11.0 PRACTICAL CONSIDERATIONS

Several issues are taken into account when assessing the practicality of using an *in vitro* test method in place of an *in vivo* test method. In addition to reliability and accuracy evaluations, assessments of the equipment and supplies needed for the *in vitro* test method, the level of personnel training, costs of the *in vitro* test method, and time to complete the method, are necessary. This consideration provides additional information as to whether the time, personnel cost, and effort required to conduct the test method are considered reasonable.

11.1 Transferability of the ICE Test Method

Test method transferability addresses the capacity of a method to be accurately and reliably performed by multiple laboratories (ICCVAM 2003). Issues of transferability include laboratories experienced in the particular type of procedure, and otherwise competent laboratories with less or no experience in the particular procedure. The degree of transferability of a test method affects its interlaboratory reproducibility.

11.1.1 Facilities and Major Fixed Equipment Required to Conduct the ICE Test Method

The capital requirements needed to outfit a laboratory to conduct the ICE test method are relatively minor, with the exception of the need for a slit-lamp biomicroscope equipped with an optical pachymeter. Along with the superfusion apparatus and eye clamps, these are the major items required in setting up an ICE test method-capable facility. It is quite possible that a facility that is presently involved in ocular toxicology would already possess or have access to a slit-lamp microscope. If necessary, such a set-up could be purchased through used equipment vendors for as little as $2,500. Due to their novelty, the superfusion apparatus and eye clamps would most likely require fabrication based on diagrams and or photographs provided by the test method developer. The actual cost of these components is not readily available. While the requisite peristaltic and vacuum pumps are typically commonplace in the laboratory, if necessary they could be purchased commercially for less than $1,000. There are no specific requirements regarding the facility at which the test is conducted (e.g., sterile environment). However, it would seem appropriate to conduct the assay under controlled temperature and humidity conditions. Should histopathology be included as a component of the ICE test method, standard tissue processing, sectioning, and staining equipment would be required at a significant additional cost. Most likely, if a facility is not already equipped to perform such tasks, this portion of the test method could be outsourced to an appropriate contractor.

The *in vivo* test, in contrast, requires a facility that is approved to house live laboratory animals; one that maintains constant, tightly regulated atmospheric conditions (i.e., temperature and humidity). In fact, the primary expense for equipping a facility to conduct the *in vivo* rabbit test would be the acquisition of an adequate animal room and associated housing (e.g., cages, bedding, food, water, etc.) for boarding animals during the study. There are no additional major equipment requirements as the remaining equipment and supplies necessary for conducting the *in vivo* test are readily available in most laboratories.

None of the equipment used for the ICE test method is fixed. Therefore, it is essentially a portable assay, provided adequate space is available to set up the slit-lamp microscope and the superfusion apparatus (a small table would suffice), and water and electrical access are available. All of the components of the assay can be readily transported to another facility if necessary. A sufficiently stable tabletop surface, that is free of major vibrations, is required for accurate use of the slit-lamp microscope (i.e., to avoid blurring the image under the microscope). Therefore, it has been suggested that, if a poultry slaughterhouse is not in sufficient proximity to laboratory, the ICE test method could be conducted at the slaughterhouse.

11.1.2 Availability of Other Necessary Equipment and Supplies

The remaining equipment and supplies necessary for conduct of the ICE test method are readily available in most scientific laboratories, or can be obtained from several scientific laboratory equipment vendors.

Similarly, the remaining equipment and supplies necessary for conducting the *in vivo* rabbit eye test are readily available in most toxicity testing laboratories or could be readily obtained from any of a number of scientific laboratory equipment vendors.

11.2 ICE Test Method Training Considerations

11.2.1 Required Level of Training and Expertise Needed to Conduct the ICE Test Method

Conducting the ICE test method (i.e., set-up and dosing of the eyes) appears to involve minimal training of technical staff, and could likely be mastered in a short period of time. However, mastering the evaluation of results at the requisite time points may require additional training. Both the *in vivo* rabbit eye test and ICE test methods incorporate the qualitative assessment of corneal opacity as an endpoint in the evaluation of ocular irritancy. The ICE test method also includes a qualitative measurement of fluorescein retention. Therefore, it is essential in both cases that laboratory personnel be adequately trained to accurately and consistently identify these endpoints. Lastly, the use of a slit-lamp microscope is necessary to evaluate corneal thickness. Accurate recording of this quantitative measurement requires that the technician be trained in the proper use of this instrument. There is no precise level of training that defines when a technician is adequately trained. Rather, this must be demonstrated through experience with the oversight of an experienced supervisor. Once the technician has demonstrated competence in identifying the study endpoints, it would seem appropriate for routine assessments of observations among trained personnel using benchmark control test substances to ensure consistency. A training video or other visual media to provide guidance on the development of endpoints may be considered for use.

11.3 Cost Considerations

As it is currently used at TNO (TNO Nutrition and Food Research, Toxicology and Applied Pharmacology, Zeist, The Netherlands), the ICE test is incorporated as a prescreen for the *in vivo* rabbit test without additional costs. If the prescreen shows that severe irritancy (as defined by the EU classification system) is expected, a full ICE test is performed without

further *in vivo* testing at the price of the *in vivo* test. If a full ICE test is used as a stand-alone assay (as mandated in EU countries for cosmetics/household products), depending on the number of samples tested, the 2004 cost of the ICE ranges from $847 to $1,694 (Prinsen M, personal communication). However, these costs do not include the inclusion of a positive control, which would increase the cost of the assay. In comparison, a GLP-compliant EPA OPPTS Series 870 Acute Eye Irritation test in the rabbit ranges from $765 for a 3 day/3 animal study up to $1665 for a 21 day/3 animal study at MB Research Laboratories (MB Research laboratories, personal communication). Therefore, it would appear that the cost, based on conducting Good Laboratory Practice (GLP) compliant studies, of an ICE test is comparable to, if not less expensive than, that of an *in vivo* rabbit test.

11.4 Time Considerations

Use of the ICE test method would significantly reduce the time needed to assess the ability of a test substance to induce ocular corrosivity or severe irritancy, when compared to the currently accepted *in vivo* rabbit eye test method. The *in vivo* Draize rabbit eye test is typically carried out for a minimum of one to three days (although it is recognized that a corrosive response could be determined in less than 24 hr). Depending upon the severity of ocular effects produced by a test substance, the method can be extended for up to 21 days. Comparatively, the standard ICE test method can be completed, from the onset of treatment, in about four hours.

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