaluation Report - In Vitro Ocular Toxicity Test Methods for Identifying Ocular Severe Irritants and Corrosives. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). NIH Publication No.: 07-4517. Appendix H: ICCVAM Recommended Reference Substances List Available: [http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_tmter.htm]

² ICCVAM (2006) Background review document, Current Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants: Bovine Corneal Opacity and Permeability (BCOP) Test Method.

Available : [http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_brd_bcop.htm]

³ ICCVAM (2006) Background review document, Current Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants Isolated Chicken Eye (ICE) Test Method.

Available : [http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_brd_bcop.htm]

Table 1. Recommended substances for demonstrating technical proficiency with BCOP or ICE

Chemical	CASRN	Chemical Class ¹	Physical Form	<i>In Vivo</i> Classification ²	<i>In Vitro</i> Classification ³
Benzalkonium chloride (5%)	8001-54-5	Onium compound	Liquid	Category 1	Corrosive/ Severe Irritant
Chlorhexidine	55-56-1	Amine, Amidine	Solid	Category 1	Corrosive/ Severe Irritant
Dibenzoyl-L-tartaric acid	2743-38-6	Carboxylic acid, Ester	Solid	Category 1	Corrosive/ Severe Irritant
Imidazole	288-32-4	Heterocyclic	Solid	Category 1	Corrosive/ Severe Irritant
Trichloroacetic acid (30%)	76-03-9	Carboxylic Acid	Liquid	Category 1	Corrosive/ Severe Irritant
2,6-Dichlorobenzoyl chloride	4659-45-4	Acyl halide	Liquid	Category 2A	Noncorrosive/ Nonsevere irritant
Ethyl-2-methylacetoacetate	609-14-3	Ketone, Ester	Liquid	Category 2B	Noncorrosive/ Nonsevere irritant
Ammonium nitrate	6484-52-2	Inorganic salt	Solid	Category 2B	Noncorrosive/ Nonsevere irritant
Glycerol	56-81-5	Alcohol	Liquid	Not Labeled	Noncorrosive/ Nonsevere irritant
n-Hexane	110-54-3	Hydrocarbon (acyclic)	Liquid	Not Labeled	Noncorrosive/ Nonsevere irritant

Abbreviations: CASRN = Chemical Abstracts Service Registry Number

¹ Chemical classes were assigned to each test substance using a standard classification scheme, based on the National Library of Medicine Medical Subject Headings (MeSH) classification system (available at: <http://www.nlm.nih.gov/mesh>)

² Based on results from the *in vivo* rabbit eye test (OECD TG 405) and using the UN GHS.

³ Based on results from the BCOP and ICE.