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, level of personnel training, costs of the *in vitro* test method, and time to complete the method are necessary. This information provides additional information as whether the time, personnel cost, and effort required to conduct the test method are considered reasonable

11.1 Transferability of the IRE Test Method

Test method transferability addresses the ability of a method to be accurately and reliably performed by different, competent 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

If standard laboratory rabbits are to be used to provide the eyes for the IRE test method, then a standard animal housing facility approved by IUCAC and approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and in accordance with the Animal Welfare Act (7 U.S.C. 2131 et. seq.) or through equivalent accreditation/ legislation in other countries is needed. This same facility would be needed to conduct in-house *in vivo* rabbit eye tests. Such facilities require strict adherence to animal welfare considerations with controlled temperature and humidity, cage size and construction, feed and watering requirements, and are likely to be available in any toxicology-testing laboratory that involves standard animal testing. The IRE test method does not require an animal facility unless the animals are purchased specifically for use in the assay. However, holding cages or boxes may be required for temporary storage of live animals. The capital or fixed item equipment required for the IRE test method include a slit-lamp (e.g., Haag-Streit) with a depth-measuring device (Haag-Streit #1 attachment) or ultrasonic pachymeter, a vibration-free table for the slit-lamp observation, and a water-jacketed, Perspex superfusion chamber with black-walled cells to hold a sufficient number of eyes (Burton et al. 1981).

11.1.2 General Availability of Other Necessary Equipment and Supplies

Noncapital equipment includes a water bath and peristaltic pump to recirculate the water in the superfusion jacket at a rate of approximately 4 liters/minute for maintenance of a temperature in the chamber cells of 32 ± 1.5°C, an additional peristaltic pump for saline infusion at a rate of 0.1 to 0.4 mL/minute, Perspex or stainless steel eye holders that fit into the superfusion chamber, stainless steel saline drip tubes, and surgical equipment for enucleation and fine dissection (scissors, forceps). Other items such as syringes, weighing boats, physiological salt solutions, fluorescein solution, sodium pentobarbital and other items are readily available for purchase commercially.

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 Training Considerations

Training considerations are defined as the level of instruction needed for personnel to conduct the test method accurately and reliably (ICCVAM 2003). Evaluation of the level of training and expertise needed to conduct the test method reliably and accurately, as well as the training requirements needed to ensure that personnel are competent in the test method, are discussed below.

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

The most important difference between the *in vivo* and *in vitro* assays is the training required for administration of anesthetic for euthanasia of the rabbits and for enucleation and dissection of the eyes in the IRE test method. Although procurement of animals and administration of anesthetic to and dissection of animals at necropsy is standard practice in a toxicology-testing laboratory, proper training is required to understand shipment requirements, proper storage of the eyes if received from a vendor, or actual enucleation and dissection of the eye in a manner that prevents loss of intraocular pressure. Personnel familiar with the use of state-of-the-art procedures should train the laboratory personnel conducting the experiment. A training video or other visual media to provide guidance on the development of endpoints may be considered for use.

Once the Perspex superfusion apparatus and associated equipment is set up and running in a laboratory, minimal training is needed to place the enucleated eyes in the holders without damaging the cornea or affecting intraocular pressure, to control the temperature in the superfusion cells using the water bath, and to control the temperature and drip rate of saline flowing over the isolated cornea in the IRE test method. Some additional training in maintenance and changing of peristaltic tubing may be required.

To carry out the IRE test method, additional training principally involves the ability to measure and/or score the appropriate ocular parameters (i.e., corneal opacity and area of involvement, corneal thickness and swelling, fluorescein retention or penetration, epithelial cellular effects). Corneal opacity and area measurements and/or observations are performed with a slit-lamp and are similar to those performed *in vivo*. Personnel experienced in the state-of-the-art use of this equipment should be used to train new personnel in the use of the instrument, as well as in corneal observation and scoring methods. However, iridal and conjunctival observations needed for the *in vivo* test method are not required for the *in vitro* test method, since these tissues are removed or inoperative in the isolated eye due to lack of perfusion or muscular activity. Measurement of corneal thickness, calculation of corneal swelling and fluorescein retention are performed both *in vivo* and *in vitro* in some laboratories as additional endpoints to the Draize system. Training for the *in vitro* IRE test method is therefore no more complex than that for the *in vivo* assay. In fact, those trained to perform the *in vivo* test method could easily adapt to the *in vitro* assay

In general, personnel performing the IRE test method should be as proficient as possible. The trainers should insure that new laboratory personnel carry out their *in vitro* ocular testing appropriately, particularly when using the slit-lamp for observation and measurement. Personnel should demonstrate proficiency in the ability to procure laboratory animals and work with live animals if necessary, to administer anesthetic, to perform dissection procedures such as enucleation with reasonable speed while keeping the eyes free of corneal damage during the process. Personnel should be able to maintain the *in vitro* superfusion testing apparatus in an appropriate state by regulation of the temperature in the holding cells and the flow rate and temperature of the saline drip. Benchmark and standard ocular irritants with varying degrees of severity that represent various types of chemical substances should be scored by the trainee. The irritation scores obtained by the trainee should approximately match those obtained by someone trained in state-of-the-art techniques. For example, surfactants could produce a different type of corneal opacity than alcohols or acids and bases (e.g., diffuse rather than punctate lesions) and the testing personnel should be trained to understand the differences, particularly in how these various types of lesions are scored. Furthermore, the laboratory personnel should be proficient in applying fluorescein solutions to the eye and in scoring the degree of penetration using benchmark or standard irritants. Additionally, laboratory personnel involved in the IRE test method should demonstrate proficiency in standard laboratory procedures such as preparation and handling of solutions, weighing solids, sterile technique if required (e.g., media preparation), safe laboratory procedures, safe and appropriate storage practices, and other standard laboratory practices.

11.3 Cost Considerations

The current cost for a GLP compliant IRE assay (without the inclusion of a concurrent positive control) at SafePharm Laboratories, Ltd. (United Kingdom) is approximately $1070 per test substance (Guest R, personal communication). In comparison, a GLP-compliant EPA OPPTS Series 870 Acute Eye Irritation test (EPA 1996) in the rabbit ranges from $765 for a three day/three animal study up to $1665 for a 21 day/three animal study at MB Research Laboratories (MB Research laboratories, personal communication).

11.4 Time Considerations

Use of the IRE 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. 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 IRE test method can be completed, from the onset of treatment, in about four hours.

[This Page Intentionally Left Blank]