1.0 INTRODUCTION AND RATIONALE FOR THE PROPOSED USE OF *IN VITRO* TEST METHODS TO IDENTIFY OCULAR CORROSIVES AND SEVERE IRRITANTS

1.1 Introduction

1.1.1 Historical Background of *In Vitro* Ocular Irritation/Corrosion Test Methods and Rationale for Their Development

The location of the eye and its anatomy predisposes it to exposure to a variety of environmental conditions (e.g., ozone, pollen) and substances on a daily basis. Injury from ocular exposure to a variety of chemical agents can lead to a range of adverse effects with the most extreme being blindness. Societal concern for evaluating consumer products for ocular irritation and/or corrosion was heightened in 1933 when a 38 year old woman went blind after her eyelashes and eyebrows were tinted with a product containing paraphenylenediamine, a chemical with the potential to cause allergic blepharitis, toxic keratoconjunctivitis, and secondary bacterial keratitis[1] (Wilhelmus 2001).

In 1938, the U.S. Congress responded to these concerns by enacting the Federal Food, Drug, and Cosmetic Act of 1938, which included extending the regulatory control of the U.S. Food and Drug Administration (FDA) to cosmetics (FDA 1938). This legislation required manufacturers to evaluate product safety before marketing their products (Wilhelmus 2001). Several additional legislative statutes were later enacted to enable government agencies to regulate a variety of substances that could pose a risk to ocular health. **Table 1-1** provides a synopsis of current U.S. regulatory laws that pertain to eye irritation and corrosion.

Table 1-1 Summary of Current U.S. Legislation Related to Ocular Health[1]

Legislation (Year of Initial Enactment)	Agency	Substance
Food, Drug and Cosmetic Act (1938)	FDA	Pharmaceuticals and cosmetics
FIFRA (1947) and Federal Environmental Pesticide Control Act (1972)	EPA	Pesticides
FHSA (1964)	CPSC	Household products
FHSA (1964) and TSCA (1976)	Department of Agriculture and EPA	Agricultural and industrial chemicals
Occupational Safety and Health Act (1970)	OSHA	Occupational materials
Clean Air Act Amendments (1990)	Chemical Safety and Hazard Investigation Board and EPA	Accidentally released chemicals and air pollutants

[1]Adapted from Wilhelmus (2001).
Abbreviations: CPSC = U.S. Consumer Product Safety Commission; EPA = U.S. Environmental Protection Agency; FDA = U.S. Food and Drug Administration, FHSA = Federal Hazardous Substances Act; FIFRA = Federal Insecticide, Fungicide, and Rodenticide Act; TSCA = Toxic Substances Control Act.

---

[1] Allergic blepharitis (also referred to as blepharitis): inflammation of the eyelids; Toxic keratocojunctivitis (also referred to as contact, irritative, or chemical keratoconjuctivitis): inflammation of the cornea and conjunctiva due to contact with an exogenous agent; Secondary bacterial keratitis: inflammation of the cornea that occurs secondary to another insult that compromised the integrity of the eye (Vaughn et al. 1999; Chambers W, personal communication).

Exposure of the eye of a rabbit to a test substance is the primary method for assessing the hazard potential of substances that may come in contact with or be placed near the eye of a human. The rabbit eye test method currently accepted by U.S. Federal and international regulatory agencies (CPSC 1995; EPA 1998; OECD 2002) is based on a method developed by Draize and colleagues in 1944 (Draize et al. 1944). This technique involves placing a test substance into the lower conjunctival sac of one eye of a rabbit. The contralateral eye serves as a negative control. The rabbit is then observed at selected intervals for up to 21 days after exposure for adverse effects to the conjunctiva, cornea, and iris.

The current rabbit eye test method identifies both irreversible (e.g., corrosion) and reversible ocular effects. It also provides scoring that allows for relative categorization of severity for reversible effects such as mild, moderate, or severe irritants (e.g., see U.S. Environmental Protection Agency [EPA] Ocular Classification System discussed below). Current EPA ocular testing guidelines and the United Nations (UN) Globally Harmonized System (GHS) of Classification and Labeling of Chemicals (UN 2003) indicate that if serious ocular damage is anticipated (e.g., irreversible adverse effects on day 21), then a test on a single animal may be considered. If serious damage is observed, then no further animal testing is necessary (EPA 1998; UN 2003). If serious damage is not observed, additional test animals (1 or 2 rabbits) may be evaluated sequentially until concordant irritant or nonirritant responses are observed (UN 2003).

Depending on the legislative mandate of various regulatory agencies and their goals for protecting human health, the classification of irritant responses evaluated by each agency varies (**Table 1-2).** The EPA ocular irritation classification regulation and testing guidelines (EPA 1996, 1998) are based on the most severe response in one animal in a group of three or more animals. This classification system takes into consideration the kinds of ocular effects produced, as well as the reversibility and the severity of the effects. The EPA classifies substances into four ocular irritant categories, ranging from I to IV (**Table 1-2**) (EPA 1996). Category I substances are defined as corrosive or severe irritants, while classification from II to IV is based on decreasing irritation severity, as well as the time required for irritation to clear. Irritation that clears in 8 to 21 days is classified as Category II, while irritation that clears within seven days is classified as Category III. For Category IV substances, irritation clears within 24 hours. The U.S. Federal Hazardous Substances Act (FHSA) guideline for ocular irritation classification (CPSC 1995) categorizes a test substance as corrosive, irritant, or nonirritant. The definition of a corrosive, according to the FHSA, is a substance that causes visible destruction or irreversible alterations in the tissue at the site of contact (CPSC 2004). FHSA classification depends on the incidence of test animals exhibiting a positive ocular response within 72 hours after application of the test substance in the conjunctival sac. Hazard classification of ocular irritants in the European Union (EU) corresponds to two risk phrases: 1) R36 denotes “Irritating to eyes”; 2) R41 denotes “Risk of serious damage to the eyes” (EU 2001). These risk phrases are based on whether the levels of damage, averaged across the 24-, 48- and 72-hour observation times for each ocular lesion, fall within or above certain ranges of scores. For the purpose of harmonizing the classification of ocular irritants internationally, the GHS (UN 2003) includes two harmonized categories, one for irreversible effects on the eye/serious damage to the eye (Category 1), and one for reversible effects on the eye (Category 2). Reversible effects are further subclassified, based on the duration of

Table 1-2 *In Vivo* Ocular Irritancy Classification Systems

Regulatory Agency (Authorizing Act)	Number of Animals	Minimum Observation Times (after treatment)	Mean Score Taken?	Positive Response	Irritant/Nonirritant Classification
EPA (FIFRA; TSCA; and The Federal Environmental Pesticide Control Act)	At least 3	1 hour, 1, 2, 3, 7, 14, and 21 days	No	- Maximum score in an animal used for classification - Opacity or Iritis ≥ 1 or Redness or Chemosis ≥ 2	One or more positive animals needed for classification in categories below. <u>Category</u>: I = Corrosive, corneal involvement, or irritation persisting more than 21 days II= Corneal involvement or irritation clearing in 8-21 days III = Corneal involvement or irritation clearing in 7 days or less IV = Minimal effects clearing in less than 24 hours
European Union	**Current Directive:** 1 if severe effects are suspected or 3 if no severe effects are suspected **Prior Directive:** 3 or 6 animals used to assign risk phrases	1, 2, 3 days (observation until Day 21)	Yes	(1) <u>6 animals</u> Mean study values (scores averaged over all animals in study over Days 1, 2, and 3) of: Opacity or Chemosis ≥ 2, Redness ≥ 2.5, or Iritis ≥ 1 **OR** (2) <u>3 animals</u> Individual animal mean values (scores for each endpoint are averaged for each animal over Days 1, 2, and 3) of: Opacity or Chemosis ≥ 2, Redness ≥ 2.5, or Iritis ≥ 1	**R36 Classification** (1) Mean study value (when more than 3 animals are tested) where: 2 ≤ Opacity < 3 or 1 ≤ Iritis < 1.5 or Redness ≥ 2.5 or Chemosis ≥ 2 (2) If 2 of 3 tested animals have individual animal mean values that falls into one of the following categories: 2 ≤ Opacity < 3 1 ≤ Iritis < 2 Redness ≥ 2.5 Chemosis ≥ 2 **R41 Classification** (1) Mean study value (when more than three animals are tested) where: Opacity ≥ 3 or Iritis > 1.5 (2) If 2 of 3 tested animals have individual animal mean values that fall into one of the following categories: Opacity ≥ 3 or Iritis = 2 (3) At least one animal where ocular lesions are still present at the end of the observation period, typically Day 21.
GHS-Irreversible	3	1, 2, 3 days	Yes	Mean animal values (over	- At least 2 positive response animals = Eye Irritant Category 1

Regulatory Agency (Authorizing Act)	Number of Animals	Minimum Observation Times (after treatment)	Mean Score Taken?	Positive Response	Irritant/Nonirritant Classification
Eye Effects		(observation until Day 21)		Days 1, 2, and 3) of: Opacity ≥ 3 and/or Iritis ≥ 1.5	- At least 1 animal where Opacity, Chemosis, Redness, or Iritis > 0 on Day 21 = Eye Irritant Category 1
GHS-Reversible Eye Effects	3	1, 2, 3 days (observation until Day 21)	Yes	Mean animal values (over Days 1, 2, and 3) of: Opacity or Iritis ≥ 1 or Redness or Chemosis ≥ 2 and the effect fully reverses in 7 or 21 days	- At least 2 positive response animals and the effect fully reverses in 21 days = Eye Irritant Category 2A - At least 2 positive response animals and effect fully reverses in 7 days = Eye Irritant Category 2B
CPSC (FHSA [provided under the authority of the Consumer Products Safety Act]), FDA (Food, Drug, and Cosmetics Act), and OSHA (Occupational Safety and Health Act)	6 (12, 18 possible)	1, 2, 3 days (observation may be extended to 7 days)	No	Opacity or Iritis ≥ 1 or Redness or Chemosis ≥ 2 for any animal on any day	1 or more animals with destruction or irreversible alterations in the tissue at the site of contact = Corrosive 1st Tier: 4 or more positive animals = Irritant 2-3 positive animals = Go to 2nd Tier 1 positive animal = Negative 2nd Tier 3 or more positive animals = Irritant 1-2 positive animals = Go to 3rd Tier 3rd Tier 1 positive animal = Irritant

Abbreviations: CPSC = U.S. Consumer Products Safety Commission; EPA = U.S. Environmental Protection Agency; FDA = U.S. Food and Drug Administration; FIFRA = Federal Insecticide, Fungicide, and Rodenticide Act; GHS = United Nations Globally Harmonized System; OSHA = Occupational Safety and Health Administration; TSCA = Toxic Substances Control Act persistence as Category 2A ("irritating to eyes") (reverses within 21 days) and Category 2B ("mildly irritating to eyes") (reverses within seven days). The GHS (UN 2003) categories are based on severity of the lesions and/or the duration of persistence. The GHS, the U.S., and the EU *in vivo* ocular irritancy classification systems are described in greater detail in **Section 4.1.3**.

Concerns about animal welfare, the cost and time to conduct ocular irritation assessments, the reproducibility of the currently used *in vivo* rabbit eye test, as well as scientific interest in understanding eye injury at the tissue and cellular level have led researchers to develop and evaluate alternative *in vitro* test methods. Recently, the EPA requested the evaluation of four *in vitro* test methods -- Isolated Chicken Eye (ICE), Isolated Rabbit Eye (IRE), Hen's Egg Test – Chorioallantoic Membrane (HET-CAM), and Bovine Corneal Opacity and Permeability (BCOP) -- for their ability to identify ocular corrosives and severe irritants. As part of this evaluation process, a Background Review Document (BRD) has been prepared for each test method that describes the current validation status of the *in vitro* test method, including what is known about its reliability and accuracy, its applicability domain, the numbers and types of substances tested, and the availability of a standardized protocol.

This BRD evaluates existing data to determine the accuracy and reliability of the BCOP test method for identifying ocular corrosives and severe irritants. The BCOP assay is an *in vitro* eye irritation test method developed by Gautheron et al. (1992) as a modification of an earlier ocular irritation assay using isolated bovine eyes from cattle that have been slaughtered for meat or other purposes (Muir 1985). Gautheron et al. (1992) was interested in developing a reproducible, predictive *in vitro* test to evaluate the ocular irritancy of substances representing a variety of chemical and product classes. This test method developer focused on a cornea-based assay because the cornea is one of the main targets during accidental eye exposures, and damage to the cornea can result in visual impairment or loss. In addition, corneal effects are weighted heavily in the original *in vivo* ocular irritancy scoring systems (e.g., 80 out of a possible 110 points in the Draize eye test scoring system), and continue to be an ocular tissue observation on which current ocular hazard classification systems are based. Measurement of opacity in the isolated bovine cornea was initially investigated since it is the only corneal endpoint graded in many *in vivo* ocular irritancy assays. Opacity in the cornea, which is normally a transparent tissue, is a significant adverse effect of some irritants that can lead to a loss of vision. However, some known irritant substances, such as sodium lauryl sulfate and certain medium-length chained alcohols, destroy the corneal epithelium without producing significant opacity. Damage to the epithelium was subsequently quantified for these substances by measuring penetration of the dye sodium fluorescein through the isolated cornea, which is an adaptation of an *in vitro* technique previously described by Tchao (1988). Gautheron and colleagues refined the BCOP assay to measure both opacity and permeability, two important components of ocular irritation, and concluded that use of the two endpoints better predicted ocular irritancy (Gautheron et al. 1992; see **Section 9.0** for a review of these data).

In the BCOP assay, opacity is determined by the amount of light transmission through the cornea, and permeability is determined by the amount of sodium fluorescein dye that passes through all corneal cell layers. While these *in vitro* toxicity measurements using the isolated cornea are correlated with *in vivo* ocular irritation corneal effects, they represent only one aspect of the overall complex response of the eye to irritants, which involves other tissues such as the iris and conjunctiva. More recent additions/endpoints to the BCOP assay are assessment of corneal swelling or hydration, and histological assessment of morphological alterations in the cornea (Bruner et al. 1998; Ubels et al. 1998; Cooper et al. 2001; Jones et al. 2001). When histological assessment is added to the BCOP assay, the type and depth of corneal injury can be evaluated, as well as whether the tissue damage is permanent (e.g., damage to the endothelium) (Curren et al. 2000).

For current regulatory applications, the BCOP test method could potentially be used to identify the irreversible, corrosive, and severe irritation potential of products, product components, individual chemicals, or substances in a tiered testing strategy (e.g., GHS; UN 2003). In the GHS stepwise approach, substances that are predicted by BCOP as ocular corrosives or severe irritants could be classified as Category 1 eye irritants without the need for animal testing. Substances that are negative in BCOP for severe/irreversible effects would then undergo additional testing to confirm that they are not false negatives, and to determine the type, if any, of reversible effects that may occur. The test method also may be useful in a battery of *in vitro* eye irritation methods that collectively predicts the eye irritation potential of a substance *in vivo*. However, the predictivity of a battery approach will first require the assessment of the performance of each individual component.

The BCOP assay is currently used by some U.S. and European companies (e.g., pharmaceutical, cosmetic, and personal care product companies) as an in-house method to assess the ocular irritation potential of a wide range of substances or products (Gautheron et al. 1994; Sina 1994; Sina et al. 1995; Casterton et al. 1996; Chamberlain et al. 1997; Bailey et al. 2004; Cuellar et al. 2004; Swanson et al. 2004). For example, in some companies, materials that induce a high BCOP score are labeled as severe irritants (based on an internal hazard classification scheme) with no further testing. Materials that are predicted as nonirritants based on the BCOP assay are tested *in vivo* to confirm the *in vitro* results (Chamberlain et al. 1997). In another company, the BCOP assay is used to evaluate non-registered household products and registered household disinfectants, pesticides and repellents (Cuellar N and Swanson J, personal communication). For non-registered household products, the BCOP assay is used to predict the relative eye irritation potential of new consumer product formulations compared to benchmark substances, such as products on the market or substances for which the eye irritation potential is well characterized; *in vivo* confirmatory testing is generally not performed. For registered products, use of the BCOP assay is limited to product development issues and worker safety at this company.

Although the BCOP test method is not yet validated, the EU national regulatory authorities accept positive outcomes from this eye irritation test method for classifying and labeling severe eye irritants (R41). Where a negative result is obtained, an *in vivo* test is subsequently required, as BCOP has not been shown to adequately discriminate between eye irritants and nonirritants (EU 2004).

1.1.2 Peer Reviews of the BCOP Test Method

Studies have been conducted in recent years to assess the validity of the BCOP test method as a complete replacement for the *in vivo* ocular irritation and corrosion test method (e.g., Balls et al. 1995). Additionally, Gautheron et al. (1994) assessed the ability of the BCOP test method to identify severe ocular irritants as classified by the European Economic Community (EEC 1984) classification system. Previous validation efforts may have failed because: 1) they attempted to support the utility of an *in vitro* alternative as a full replacement for the *in vivo* rabbit test, rather than as a component in a tiered testing strategy; and/or, 2) data generated with the *in vitro* test method(s) have typically been compared to *in vivo* maximum average scores (MAS).

However, there have been no formal evaluations of the ability of the BCOP test method to identify ocular corrosives and severe irritants, as defined by the GHS and the EPA. This BRD was prepared for use by an Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) expert panel review of the BCOP assay as a method to identify ocular corrosives and severe irritants. Parallel reviews of the ICE, IRE, and HET-CAM test methods were also conducted. Results of the Expert Panel Report, combined with the analyses presented in the BRDs, were used to support ICCVAM recommendations on the proposed standardized test method protocols, proposed list of recommended reference substances, and additional optimization and/or validation studies that may be necessary to further develop and characterize the usefulness and limitations of these methods.

1.2 Scientific Basis for the BCOP Test Method

1.2.1 Purpose and Mechanistic Basis of the BCOP Test Method

The BCOP is an organotypic model (i.e., isolated whole organ, or component thereof) that provides short-term maintenance of normal physiological and biochemical function of the cornea in an isolated system (Chamberlain et al. 1997). As noted above, the BCOP was developed as an alternative eye irritation test method in order to obviate the need for laboratory animals as the source for test eyes.

The most commonly used endpoints evaluated in the BCOP assay to measure the extent of damage to the cornea following exposure to a chemical substance are corneal opacity and permeability. Opacity is quantitatively measured by the amount of light transmission through the cornea, and permeability is quantitatively measured as the amount of the small molecule, sodium fluorescein, that penetrates all corneal cell layers. Irritant-induced opacity in the cornea indicates denaturation/precipitation of proteins in the epithelial or stromal layers and/or swelling, vacuolization, or damage to the cells in the stromal layer (Millichamp 1999). Development of opacity in the cornea, which is normally a transparent tissue, is a significant adverse effect of some irritants that can lead to vision loss. Increased corneal permeability results from damage to the corneal epithelium, which normally serves a barrier function. In addition, histopathological evaluation of the treated cornea provides useful descriptive information of corneal damage (Curren et al. 2000; Cooper et al. 2001).

Histopathology or confocal microscopy would allow for a more accurate assessment of the extent of corneal injury. Maurer et al. (2002) proposed that the extent of ocular injury, as measured by confocal microscopy, has the greatest impact on the outcome of such an injury. Live/dead cell staining methods evaluated with confocal microscopy have also been used to determine the extent or depth of corneal injury *in vivo* (Maurer et al. 1997) and in an *ex vivo* corneal button assay (Jester et al. 2001). These studies prompted the authors to suggest that the extent of corneal injury could be used as the basis for developing alternative methods to predict the level of damage produced by ocular irritants.

1.2.2 Similarities and Differences of Modes of Action Between the BCOP Test Method and Ocular Irritancy in Humans and/or Rabbits

1.2.2.1 *The Mammalian Eye: Common Anatomy of the Human, Rabbit and Bovine Eye*

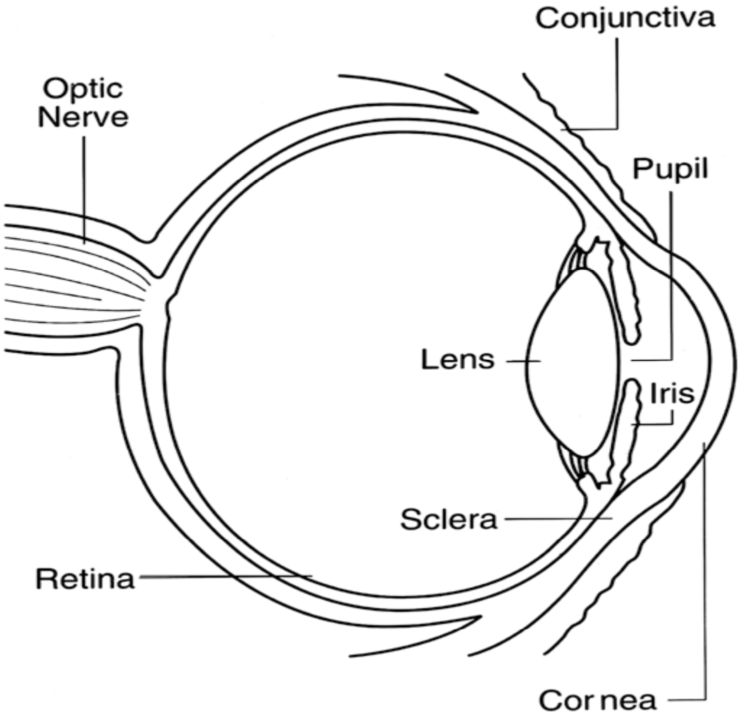
The eyeball is a fibrovascular globe, which is surrounded by a bony orbit that is impenetrable to light (Bruner 1992). The anterior portion of the eyeball is the only portion that is exposed to the environment, while the remainder of the eye is protected by the eyelids and the bony orbit. The eyeball is composed of three concentric tunics (the fibrous tunic, the vascular tunic, and the neuroectodermal tunic) that can be further subdivided. The fibrous tunic is the outermost layer of the eye comprised of the transparent cornea and the opaque sclera. The middle vascular tunic is comprised of the choroids, the ciliary body, and the iris (which can be referred to as the uvea). The neuroectodermal tunic is the innermost layer and is comprised of the retina, which contains photoreceptors and is connected to the central nervous system (Wilkie and Wyman 1991; Bruner 1992).

The fibrous tunic provides the primary framework for the eye. The cornea is the transparent surface of the eye, and is comprised of three major layers: the epithelium, the stroma, and the endothelium (**Figure 1-1**). The human cornea is a hydrated, nonvascularized structure. The corneal stroma contains 78% water and hydration is a requisite for the capacity of the stroma to swell in response to an irritant (Duane 1949). The cornea is nutritionally maintained in a homeostatic state by the aqueous humor, tear film, and the surrounding vascularized tissues. Proper function of squamous or cuboidal cells in the endothelial layer is required to remove water from the cornea.

The cornea is the major refracting element in the optical path, which flows from the light source through the cornea (70% of refractive power) to the lens (30% of refractive power) and into the retina (Duane 1949; Mishima and Hedbys 1968a). Therefore, corneal transparency is an important factor in optimal eye functioning. For maximum refractive power, the anterior surface of the cornea, composed of layers of translucent epithelial cells, is maintained in a smooth configuration by the tear film. The corneal stroma, composed of translucent keratocytes interspersed with collagen fibrils, requires uniformity and proper spacing of the collagen fibrils to maintain an appropriate corneal refractive index with minimal light scattering (Maurice 1957). This combination of structure and cellular morphology serves to maintain corneal transparency.

The eye is critically dependent on the highly vascularized middle coat (uvea) for regulation of blood and ocular permeability barriers, maintenance of intraocular pressure in the aqueous humor, and drainage of ocular fluid (Unger 1992). The uveal tract is richly innervated by somatic sensory neurons, derived from the ophthalmic division of the trigeminal nerve. Importantly, alterations to any of these features (e.g., edema, cell destruction, vascularization,

Figure 1-1 Anatomy of the Human Eye



Figured obtained at http://www.nei.nih.gov/photo/eyean/index.asp

cell proliferation) can cause corneal opacity and concomitant loss of function (Parish 1985; Wilkie and Wyman 1991; Bruner 1992).

The sclera is comprised primarily of three layers of irregularly arranged collagen fibrils of varying diameter. The irregular arrangement of the fibrils produces the white color that is seen on eyeballs. The conjunctiva is a mucous membrane that covers the exposed scleral surface (bulbar conjunctiva) and the inner surface of the eyelids (palpebral conjunctiva). The conjunctiva contains blood vessels, nerves, conjunctival glands, and inflammatory cells. As part of the inflammatory response in the conjunctiva, dilation of the blood vessels, fluid leakage, and cellular leakage occurs (Bruner 1992).

The major component of the vascular tunic is the iris. The iris sits in front of the lens and the cilliary body, which also are considered part of the vascular tunic. Contraction of the iridal muscles alters the diameter of the pupil and thus regulates the amount of light entering the eye (Bruner 1992).

1.2.2.2 *Differences Between Human, Rabbit and Bovine Eyes*

There are several anatomical and physiological differences between the rabbit eye and the human eye. One difference is the presence of a nictitating membrane, or third eyelid, in the rabbit. As this membrane slides horizontally across the eye, it is proposed that it aids removing and/or excluding irritating substances from the corneal surface (Calabrese 1983). It also is proposed that the kinetic removal of a substance from a rabbit eye may occur at a rate different than in humans, due to the presence of the nictitating membrane, although this has not been documented in comparative studies (Curren and Harbell 1998). Another difference is the larger conjunctival sac in the rabbit, which allows for larger test volumes to be instilled, perhaps more than could be accounted for on accidental exposure (Curren and Harbell 1998).

There are also some species differences in morphology of the cornea that could have an effect on the response of the isolated cornea to irritants. In different species, the cornea is known to vary in thickness. For example, the corneal thickness of the bovine eye is 0.8 mm, while that of the human eye is approximately 0.5 mm , and the rabbit eye is about 0.37 mm (Chan and Hayes 1985). The number of epithelial cell layers in the cornea ranges from five to seven in rabbits, compared to an average of five in humans and 10 to 14 in cattle (Cooper et al. 2001). The thicknesses of structural components of the cornea also are different between species. For example, Descemet's membrane is proposed to be about 5 to 10 μm in humans and 7 to 8 μm in rabbits (Calabrese 1983). Furthermore, the area of the cornea in relation to the total surface of the globe varies significantly between species; in humans, the relationship is 7%, while in rabbits the relationship is 25% (Swanston 1985). The Bowman's layer is well developed in humans, but it is not present to any great degree in cattle or rabbits. Finally, young rabbits have the ability to regenerate damaged corneal endothelium, while humans do not (Chambers W, personal communication). While there are known anatomical differences between human, rabbit, and bovine corneas, studies have not been found that compare the response of bovine, rabbit, and human corneas to irritants.

The relationship between species differences in eye anatomy and physiology and the sensitivity to ocular irritants has not been clearly established. It has been proposed that the larger conjunctival sac, thinner cornea, larger proportion of the cornea to the eyeball, as well as other differences in the rabbit eye, lead to an increased sensitivity to irritants (Calabrese 1983; Swanston 1985). However, other differences (e.g., the presence of the nictitating membrane, low blink frequency rate) indicate that the rabbit is as sensitive as humans to irritants. Comparisons of human exposure experiences to results in the *in vivo* test method indicate that in some cases the rabbit eye is more sensitive to some irritants, while in other cases the human eye is more sensitive (McDonald et al. 1987).

1.2.2.3 *The In Vivo Rabbit Eye Test Method*

The current *in vivo* rabbit eye irritation test method evaluates the cornea, the iris, and the conjunctiva for adverse effects after exposure to a potential irritant (see **Section 4.0** for a discussion of the *in vivo* scoring system for lesions at these sites). The cornea is visually observed both for the degree of corneal opacity and the area of the cornea in which opacity is involved. The iris is assessed for inflammation, iridal folds, congestion, swelling, circumcorneal injection, reaction to light, hemorrhage, and gross destruction. The conjunctiva is evaluated for the degree of redness, chemosis (swelling), and discharge (Draize et al. 1944). Draize and colleagues (1944) developed an analysis method where the severities of the effects are weighted differently; with corneal effect being weighted the most. The effects of a test substance on the cornea, conjunctiva, and iris play a role in severe ocular irritant and corrosive labeling and classification of severe ocular irritants and corrosives in the hazard classification systems used by some regulatory agencies (CPSC 1995; EPA 1998; EU 2001; UN 2003).

Irritation responses and the degree of the response in the cornea, iris, and conjunctiva differ due to the specific functions and anatomy of each structure. Development of slight corneal opacity can be due to loss of superficial epithelial cells and epithelial edema. Comparatively, more severe corneal opacity may be observed if an ocular irritant produces its effects deeper in the cornea. The ensuing repair process can lead to scar development in the cornea and vision impairment. Irritation responses in the iris are typically due to direct exposure to a substance, which has passed through the cornea and sclera, or due to extension of significant surface inflammation. Acute inflammation of the uvea tract is characterized by edema, vessel dilation, and the presence of exudates, while severe inflammation of the uvea tract is characterized by accumulation of blood or leukocytes in the anterior chamber. Conjunctival inflammatory responses can produce vasodilation, edema, subconjunctival hemorrhage, and lacrimal secretions (Bruner 1992).

The extent of corneal injury resulting from an ocular irritant also is dependent on the physicochemical characteristics (e.g., acids and bases with pH extremes, solvent-induced protein or DNA precipitation, surfactant-induced saponification of membranes), and chemical reactivity of the substances when in contact with individual ocular cells or structures (e.g., alkylation, hydrolysis, oxidation, reduction, hydroxylation, etc.) (Grant 1974; McCulley 1987; Berta 1992; Nourse et al. 1995; Fox and Boyes 2001). Direct or indirect ocular injury may result from the impact of these physicochemical effects on normal homeostatic cellular mechanisms and from consequent edema, inflammation, apoptosis, necrosis, and reparative processes (e.g., collagen deposition and scarring) (Unger 1992; Pfister 2005). In the normal eye, test substances may disrupt the tear film, reach the epithelium, and penetrate through Bowman's layer into the stroma, through Descemet's membrane, and into the endothelium (Pasquale and Hayes 2001). Damage to the endothelium may be irreparable.

The tear film consists of an inner layer of mucous, a middle layer of water, and an outer film of oil. The tear film contains lactoferrin, peroxidase, lysozyme, immunoglobulins and complement factors to eliminate potentially offensive material (Unger 1992). In conjunction with the neurogenically controlled blink reflex and tear producing cells, the tear film serves as a protective barrier against an ocular irritant for the corneal epithelium. The physicochemical properties (e.g., hydrophilicity, hydrophobicity, hypertonicity, hypotonicity, oxididation, reduction) in addition to the chemical and biochemical properties of an applied test substance impact its ability to breach the tear film, or interact with its components and impact the corneal epithelium. The tear film and the aqueous humor also provide nourishment (e.g., glucose and oxygen) to the nonvascularized cornea. The extent of damage to the tear film by an applied substance therefore impacts the ability of the tear film to nourish dependent corneal tissue. Changes in the distribution, physical structure, or secretion rate of the tear film by an applied test substance might have significant nutritional, refractory, chemical and physical impacts on corneal tissue (Mishima and Hedbys 1968a, 1968b).

Either direct (e.g., caustic or corrosive) or indirect (e.g., inflammatory mediator release) effects of chemicals in contact with the anterior corneal surface may result in perturbation of the optical elements needed to maintain the appropriate index of refraction in the cornea (e.g., uniformity and proper spacing of collagen fibrils), resulting in significant light scattering and impairment of vision (McCulley 1987; Berta 1992; Nourse et al. 1995; Wilson et al. 2001). Corneal injury may result in opacification, swelling, damage extending from the epithelium into the stroma or possibly through the endothelium, and changes in corneal morphology (e.g., ulceration, scarring, pitting, mottling).

Opacification of the cornea may result from: 1) direct or indirect damage to the epithelial cells with or without penetration into the stroma; 2) protein denaturation of the epithelial cells such as that produced by alcohols, alkalis, or organic solvents; 3) alkylation of protein or DNA; 4) membrane saponification by surfactants, 5) inflammatory cell infiltration; 6) collagen deposition; 7) swelling of corneal epithelial cells or corneal stroma; 8) displacement or rearrangement of collagen fibrils; or 9) degradation of the extracellular matrix (Grant 1974; Thoft 1979; York et al. 1982; McCulley 1987; Fox and Boyes 2001; Kuckelkorn et al. 2002; Eskes et al. 2005; Pfister 2005).

Corneal swelling results from disruption of the anterior barrier membrane formed by the epithelial cell layer and Bowman's layer. This results in disruption of stromal collagen fibril uniformity, loss of proteoglycans, cell death, which leads to bullae formation, stromal cloudiness, and increased hydrostatic pressure (which may extend posteriorly throughout the corneal stroma, penetrating into Descemet's layer and into the endothelium) (Mishima and Hedbys 1968a, 1968b). Osmotic changes induced by these effects may further damage keratocytes and the collagen matrix.

Corneal damage also may be characterized by morphological changes (e.g., described as stippling, ulceration, mottling, pannus, neovascularization). Corneal injury also is dependent on the type and concentration of applied chemical. Alkalis penetrate more readily than acids do, and the depth of penetration is dependent on alkali concentration (McCulley 1987). With alkali injury, the hydroxyl ion saponifies the fatty acid components of the cell membrane, disrupting cellular contents and resulting in cell death. The cation is responsible for the penetration process (Grant 1974). Acids tend to penetrate less deeply than alkalis, with the exception of hydrofluoric and sulfuric acids. The hydrogen ion causes damage due to pH alteration, while the anion precipitates and denatures protein in the corneal epithelium and superficial stroma (Freidenwald et al. 1946). Limbal ischemia is a significant consequence of even mild alkali or acid burns (Kuckelkorn et al. 2002).

While not in the direct optical path, the Palisades of Vogt, located in the sclero-corneal limbus, are thought to house corneal stem cells and serve as a generative organ for normal replacement of dead corneal epithelial cells for re-epithelialization during repair of corneal injury. Depletion or partial loss of the limbal stem cell population may result in corneal vascularization due to loss of the barrier function of the limbus, which serves to prevent conjunctival epithelial cells from migrating to the corneal surface (Dua and Azuara-Blano 2000).

Neutrophils are recruited in response to acid and alkali injury as well as in response to other ocular toxicants (Pfister 2005). Neutrophil migration is stimulated by the release of chemotatic factors (e.g., interleukins, growth factors, etc.) from damaged or chemically activated local resident epithelial cells or stromal keratocytes (Wilson et al. 2001). Loss of keratocytes following either chemical or mechanical epithelial injury may be mediated by apoptosis, perhaps by release of IL-1 and TNFα (Wilson et al. 2001). Resident mast cells may release biogenic amines that perturb the hydrostatic balance and permit inflammatory or edemagenic mediators into the locally inflamed area. Migrated neutrophils release additional cytokines (e.g., IL-1 and TNF-α) and enzymes such as proteases, collagenases, kinases, and phospholipaseA2 (PLA2). PLA2 produces edemagenic and vasoactive mediators such as prostaglandins and leukotrienes from arachidonic acid in cellular membranes.

This cascade of events ultimately facilitates repair by stimulating fibrin deposition and granuloma formation. However, migrating inflammatory cells such as neutrophils also may be involved in the release of collagenases (e.g., matrix metalloproteinases [MMPs]), which have been implicated in corneal ulcer formation. Acetylcysteine, L-cysteine, and EDTA have been shown to reduce corneal ulceration in response to alkali injury while inhibiting MMPs (Pfister 2005). Other inflammatory cells such as macrophages and T-lymphocytes may be found up to 24 hours after injury. Once an area is damaged and devoid of keratocytes, proliferation and migration occurs as part of the wound healing process. This process may be mediated in part by numerous growth factors (Wilson et al. 2001).

Although variable responses occur among species, neuropeptides (e.g., Calcitonin Gene Related Peptide [CGRP] and substance P) have profound effects on the anterior portion of the highly innervated eye, particularly in lower mammals such as the rabbit (Unger 1992). CGRP appears to affect vascular smooth muscle (Oksala and Stjernschantz 1988), whereas substance P may be involved in meiosis (Unger 1990). Loss of functional sympathetic innervation reduces or eliminates presynaptic catecholamine reuptake sites resulting in denervation supersensitivity. This also may result in enhanced sensitivity to noxious stimuli.

Applied test substances also can adversely affect homeostasis within the cornea. As oxygen is absorbed into the cornea from the atmosphere, interference with oxygen uptake may lead to corneal swelling (Mishima and Hedbys 1968a, 1968b). The cellular respiratory needs of the endothelium and epithelium are similar, both requiring carbohydrate metabolism. Glucose metabolism in the cornea occurs by glycolysis and oxidation through the tricarboxylic acid cycle as well as through the hexose-monophosphate shunt (Kinoshita 1962). Glucose within the cornea is used to supply glycogen, which is stored in the epithelium. Applied substances that modulate any of these processes may be associated with ocular toxicity.

1.2.2.4 *Comparison of BCOP Test Method with the In Vivo Rabbit Eye Test Method*

In the BCOP test method, damage to the isolated cornea is assessed by measuring corneal opacity and permeability in a short-term test that typically takes less than 8 hours to perform. The two endpoints are measured quantitatively with an opacitometer and an ultraviolet/visible (UV/VIS) spectrophotometer, respectively, at two or four hours after exposure to a test substance, depending on the physical properties of the substance tested.

Depending on the physicochemical properties of the test substance, post-exposure measurements may be extended to 24 hours (e.g., for substances with delayed responses). In contrast, the *in vivo* rabbit eye test involves a qualitative visual evaluation of the severity of adverse effects on the cornea, the iris, and the conjunctiva, as well as the reversibility of any ocular effects detected at selected intervals up to 21 days after exposure. In BCOP, liquids are usually applied undiluted for 10 minutes, then rinsed off the cornea, followed by a 2-hour incubation of the cornea in assay medium. Solids are usually applied as a suspension or solution (20%) for four hours, then rinsed off the cornea before opacity and permeability measurements are performed. Whether the test substance is a liquid or a solid, the entire cornea is exposed for a specified duration. In the *in vivo* rabbit eye test, liquid and solid test substances are applied to the conjunctival sac, usually in an undiluted form. Because the rabbit eye can blink and/or tear, exposure of the cornea to the test substance will be affected by these factors in terms of coverage or duration. The neurogenic components that drive tear film production are not present in the BCOP. When compared with an *in vivo* rabbit eye study, application of a test substance in the absence of this protective barrier might be expected to cause an increase in false positive outcomes. One of the conclusions from a workshop on mechanisms of eye irritation highlighted the need for additional research on the impact of chemicals on tear film and the consequences of tear film disruption (Bruner et al. 1998). Protective mechanisms for the eye (e.g., blinking, tear film) are built into *in vivo* testing, but are absent in *in vitro* testing. However, note that for some test substances (e.g., solids), blinking can also induce mechanical damage *in vivo*, contributing to a higher degree of irritation. Thus, the BCOP test method differs from the *in vivo* rabbit eye test method in the following significant ways:

- The BCOP evaluates only corneal effects and does not assess effects on the iris and the conjunctiva as performed in the *in vivo* rabbit eye test. Measurements are performed quantitatively in the BCOP assay, while they are assessed with qualitative observations in the *in vivo* rabbit eye test.
- Corneal exposure conditions, including test substance concentration and exposure duration, are well controlled in the BCOP assay, but subject to potentially greater variation *in vivo,* due in part to the blink response and natural tearing of the eye in a live animal.
- Reversibility/irreversibility of corneal effects induced by a test substance cannot be observed in the BCOP assay, *per se*, but histological evaluation of the exposed cornea may provide additional information about the depth and type of injury that could aid predictions, as to whether damage is irreversible (Harbell J, personal communication). Maurer et al. (2002) have shown that that type and depth of ocular injury are good predictors of the degree and duration of injury.
- The observation period of the BCOP assay is typically less than 24 hours, whereas ocular effects are typically evaluated in the *in vivo* rabbit eye test for a minimum of 72 hours and can extend up to 21 days.
- Protective mechanisms of the eye, such as tear production and blinking, are built into *in vivo* testing, but are absent in *in vitro* testing.
- The BCOP assay does not account for systemic effects following ocular instillation that may be noted with the *in vivo* rabbit eye test (e.g., toxicity or lethality as in the case of certain pesticides). However, these effects are

typically predicted from other acute toxicity test methods, and may not be relevant for the many consumer products that are formulated with well-characterized raw materials of known systemic toxicity.

1.2.3 Intended Range of Substances Amenable to the BCOP Test Method and/or Limits of the BCOP Test Method

Studies indicate that the BCOP test method is amenable to use with a broad range of substances with a few limitations. Substances amenable to testing include, but are not limited to, inorganic chemicals; aliphatic, aromatic, and heterocyclic chemicals; and mixtures/formulations (Gautheron et al. 1994; Balls et al. 1995; Sina et al. 1995; Gettings et al. 1996). While a wide range of substances with various physicochemical characteristics can be tested in the BCOP assay, water insoluble solid substances that are less dense than water (i.e., float on top of the solvent) do not adequately contact the cornea during treatment (Sina and Gautheron 1998). Colored test substances may be problematic as they could interfere with the opacity and/or permeability measurements.

Chamberlain et al. (1997) noted some false negative responses in the BCOP assay for substances with a delayed onset of irritation *in vivo.* However, these BCOP data were obtained using a 10-minute exposure/2-hour post-exposure protocol for liquids and a 4-hour exposure/post-exposure protocol for solids. It has been noted by some investigators that extending the post-exposure incubation time of the BCOP assay to 24 hours, and adding histopathological evaluation identifies some chemicals and formulations that produce a delayed onset of corneal damage (e.g., reactive chemicals, such as sodium percarbonate and hydrogen peroxide; Gran et al. 2003).

Additionally, some false positive responses have been noted for certain highly volatile solvents when tested using a 10-minute exposure/2-hour post-exposure protocol for liquids (Gautheron et al. 1994). More recent studies show that using a 3-minute/2-hour post-exposure protocol for volatile solvents provides a better prediction of *in vivo* results for some of these substances (Cuellar et al. 2004). Thus, as experience has been gained with the BCOP assay, practitioners have found that modifying the exposure and post-exposure times for certain substances improves the assay's predictive capability relative to results from the *in vivo* rabbit eye test.

1.3 Regulatory Rationale and Applicability

1.3.1 Current Regulatory Testing Requirements and ICCVAM Prioritization Criteria

The following section reviews and summarizes the extent to which the five ICCVAM prioritization criteria apply to the BCOP assay (ICCVAM 2003).

Criteria 1. The extent to which the proposed test method is (a) applicable to regulatory testing needs, and (b) applicable to multiple agencies/programs.

The BCOP assay has been proposed as a method to identify ocular corrosives or severe irritants, as is required by several U.S. laws. **Table 1-1** identifies the U.S. agencies and programs, which classify and label substances for eye irritation and corrosion. These agencies are the FDA, the EPA, Department of Agriculture, Department of Labor, the

Consumer Products Safety Commission (CPSC), and the Chemical Safety and Hazard Investigation Board. Therefore, the proposed use of the BCOP test method is applicable to the regulatory testing needs of multiple U.S. Federal agencies and programs.

Criteria 2. Warranted, based on the extent of expected use or application and impact on human, animal, or ecological health.
Current regulatory testing needs require the *in vivo* assessment of the eye irritancy or corrosivity hazard associated with the use of chemicals/products for labeling purposes. These testing needs require the use of laboratory rabbits. Alternative *in vitro* eye irritation and corrosion test methods could be applied to these testing needs.

Criteria 3. The potential for the proposed test method, compared to current test methods accepted by regulatory agencies, to (a) refine animal use (decreases or eliminates pain and distress), (b) reduce animal use, or (c) replace animal use.[2]
The BCOP test method has the potential to refine or reduce animal use in eye irritation testing. The BCOP test method was designed to use an animal species that is routinely used in the food industry (cattle) and that are routinely slaughtered for other purposes (e.g., food consumption). Substances that are identified as ocular corrosives or severe irritants would be excluded from testing *in vivo*, which would reduce the number of rabbits used for ocular testing and also spare animals the pain and distress of exposure to severe eye irritants.

Criteria 4. The potential for the proposed test method to provide improved prediction of adverse health or environmental effects, compared to current test methods accepted by regulatory agencies.
Based on its long history of use and acceptance by U.S. Federal and international regulatory agencies, the current system of ocular hazard assessment, which is based on the rabbit eye test (i.e., CPSC 1995; EPA 1998; OECD 2002), appears to have adequately protected public health. However, use of the rabbit eye test to predict the ocular irritation potential of substances for humans is not without controversy (e.g., intra- and inter-laboratory variability, qualitative evaluation of ocular lesions). The accuracy of the currently used *in vivo* rabbit eye test for predicting severe eye irritants in humans and the limitations of the method for predicting the irritancy of specific chemical and/or product classes are not known due to the lack of comparative data. Therefore, the potential of the proposed test method to provide improved prediction of adverse human health effects is unknown.

Criteria 5. The extent to which the test method provides other advantages (e.g., reduced cost and time to perform) compared to current methods.
Under certain circumstances, the BCOP test method could reduce the time needed to assess a substance, 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 and can

---

[2] Refinement alternative is defined as a new or revised test method that refines procedures to lessen or eliminate pain or distress to animals, or enhances animal well-being; Reduction alternative is defined as a new or revised test method that reduces the number of animals required; Replacement alternative is defined as a new or revised test method that replaces animals with nonanimal systems or one animal species with a phylogenetically lower one (e.g., a mammal with an invertebrate) (ICCVAM 1997).

be extended up to 21 days, while the standard BCOP test method can be completed in about five hours for liquid substances and seven hours for solids. However, it should be noted that the rabbit eye test may be completed within four hours for corrosive or severe irritants that produce severe lesions shortly after application to the rabbit eye, since animals should be killed for humane reasons. Additionally, the time required to perform the BCOP test method may be increased up to 24 hours when extended exposure or post-exposure times are used, or up to a week or more when histopathology is conducted. Histopathology significantly increases the time required to complete the BCOP assay, since additional time is needed for technicians to fix, process, section, and stain the corneal tissue, and for a qualified pathologist to evaluate and grade the corneal lesions.

Regarding comparative costs (based on conducting GLP compliant studies), the standard BCOP assay conducted with concurrent positive and negative controls costs $1400 per test substance at IIVS (Harbell J, personal communication). A histological evaluation, which includes photographs of tissue sections of treated corneas, as well as negative and control corneas, can be added for an additional $650-$850 per sample. A more involved GLP compliant BCOP study for one sample with benchmarks and histology costs about $4,500, which includes two time courses and one benchmark (Cuellar N and Swanson J, personal communication). The current cost of a GLP compliant EPA OPPTS Series 870 Acute Eye Irritation test (EPA 1998) or OECD Test Guideline 405 test (OECD 2002) at MB Research Laboratories (Spinnerstown, Pennsylvania) ranges from $765 for a 3 day/3 animal study up to $1665 for a 21 day/3 animal study (MB Research Laboratories, personal communication). While the cost of the BCOP assay includes concurrent positive controls, the *in vivo* rabbit test method does not include equivalent controls. One company notes that the turnaround time from initiation of the study to receipt of the final report is similar for the BCOP assay and the *in vivo* rabbit eye test (Cuellar N and Swanson J, personal communication).

1.3.2 Intended Uses of the Proposed BCOP Test Method

In vitro ocular irritation testing methods (e.g., ICE, IRE, BCOP, and HET-CAM) have been proposed for identification of ocular corrosives and severe irritants (e.g., Ocular Irritant Class I per the EPA classification system [EPA 1996], Ocular Irritant Class R41 per the EU classification system [EU 2001], or Ocular Irritant Class 1 per the GHS classification system [UN 2003]).

1.3.3 Similarities and Differences in the Endpoints Measured in the Proposed Test Method and the *In Vivo* Reference Test Method

As mentioned in **Section 1.1.1**, the *in vivo* rabbit eye test method in current use by U.S. Federal and international agencies is based on a method developed by Draize and colleagues in 1944. This test method involves instillation of the test substance into the lower conjunctival sac of the rabbit eye, and evaluates the cornea, the iris, and the conjunctiva for adverse effects after exposure to the potential irritant. The cornea is evaluated both for the degree of corneal opacity and the area of the cornea in which opacity is involved. The iris is assessed for inflammation, iridal folds, congestion, swelling, circumcorneal injection, reaction to light, hemorrhage, and gross destruction. The conjunctiva is evaluated for the degree of redness, chemosis (swelling), and discharge (Draize et al. 1944).

As detailed in **Section 1.2.1**, the BCOP test method evaluates only corneal effects to measure the extent of an irritant response. Corneal opacity is the only common endpoint shared between the BCOP and the *in vivo* rabbit eye test. However, this shared endpoint is evaluated differently in the two test methods. Corneal opacity is measured quantitatively with the aid of instrumentation (i.e., opacitometer or spectrophotometer) in the BCOP assay, while it is evaluated qualitatively by trained laboratory personnel in the *in vivo* rabbit eye test method. For the BCOP test method, opacity is measured on a continuous scale (e.g., 0 to 500), while for the *in vivo* rabbit eye test, opacity is graded on a discrete scale for which the only possible values are 0 for no opacity, 1 for scattered or diffuse areas of opacity, 2 for easily discernible translucent areas, 3 for nacreous areas, and 4 for complete corneal opacity.

1.3.4 Use of Proposed Test Method in Overall Strategy of Hazard or Safety Assessment

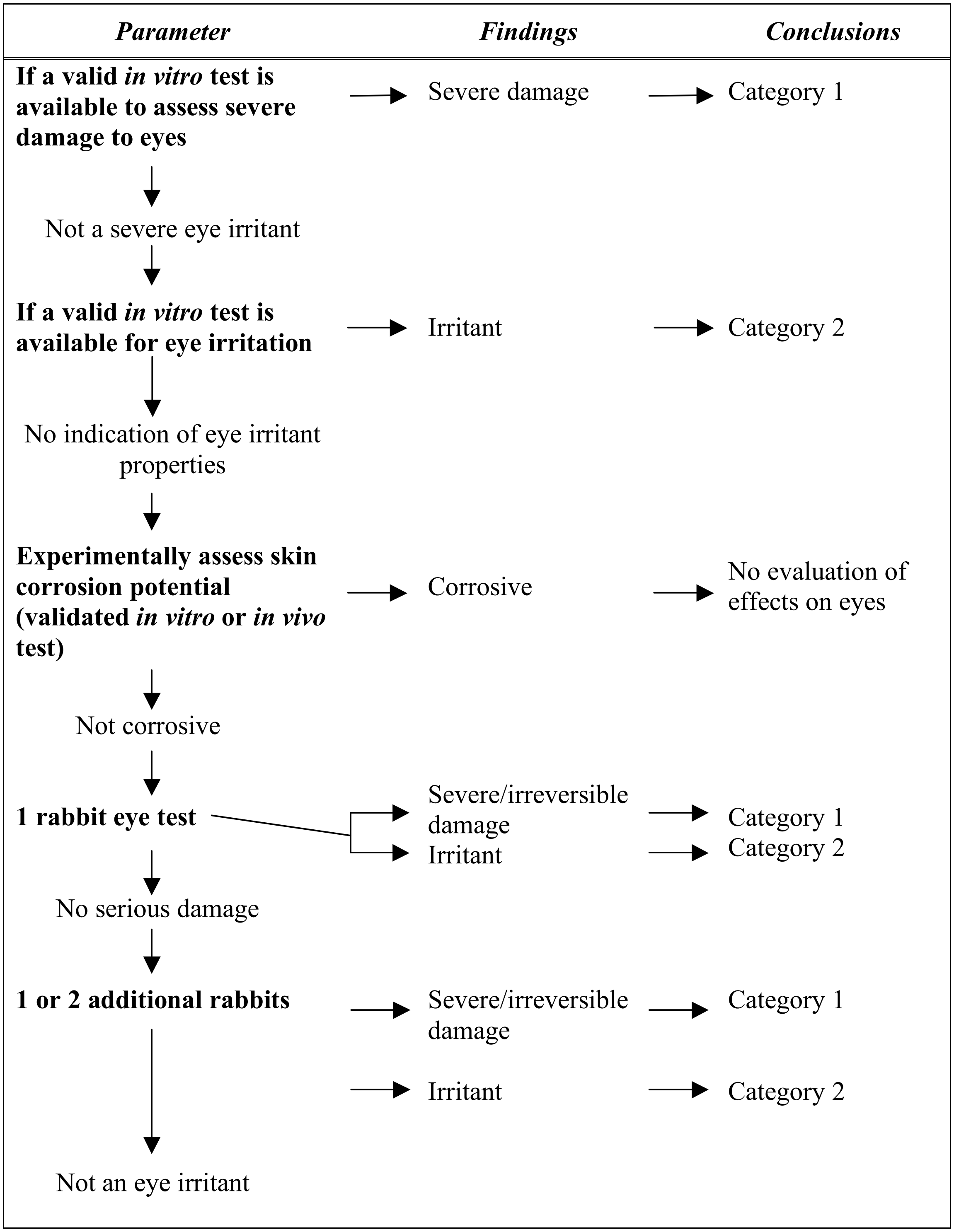
The BCOP test method is being considered for use in the identification of ocular corrosives and severe irritants in a tiered testing strategy (e.g., GHS; UN 2003). The GHS proposes a tiered testing and evaluation strategy for serious eye damage and eye irritation using available data from dermal irritation studies, knowledge of structure activity relationships, and pH screening. As shown in **Figure 1-2**, the GHS also allows for use of validated and accepted *in vitro* methods to identify severe ocular irritants/corrosives without further testing. If a test substance is classified in a validated *in vitro* method as an ocular corrosive or severe irritant, then no further testing would be required and the test substance would be appropriately labeled. If a test substance is not classified as an ocular corrosive or severe irritant using a validated *in vitro* method (i.e., the test substance remains unclassified), then current regulatory agency regulations for ocular testing would be followed. It is noted that the current testing strategy is proposed for use for regulatory classification and labeling purposes.

1.4 Validation of the *In Vitro* BCOP Test Method

The ICCVAM Authorization Act (Sec. 4(c)) mandates that "[e]ach Federal Agency … shall ensure that any new or revised … test method … is determined to be valid for its proposed use prior to requiring, recommending, or encouraging [its use]." (Public Law [P.L.] 106-545).

Validation is the process by which the reliability and relevance of an assay for a specific purpose are established (ICCVAM 1997). Relevance is defined as the extent to which an assay will correctly predict or measure the biological effect of interest (ICCVAM 1997). For the BCOP test method described in this BRD, relevance is restricted to how well the assay identifies substances that are capable of producing corrosive or severe irritant effects to the eye. Reliability is defined as the reproducibility of a test method within and among laboratories and should be based on performance with a diverse set of substances that are representative of the types of chemical and product classes that are expected to be tested and the range of responses that needs to be identified. The validation process will provide data and information that will allow U.S. Federal agencies to develop guidance on the development and use of the BCOP test method as part of a tiered testing approach to evaluating the eye irritation potential of substances.

Figure 1-2 GHS Testing Strategy for Serious Eye Damage and Eye Irritation



Adapted from UN (2003).

The first stage in this evaluation is the preparation of a BRD that presents and evaluates the relevant data and information about the assay, including its mechanistic basis, proposed uses, reliability, and performance characteristics (ICCVAM 1997). This BRD summarizes the available information on the various versions of the BCOP test method that have been published. Where adequate data are available, the qualitative and quantitative performances of the assays are evaluated and the reliability of each version of the test method is compared with the reliability of the other versions. If there are insufficient data to support the recommendation of a standardized protocol for BCOP, this BRD will aid in identifying essential test method components that should be considered during its development and validation.

1.5 Search Strategies and Selection of Citations for the BCOP BRD

An online search of entries in MEDLINE, TOXLINE, Web of Science, and STN International was conducted to retrieve database records on publications reporting on *in vitro* testing of substances for their ocular irritancy potential using the BCOP test method. The search was conducted in the database basic index, which includes words in the title and abstract, and indexing words. Specifically, records were sought containing the keywords "bovine" and "cornea or corneal" and "opacity" and "permeability" or "BCOP". Each database record included authors, bibliographic citation, and indexing terms. Most records also included abstracts. Of the 58 records obtained from the literature search in November 2003 (last updated in October 2004), 18 contained results and protocol information from a BCOP test method, nine were review articles, and seven were background articles related to the BCOP test method. Abstracts of selected titles were reviewed, and the relevant articles were selected and retrieved from the literature for analysis. A database of the literature citations was established using bibliographic database software. Subsequent to the initial search, additional articles with relevant information were identified and retrieved; many of these were identified from the bibliographies of the articles that were selected initially.