# 6.0 COMPARISON OF PERFORMANCE CHARACTERISTICS AND GENERAL RECOMMENDATIONS FOR FOUR EVALUATED *IN VITRO* TEST METHODS

In addition to the test method specific recommendations discussed in **Sections 2.0** through **5.0**, ICCVAM also makes some general recommendations that relate to all the *in vitro* test methods discussed.

**Table 6-1** provides a comparison of the accuracy, false positive, and false negative rates for all four *in vitro* ocular toxicity test methods evaluated for each of the regulatory hazard classification systems evaluated (EPA, EU, and GHS). As noted in the sections discussing each of the test methods individually (**Sections 2.0** through **5.0**), these performance characteristics are similar among the three hazard classification systems.

Although both BCOP and ICE can be used as screens for the detection of ocular corrosives and severe irritants in a tiered-testing strategy, as part of a weight-of-evidence approach, both test methods as well as HET-CAM and IRE have limitations. As shown in **Table 6-1**, exclusion of specific chemical and physical classes increases the accuracy and decreases the false positive and false negative rates for BCOP and ICE. ICCVAM recommends that users consider, to the extent possible, the chemical classes and physical structures of the substances to be tested to determine whether either of these test methods would be appropriate to use as a screening test for ocular corrosion or severe irritation. Also, additional studies with each test method are recommended to determine if modification of the test method standardized protocol and/or the decision criteria for classification of a test substance as a corrosive/severe irritant or as a nonsevere irritant/nonirritant can improve test method sensitivity and specificity.

Results from appropriately validated *in vitro* ocular toxicity test methods are recommended for use in a weight-of-evidence decision making process in accordance with the EPA and EU ocular testing regulations (EPA 1996, EU 2004) and the GHS tiered-testing strategy (UN 2003).[20] In these testing schemes, when a positive result is obtained in an appropriately validated *in vitro* test, a test substance may be classified as an ocular hazard without testing in rabbits. A substance that tests negative in the *in vitro* ocular toxicity test would need to be tested in the *in vivo* ocular test to identify possible *in vitro* false negatives and to identify moderate and mild ocular irritants. As is appropriate for any test system, there is the opportunity for confirmatory testing if false positive results are indicated based on a weight-of-evidence evaluation of supplemental information (e.g., structure-activity relationships, other testing data). Use of a weight-of-evidence decision making process and a tiered-testing strategy for classification of substances as ocular corrosives or severe irritants will eliminate the pain and distress that might be experienced by rabbits who otherwise would have been administered these test substances.

[20]A tiered-testing strategy approach may not be applicable to purposes other than regulatory classification and labeling.

**Table 6-1 Comparison of Performance Characteristics of Four *In Vitro* Ocular Test Methods for the Identification of Severe Ocular Irritants or Corrosives, for Three Hazard Classification Systems**

| Test Method | Database | EPA Classification System | | | | EU Classification System | | | | GHS Classification System | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N[1] | Accuracy (%)[2] | False Positive Rate[3] (%) | False Negative Rate[4] (%) | N[1] | Accuracy (%)[2] | False Positive Rate[3] (%) | False Negative Rate[4] (%) | N[1] | Accuracy (%)[2] | False Positive Rate[3] (%) | False Negative Rate[4] (%) |
| **BCOP** | All | 143 | 79 (113/143) | 19 (20/103) | 25 (10/40) | 143 | 80 (114/143) | 21 (22/103) | 18 (7/40) | 147 | 81 (119/147) | 20 (21/104) | 16 (7/43) |
| | Excluding alcohols, ketones, and solids | 83 | 87 (72/83) | 14 (8/57) | 12 (3/26) | 82 | 88 (72/82) | 16 (9/56) | 4 (1/26) | 85 | 92 (78/85) | 12 (7/58) | 0 (0/27) |
| **ICE** | All | 145 | 84 (122/145) | 8 (9/116) | 48 (14/29) | 154 | 87 (134/154) | 6 (7/122) | 41 (13/32) | 144 | 83 (120/144) | 8 (9/114) | 50 (15/30) |
| | Excluding alcohols, surfactants, and solids | 79 | 91 (72/79) | 6 (4/70) | 33 (3/9) | 82 | 91 (75/82) | 5 (4/73) | 33 (3/9) | 75 | 92 (69/75) | 6 (4/68) | 29 (2/7) |
| **IRE** | Pooled Data Set | 107 | 64 (68/107) | 40 (25/62) | 31 (14/45) | 114 | 69 (79/114) | 35 (23/65) | 24 (12/49) | 107 | 65 (70/107) | 38 (23/60) | 30 (14/47) |
| **HET-CAM** | IS(B)-10 | 98 | 65 (64/98) | 36 (24/67) | 32 (10/31) | 95 | 67 (64/95) | 34 (21/62) | 30 (10/33) | 101 | 68 (69/101) | 33 (20/61) | 30 (12/40) |
| | IS(B)-100 | 133 | 52 (69/133) | 58 (61/105) | 11 (3/28) | 164 | 57 (94/164) | 52 (68/131) | 6 (2/33) | 138 | 54 (75/138) | 59 (58/99) | 13 (5/39) |

Abbreviations: BCOP = Bovine Corneal Opacity and Permeability; EPA = U.S. Environmental Protection Agency (EPA 1996); EU = European Union (EU 2001); GHS = Globally Harmonized System (UN 2003); HET-CAM = Hen's Egg Test – Chorioallantoic Membrane; ICE = Isolated Chicken Eye; IRE = Isolated Rabbit Eye.

[1]N=number of substances.

[2]Numbers in parentheses represent data used to calculate percentages.

[3]False Positive Rate = the proportion of all negative substances that are falsely identified as positive *in vitro*.

[4]False Negative Rate = the proportion of all positive substances that are falsely identified as negative *in vitro*.

Additional research and development, optimization, and/or validation efforts should use reference substances with existing rabbit data. Additional rabbit studies should be conducted only if important data gaps are identified. If such studies are conducted, they should be designed to minimize the number of rabbits tested, to minimize or avoid pain and distress, and to maximize the information collected. Designing and conducting such studies should be in accordance with the recommendations from the Scientific Symposium on Mechanisms of Chemically-Induced Ocular Injury and the Scientific Symposium on Minimizing Pain and Distress in Ocular Safety Testing (see http://iccvam.niehs.nih.gov/methods/ocudocs/ocumeet/sympinfo.htm). These symposia were organized by ICCVAM, NICEATM, and ECVAM.

All raw data generated using any of the recommended standardized *in vitro* ocular testing protocols and the *in vivo* rabbit eye test on the same substance should be submitted to NICEATM to expand the available validation database for these four test methods. The availability of such data will allow for additional retrospective evaluations of test method accuracy and/or reliability. Ideally, all substances should be completely identified (e.g., chemical name, chemical class, physicochemical properties). However, if this is not possible for proprietary reasons, data may be submitted using coded labels for each substance tested. If such coding is used, as much information as possible on physical and chemical properties should be provided to NICEATM.

Although the IRE and HET-CAM test methods cannot currently be recommended for meeting regulatory testing requirements, there may be non-regulatory uses for these two test methods. Accordingly, the four *in vitro* test methods should be considered prior to conducting *in vivo* ocular testing and an alternative test method should be used where determined appropriate for the specific testing situation. Since ocular irritancy testing frequently involves more than slight or momentary pain or distress, consideration of alternative test methods prior to the use of animals is necessary to comply with provisions of U.S. Animal Welfare Act regulations (9 CFR, Part 2, Section 2.31 and 9 CFR, Part 2, Section 2.32), the Public Health Service Policy on the Humane Care and Use of Laboratory Animals (PHS 2002), and the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (National Research Council 1996).

The potential usefulness of combining two or more *in vitro* test methods in a battery to identify ocular corrosives and severe irritants should be evaluated. Currently, there is insufficient guidance on the utility of a battery approach for such determinations.

Interested stakeholders are encouraged to support research and development of alternative test methods and technologies that may provide for a more accurate assessment of ocular toxicity and/or advantages in terms of time and cost.