Appendix B

ICCVAM Summary Review Document:
The Low Volume Eye Test

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ICCVAM Summary Review Document:

The Low Volume Eye Test

Interagency Coordinating Committee on the Validation of Alternative Methods

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

2010

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List of Abbreviations and Acronyms

A.I.S.E.	International Association for Soaps, Detergents and Maintenance Products
ATSDR	Agency for Toxic Substances and Disease Registry
BRD	Background review document
CASRN	Chemical Abstracts Service Registry Number
Conj	Conjunctiva
CPSC	(U.S.) Consumer Product Safety Commission
CR	Conjunctival redness
DER	Data Evaluation Report
EC	European Commission
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EC/HO	European Commission/British Home Office
ECVAM	European Centre for the Validation of Alternative Methods
EEC	European Economic Community
EI	Extremely irritating
EPA	(U.S.) Environmental Protection Agency
EU	European Union
FDA	(U.S.) Food and Drug Administration
FR	*Federal Register*
FHSA	U.S. Federal Hazardous Substances Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GLP	Good Laboratory Practices
hr	Hour
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
JaCVAM	Japanese Center for the Validation of Alternative Methods
LVET	Low volume eye test
µL	Microliter
MAS	Maximum average score
MeSH	(National Library of Medicine) Medical Subject Headings
MI	Maximally irritating
Minim	Minimally irritating
MMAS	Mean maximum average score
MSDS	Material Safety Data Sheet
NA	Not applicable
NI	Nonirritating
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute of Occupational Safety and Health
NP	Not provided
NT	Not tested
NTP	(U.S.) National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances
OSHA	Occupational Safety and Health Administration
OTWG	Ocular Toxicity Working Group
PNI	Practically nonirritating
PSB	Product Safety Branch
scs	Test substance dosed on the superior conjunctival sac
SD	Standard deviation
TSCA	Toxic Substances Control Act
TG	Test Guideline
UN	United Nations

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Preface

Accidental contact with hazardous chemicals frequently causes eye injury and visual impairment. United States and international regulatory agencies currently use the Draize rabbit eye test (Draize et al. 1944) to identify potential ocular hazards associated with chemicals. The U.S. Consumer Product Safety Commission, U.S. Environmental Protection Agency, U.S. Food and Drug Administration, and U.S. Occupational Health and Safety Administration have testing regulations and/or guidelines and recommendations for assessing the ocular irritation potential of substances such as pesticides, household products, pharmaceuticals, cosmetics, and agricultural and industrial chemicals.

Although ocular safety assessment has clearly helped to protect consumers and workers, concerns have been raised about the humane aspects of the Draize rabbit eye test. Regulatory authorities have adopted various modifications that reduce the number of animals used and the potential pain and distress associated with the procedure. Significant progress has been made during the last decade. Now only one to three rabbits are required per test, compared to six rabbits in the original protocol. Provisions have been added that allow for animals with severe lesions or discomfort to be humanely euthanized.

The low volume eye test (LVET) was developed by Griffith et al. (1980) with the intent of refining the Draize rabbit eye test to reduce overlabeling of commercial products and more closely predict the human accidental response to ocular hazard. The Draize test was refined by applying the test substance to the corneal surface rather than to the conjunctival sac and by reducing the volume of exposure from 100 µL to 10 µL. However, the hypothesis that the LVET more closely predicts the human response than the Draize test for a wide applicability domain of test substances has not been clearly demonstrated yet. Thus the LVET has yet to be adopted as a reference test method by any regulatory agency.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) reviewed the validity of the LVET because LVET data was used to support the validity of a test method described in the *ICCVAM Test Method Evaluation Report: Current Validation Status of a Proposed* In Vitro *Testing Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products* (ICCVAM 2010). The ICCVAM Ocular Toxicity Working Group and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) have prepared this draft summary review document to summarize the current validation status of the LVET based on available information and data obtained by NICEATM. This draft summary review document forms the basis for draft ICCVAM test method recommendations, which are provided in a separate document.

An independent international scientific peer review panel met in public forum on May 19–21, 2009, to develop conclusions and recommendations for the LVET. The Panel included expert scientists nominated by the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods. We anticipate that these organizations will be able to use the Panel's independent report for their deliberations and development of test method recommendations. The Panel considered this summary review document and evaluated the extent to which the available information supported the draft ICCVAM test method recommendations. ICCVAM considered the conclusions and recommendations of the Panel, along with comments received from the public and the Scientific Advisory Committee on Alternative Toxicological Methods, before finalizing the summary review document and test method recommendations. These will be forwarded to Federal agencies for their consideration and acceptance decisions where appropriate.

We gratefully acknowledge the organizations and scientists who provided data and information for this document. We also acknowledge the efforts of those individuals who helped prepare this

summary review document, including the following staff from the NICEATM support contractor, Integrated Laboratory Systems, Inc.: David Allen, Jon Hamm, Nelson Johnson, Elizabeth Lipscomb, Brett Jones, Linda Litchfield, Gregory Moyer, Catherine Sprankle, and James Truax. We also thank the members of the ICCVAM Ocular Toxicity Working Group, chaired by Karen Hamernik, Ph.D. (EPA), and Jill Merrill, Ph.D. (U.S. Food and Drug Administration), and ICCVAM representatives who reviewed and provided comments throughout the process leading to this draft version. We also want to thank Valerie Zuang, Ph.D., and Dr. Hajime Kojima, Ph.D., the Ocular Toxicity Working Group liaisons from the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods, respectively, for their participation.

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Executive Summary

Accidental eye injury due to contact with hazardous chemicals is a major cause of visual impairment. United States and international regulatory agencies currently use the Draize rabbit eye test (Draize et al. 1944) to identify potential ocular hazards associated with chemicals. In the Draize rabbit eye test, 100 µL of the test substance is introduced into the conjunctival sac of each animal's eye. Alternatives to the Draize test have been explored to reduce the possibility of pain and distress during the test procedure.

Griffith et al. (1980) developed the low volume eye test (LVET) to both refine the rabbit eye test and more closely predict the human response to ocular hazard. In the LVET, the test substance is applied to the corneal surface rather than the conjunctival sac. The volume of exposure is decreased from 100 µL to 10 µL. However, the LVET has not been shown to predict the human response more closely than the Draize test for a wide array of test substances. Thus, the LVET has not yet been adopted as a reference test method by any regulatory agency. This report reviews available scientific literature and summarizes the usefulness and limitations of the LVET as an acceptable *in vivo* reference test method.

Most available LVET data were generated with surfactant-based mixtures or products, which produce only a mild ocular irritant response or no response. Gettings et al. (1996a) evaluated 25 surfactant formulations and their hazard classifications by the Environmental Protection Agency and Globally Harmonized System of Classification and Labelling of Chemicals. The authors reported several instances in which the LVET underpredicted an ocular corrosive or severe irritant response identified in the Draize test. While some claim that these data show the Draize test to be excessively overpredictive, there is limited information on the performance of known human corrosives in the LVET.

Freeberg et al. (1984) conducted both the LVET and the Draize test on 29 household cleaning products for which human accidental exposure data are available. The authors concluded that the LVET more accurately predicts the human accidental response to such substances. Similarly, Freeberg et al. (1986b) tested 14 cleaning products with both the LVET and Draize tests and compared the responses to human accidental eye exposures. They concluded that the LVET response corresponds more closely to the human experience than does the Draize rabbit eye test.

Ghassemi et al. (1993) and Roggeband et al. (2000) concluded that the smaller volume used in the LVET (10 µL) is more appropriate when compared directly with human clinical data. However, the lack of available Draize test data in these studies precludes any direct comparison with the LVET.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) reviewed the validity of the LVET because LVET data was used to support the validity of a test method described in the *ICCVAM Test Method Evaluation Report: Current Validation Status of a Proposed* In Vitro *Testing Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products* (ICCVAM 2010). LVET data are available for only limited types and numbers of substances (i.e., surfactant-containing personal and household cleaning products), precluding comprehensive evaluation of LVET performance.

Comparative human data from clinical studies and accidental exposures have been proposed to support the accuracy of the LVET. However, these data are primarily for mild or nonirritating substances. Ethical considerations have limited the severity of substances that can be tested in human clinical studies. As a result, LVET comparisons to human clinical study data are based on tests with mild irritants or substances not labeled as irritants. Regulatory agencies charged with protecting public health cannot be assured that the LVET can adequately protect against substances that may cause moderate or severe ocular injuries in humans.

The LVET may approximate experimentally the volume of a substance that could enter the human eye accidentally, but there are limited data to indicate whether it can accurately identify the ocular hazard of substances known to cause moderate, severe, or permanent human ocular injuries. In contrast, there are no documented instances in which a substance that produced a severe irritant/corrosive response in humans was not also classified as a severe irritant/corrosive in the Draize rabbit eye test.

1.0 Background on Ocular Safety Testing

Accidental eye injury is a leading cause of visual impairment in the United States. Many of these injuries occur due to contact with workplace or household chemicals. According to the National Institute of Occupational Safety and Health (NIOSH), each day about 2,000 U.S. workers have a job-related eye injury that requires medical treatment. Additional eye injuries occur in the home, with about 125,000 eye injuries a year caused by accidents involving common household products such as oven cleaner and bleach (source, American Academy of Ophthalmology). U.S. regulatory agencies such as the Consumer Product Safety Commission (CPSC), Environmental Protection Agency (EPA), Food and Drug Administration (FDA), and Occupational Safety and Health Administration (OSHA) have testing regulations and/or guidelines and recommendations to assess the hazard potential of substances that may come in contact with human eyes.

These testing requirements have effectively protected consumers and workers from potential eye injury (Wagoner 1997; Chiou 1999; McGwin et al. 2006). The primary method currently accepted by U.S. and international regulatory agencies for assessing ocular safety hazards is the Draize rabbit eye test (Draize et al. 1944). Testing guidelines describing the procedure have been published (EPA OPPTS 870.2400 [EPA 1998]), Organisation for Economic Co-operation and Development Test Guideline 405 [OECD 2002]) and several legislative statutes have been enacted that enable government agencies to regulate a variety of substances with the potential to pose a risk to ocular health and safety (see **Table 1-1**).

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

Legislation (Year of Initial Enactment)	Agency	Substance
Food, Drug, and Cosmetic Act (1938)	Food and Drug Administration	Pharmaceuticals and cosmetics
Federal Insecticide, Fungicide, and Rodenticide Act (1947) and Federal Environmental Pesticide Control Act (1972)	Environmental Protection Agency	Pesticides
Federal Hazardous Substances Act (1964)	Consumer Product Safety Commission	Household products
Federal Hazardous Substances Act (1964) and Toxic Substances Control Act (1976)	Department of Agriculture and Environmental Protection Agency	Agricultural and industrial chemicals
Occupational Safety and Health Act (1970)	Occupational Safety and Health Administration	Occupational materials
Clean Air Act Amendments (1990)	Chemical Safety and Hazard Investigation Board and Environmental Protection Agency	Accidentally released chemicals and air pollutants

Adapted from Wilhelmus (2001).

2.0 Regulatory Testing Requirements for Ocular Hazards

The classification of irritant responses evaluated by each regulatory agency varies depending on their legislative mandate and specific goals for protecting human health (**Table 2-1)**. The EPA ocular irritation classification regulation and testing guidelines (EPA 1998, 2003) 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 severity of the effects. The EPA classifies substances in ocular irritant Categories I through IV (EPA 2003). Category I substances are defined as corrosive or severe irritants, while classification from II to IV is based on decreasing severity of irritation and time required for irritation to clear. Irritation that clears in 8 to 21 days is classified as Category II, while irritation that clears within 7 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 substance not labeled as irritant. 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 1995). FHSA classification depends on the number of test animals that exhibit a positive ocular response within 72 hours after application of the test substance in the conjunctival sac.

For the purpose of harmonizing the classification of ocular irritants internationally, the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2007) includes two harmonized categories. One designates irreversible effects on the eye/serious damage to the eye (Category 1), and one designates reversible effects on the eye (Category 2). *Reversible effects* are further classified based on the duration of persistence. Category 2A (irritating to eyes) reverses within 21 days, and Category 2B (mildly irritating to eyes) reverses within 7 days. The GHS categories are based on severity of the lesions and/or the duration of persistence.

Hazard classification of ocular irritants in the European Union is characterized by 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.

Table 2-1 Ocular Toxicity Classification Systems

Regulatory Agency (Authorizing Act)	Number of Animals	Observation Days (after treatment)	Mean score taken?	Positive Response	Classification Criteria
U.S. CPSC (Federal Hazardous Substances Act) OSHA (Occupational Safety and Health Act)	6 (12, 18 possible)	1, 2, 3	No	Opacity or Iritis ≥1 or Redness or Chemosis ≥2 for any animal on any day	1st Tier: 4 or more positive animals = Irritant 2–3 positive animals = Go to 2nd Tier 2nd Tier 3 or more positive animals = Irritant 1–2 positive animals = Go to 3rd Tier 3rd Tier : 1 positive animal = Irritant
U.S. EPA (FIFRA, Federal Environmental Pesticide Control Act, and TSCA)	At least 3	1 hr, 1, 2, 3, 7, 21	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. Category: 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 **Definition of Full Reversal:** Opacity and Iritis scores = 0 and Redness and Chemosis scores ≤1

continued

Table 2-1 Ocular Toxicity Classification Systems (continued)

Regulatory Agency (Authorizing Act)	Number of Animals	Observation Days (after treatment)	Mean score taken?	Positive Response	Classification Criteria
European Union	1 if severe effects are suspected or 3 if no severe effects are suspected	1, 2, 3 (observation until Day 21)	Yes	Mean study values (scores of all animals in study averaged over Days 1, 2, and 3) of: Opacity or Chemosis ≥2, Redness ≥2.5, or Iritis ≥ 1 **OR** 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** (3) Mean study value where: 2 ≤ Opacity < 3 or 1 ≤ Iritis < 1.5 or Redness ≥2.5 or Chemosis ≥2 (2) If 2/3 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** (3) Mean study value where: Opacity ≥3 or Iritis >1.5 (2) If 2/3 tested animals have individual animal mean values that fall into one of the following categories: Opacity ≥3 Iritis = 2 (3) At least one animal (at the end of the observation period, typically Day 21) where Opacity or Chemosis ≥2, Redness ≥2.5 or Iritis ≥1
GHS: Irreversible Eye Effects	3	1, 2, 3 (observation until Day 21)	Yes	Mean animal values (over Days 1, 2, and 3) of: Opacity ≥3 and/or Iritis ≥1.5	–At least 2 positive response animals = Eye Irritant Category 1 –At least 1 animal with an Opacity, Iritis, Redness, or Chemosis score >0 on Day 21 = Eye Irritant Category 1 **Definition of Full Reversal:** Opacity, Iritis, Redness, and Chemosis scores = 0

continued

Table 2-1 Ocular Toxicity Classification Systems (continued)

Regulatory Agency (Authorizing Act)	Number of Animals	Observation Days (after treatment)	Mean score taken?	Positive Response	Classification Criteria
GHS: Reversible Eye Effects	3	1, 2, 3 (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 **Definition of Full Reversal:** Opacity, Iritis, Redness, and Chemosis scores = 0

Abbreviations: CPSC = U.S. Consumer Product 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 (UN) Globally Harmonized System of Classification and Labelling of Chemicals; OSHA = U.S. Occupational Safety and Health Administration; TSCA = Toxic Substances Control Act.

3.0 Principle of the Low Volume Eye Test

The low volume eye test (LVET) is an *in vivo* rabbit eye test that, like the Draize test, was designed to determine the extent of potential ocular hazard of a test substance. The tests evaluate the ocular irritation response when a test substance is administered as a single dose to the eye of a rabbit. Developed by Griffith et al. (1980), the LVET differs from the Draize rabbit eye test primarily by applying 10 μL (instead of 100 μL) of a test substance directly on the cornea (instead of the conjunctival sac) (**Table 3-1**). Scoring of corneal, iridal, and conjunctival lesions in the LVET is identical to that of the Draize rabbit eye test (**Table 3-2**).

Table 3-1 Comparison of LVET and Draize Rabbit Eye Test Protocols

	LVET	**Draize**
Dose volume	10 μL	100 μL
Dose location	Applied directly onto the cornea	Applied into the lower conjunctival sac
Eyelid closure	No forced eyelid closure	Eyelids held closed for one second
Scale for scoring ocular lesions	Draize	Draize

Abbreviation: LVET = low volume eye test

To date, the LVET has not been demonstrated as an adequately valid *in vivo* reference test method. It has not been formally adopted by any regulatory agency. For this reason, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is reviewing the validity of the LVET as an acceptable *in vivo* reference test method. In February 2007, the International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.) submitted a background review document to the European Centre for the Validation of Alternative Methods (ECVAM) for an independent peer review by their Scientific Advisory Committee. The A.I.S.E. background review document provides a comprehensive summary of available data and information with which to evaluate the usefulness and limitations of the LVET.

Since its original development, proponents of the LVET have suggested that it is a more appropriate *in vivo* reference test method for comparisons to *in vitro* data than is the Draize rabbit eye test. This is based primarily on the assertion that the LVET is more representative of the human response to a potential ocular hazard than the Draize rabbit eye test, given that the site (corneal surface) and volume of exposure used in the LVET more closely resemble that of accidental human exposure. As a result, a reported advantage of the LVET is that it underpredicts the Draize test and is thereby less overpredictive of the human response than the Draize test. However, definitive data to support this claim are not available.

Table 3-2 Scale of Weighted Scores for Grading the Severity of Ocular Lesions

Cornea	
Lesion	**Score[1]**
A. Opacity – Degree of density (area which is most dense is taken for reading)	
Scattered or diffuse area – details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4
B. Area of cornea involved	
One quarter (or less) but not zero	1
Greater than one quarter but less than one half	2
Greater than one half but less than three quarters	3
Greater than three quarters up to whole area	4
Score equals A x B x 5	Total maximum = 80
Iris	
Lesion	**Score[1]**
A. Values	
Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage; gross destruction (any one or all of these)	2
Score equals A x 5	Total possible maximum = 10
Conjunctiva	
Lesion	**Score[1]**
A. Redness (refers to palpebral conjunctiva only)	
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3
B. Chemosis	
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of the lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4
C. Discharge	
Any amount different from normal (does not include small amount observed in inner canthus of normal animals	1
Discharge with moistening of the lids and hairs just adjacent to the lids	2
Discharge with moistening of the lids and considerable area around the eye	3
Score equals (A + B + C) x 2	Total maximum = 20

From Draize et al. (1944).

[1] The maximum total score is the sum of all scores obtained for the cornea, iris and conjunctiva. Scores of 0 are assigned for each parameter if the cornea, iris, or conjunctiva is normal.

4.0 Performance of the Low Volume Eye Test vs. the Draize Rabbit Eye Test

In general, most of the original data generated with the LVET were from surfactant-based mixtures or surfactant-based products (Freeberg et al. 1984; Gettings et al. 1996a, 1998). A comparison of the substances that have been classified by the Draize rabbit eye test as ocular corrosives or severe irritants that have also been tested in the LVET indicates that the LVET routinely underpredicts the ocular corrosive or severe irritant response in the Draize, in many cases by more than one hazard category. Gettings et al. (1996a, 1998) illustrate this in their evaluation of 25 surfactant-containing formulations and the resulting hazard classifications according to the EPA and GHS classification systems (EPA 2003; UN 2007) (**Tables 4-1** and **4-2**).

Table 4-1 Performance of the LVET in Identifying Ocular Hazard Classification According to the EPA Classification System When Compared to Draize Rabbit Eye Test Results

EPA Category[1]		LVET Classification				
		I	II	III	IV	Totals
Draize	I	3	1	6	0	10
	II	0	0	0	0	0
	III	0	0	9	2	11
	IV	0	0	0	4	4
	Totals	3	1	15	6	25

From Gettings et. al. 1996a and 1998.

Abbreviations: EPA = Environmental Protection Agency; LVET = low volume eye test

[1] EPA classification system (EPA 2003).

Table 4-2 Performance of the LVET in Identifying Ocular Hazard Classification According to the GHS Classification System When Compared to Draize Rabbit Eye Test Results

GHS Category[1]		LVET Classification				
		1	2A	2B	Not Labeled	Totals
Draize	1	0	0	4	4	8
	2A	0	0	0	0	0
	2B	0	0	0	1	1
	Not Labeled	0	0	0	16	16
	Totals	0	0	4	21	25

From Gettings et. al. 1996a and 1998.

Abbreviations: GHS = Globally Harmonized System; LVET = low volume eye test

[1] GHS classification system (UN 2007).

Tables 4-1 and **4-2** show multiple instances of underprediction of an ocular corrosive or severe irritant response in the Draize rabbit eye test by the LVET. When the EPA hazard classification system (EPA 2003) was used, the LVET underpredicted 60% (6/10) of Draize Category I substances as Category III (mild irritant) (**Table 4-3**). When the GHS hazard classification system (UN 2007) was used, the LVET underpredicted all eight of the Draize Category 1 substances: 50% (4/8) as

Category 2B (mild irritant) and 50% (4/8) as Not Labeled (not labeled as an irritant) (**Table 4-4**). These data raise concern about the ability of the LVET to reliably detect ocular corrosives or severe irritants (i.e., EPA Category I, EU Category R41, GHS Category 1).

Table 4-3 Extent of Underprediction of LVET vs. Draize Rabbit Eye Test Results According to the EPA Classification System[1]

EPA Category	LVET Category	Product
Category I	Category II	HZY (Antidandruff shampoo)
Category I	Category III	HZA (Shampoo #7)
Category I	Category III	HZE (Gel cleanser)
Category I	Category III	HZF (Baby shampoo #2)
Category I	Category III	HZL (Foam bath)
Category I	Category III	HZR (Facial cleaning foam)
Category I	Category III	HZX (Shampoo #2)

Abbreviations: EPA = Environmental Protection Agency; LVET = low volume eye test

[1] EPA classification system (EPA 2003).

Table 4-4 Extent of Underprediction of LVET vs. Draize Rabbit Eye Test Results According to the GHS Classification System[1]

GHS Category	LVET Category	Product
Category 1	Category 2B	HZI (Skin cleanser)
Category 1	Category 2B	HZK (Bubble bath)
Category 1	Category 2B	HZS (Shower gel)
Category 1	Category 2B	HZY (Antidandruff shampoo)
Category 1	Not Classified	HZL (Foam bath)
Category 1	Not Classified	HZF (Baby shampoo #2)
Category 1	Not Classified	HZX (Shampoo #2)
Category 1	Not Classified	HZA (Shampoo #7)

Abbreviations: GHS = United Nations Globally Harmonized System; LVET = low volume eye test

[1]GHS classification system (UN 2007).

Gettings et al. (1996b) published another study investigating the relationship between the LVET and Draize eye irritation test data for 10 representative hydroalcoholic personal-care formulations. **Table 4-5** provides the eye irritation profile for each of the 10 substances tested. A range of irritancy classification was demonstrated for the LVET; however, only one of the test substances was considered moderately irritating and none severely irritating according to the criteria developed by Kay and Calandra (1962). A further comparison of the LVET using the classification scheme of Bruner et al. (1992) revealed a range of responses from nonirritating to moderately irritating. The Bruner et al. (1992) LVET classification appeared to be more consistent with the Kay and Calandra irritancy classification as determined by the Draize rabbit eye test (**Table 4-5**).

Table 4-5 Summary of Available Rabbit LVET and Draize Data from Gettings et al. (1996b)

	Rabbit LVET			Rabbit Draize	
Ethanol (%)	MAS	Category[1]	Category[2]	MAS	Category[1]
5	2.2	PNI	I	7.7	Mild
10	1.3	PNI	I	3.0	Minim
15	0.7	PNI	I	0.7	PNI
20	0.7	PNI	I	0.7	PNI
33	4.3	Minim	I	14.3	Mild
40	15.5	Mild	III	38.7	Moderate
55	14.3	Mild	II	36.7	Moderate
65	22.5	Mild	III	28.3	Moderate
83	22.5	Mild	III	36.0	Moderate
90	26.0	Moderate	III	45.7	Moderate

Modified from Gettings et al. (1996b).

Abbreviations: LVET = low volume eye test; MAS = maximum average score.

[1] Kay and Calandra (1962): PNI = practically nonirritating; Minim = minimally irritating; Mild = mildly irritating; Moderate = moderately irritating.

[2] Bruner et al. (1992): I = none to inconsequential irritation (LVET-MAS = 0–5); II = slight irritation (LVET-MAS > 5–15); III = moderate to severe irritation (LVET-MAS > 15–50); IV = severe irritation (LVET-MAS > 50–65); V = extremely irritating to corrosive (LVET-MAS > 65–110).

The authors noted a similarity between the irritant responses observed in the Draize rabbit eye test and the LVET, with both tests ranking the substances in a similar order. In addition, the observed irritation for both tests significantly increased when ethanol levels exceeded 33%. Indeed, the LVET consistently underpredicted ethanol solutions above this range when compared to the Draize rabbit eye test data (**Table 4-5**).

Maurer et al. (2001a, 2001b) used pathology to evaluate the relationship of the ocular irritation response to the extent of initial injury for several nonsurfactant materials using the LVET. In these studies, they reported maximum average score (MAS) data for the LVET and irritation classifications based on Kay and Calandra (1962) as shown in **Table 4-6**. These LVET data are compared to available Draize data obtained from the database of the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC 1998) in **Table 4-7**. Maurer et al. (2001a, 2001b) applied test substances directly to the cornea and performed macroscopic assessments for irritation 3 hours after dosing and periodically thereafter up to 35 days. The alcohols, cyclohexanol and parafluoroaniline, were moderate to severe irritants in the LVET. Only cyclohexanol was tested in the Draize test, and it was a severe irritant/corrosive. Of the aldehydes, acetone was a mild irritant in the LVET and a moderate irritant in the Draize test. Formaldehyde (37%; w/v) was a severe irritant in the LVET but was not tested in the Draize test.

Four bleaches, sodium perborate monohydrate ($NaBO_3$), sodium hypochlorite (NaOCl), 10% hydrogen peroxide (H_2O_2), and 15% H_2O_2, were evaluated in the LVET, but no corresponding Draize data were available. $NaBO_3$ and NaOCl were classified as mild and minimal irritants in LVET respectively, with corneal injuries being limited to the epithelium and superficial stroma, as determined using *in vivo* confocal microscopy. It should be noted that some Material Safety Data Sheets (MSDS) from various manufacturers label NaOCl as moderately irritating or a severe irritant/corrosive in humans at or above 5.25%, while label it corrosive in humans above 14%. The

15% H_2O_2 solution would be classified as a severe irritant based on LVET data. Both concentrations affected the epithelium and deep stroma, as determined using *in vivo* confocal microscopy. In undiluted form, H_2O_2 is a known human ocular corrosive/severe irritant.

Table 4-6 Summary of MAS Categorization Data

MAS Score	Ocular Irritation Rating
0–0.5	Nonirritating— NI
0.5–2.5	Practically nonirritating— PNI
2.5–15	Minimally irritating— Minim
15–25	Mildly irritating— Mild
25–50	Moderately irritating— Moderate
50–80	Severely irritating— Severe
80–100	Extremely irritating— EI
100–110	Maximally irritating— MI

From Kay and Calandra (1962).

Abbreviation: MAS = maximum average score.

Table 4-7 Summary of Available Rabbit LVET Data

Chemical Class	Eye Data			
	Rabbit LVET		Rabbit Draize	
	MAS	Category[1]	MMAS	Category[2]
Alcohols	-	-	-	-
Cyclohexanol	50.8	Moderate/Severe	79.8	1/I/R41
Parafluoroaniline	55.0	Moderate/Severe	69.8	
Aldehydes	-	-	-	-
Acetone	19.1	Mild	65.8	2A/II/R36
Formaldehyde, 37% (w/v)	80.0	Severe		
Bleaching Agents	-	-	-	-
Sodium Perborate Monohydrate	23 ± 31.2	Mild	-	-
Sodium Hypochlorite (2.4%)	11 ± 3.6	Minim	-	-
10% Hydrogen Peroxide	16 ± 7.5	Mild	-	-
15% Hydrogen Peroxide	58.3 ± 26.1	Severe	-	-

Data from Maurer et al. (2001a, 2001b).

Abbreviations: ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; LVET = low volume eye test; MAS = maximum average score; MMAS = mean maximum average score;

[1] MAS categorization data compiled from classification table of Kay and Calandra (1962): PNI = practically nonirritating; Minim = minimally irritating; Mild = mildly irritating; Moderate = moderately irritating; Severe = severely irritating. Eye irritancy classification scores based on *in vivo* confocal microscopy and light microscopy also available in Jester (2006).

[2] Data obtained from ECETOC database (ECETOC 1998). Hazard classifications based on the Globally Harmonized System (UN 2007)/EPA (EPA 2003)/European Union (EU 2001) were determined by NICEATM based on available ECETOC Draize data.

Maurer et al. (2001a, 2001b) concluded that results obtained on these nonsurfactant materials support their hypothesis that ocular irritation is principally defined by the extent of initial injury, despite clear differences in the means by which irritants cause tissue damage.

Jester (2006) used the LVET to investigate the ocular irritancy of 22 substances varying in type (i.e., surfactant, acid, alkali, bleach, alcohol, aldehyde, and acetone) and severity (**Table 4-8**). Jester evaluated the extent of ocular irritation using light microscopy, *in vivo* confocal microscopy, and laser scanning confocal microscopy. Of the 22 substances, five produced slight irritation, nine produced mild irritation, three produced moderate/severe irritation, and five produced severe irritation. However, of the three substances for which Draize data were identified (i.e., 10% acetic acid, cyclohexanol, and acetone), the LVET underpredicted Draize results.

Table 4-8 Summary of Available Rabbit LVET Data

Chemical Class	Eye Data					
	Human[2]		Rabbit LVET		Rabbit Draize[1]	
			MAS	Category[3]	MMAS	Category[4]
Surfactant	-	-	-	-	-	-
Nonionic	-	-	-	-	-	-
Polyoxyethylene glycol monoalkylether	-	-	0.0	NI	-	-
Polyoxyethelenesorbitan	-	-	0.0	NI	-	-
Alkyl E ethoxylate	-	-	33.0	Moderate	-	-
Anionic	-	-	-	-	-	-
Sodium lauryl sulfate, 5%	-	-	4.8	Minim	-	-
Sodium linear alkylbenzene sulfonate	-	-	49.3	Moderate	-	-
Sodium alkyl ethoxylate sulfate	-	-	31.2	Moderate	-	-
Cationic	-	-	-	-	-	-
Cetyltrimethylammonium chloride, 50%	-	-	76.3	Severe	-	-
3-Isotridecyloxypropyl-bis(polyoxyethylene) ammonium chloride	-	-	7.7	Minim	-	-
3-Decyloxypropyl-bis(polyoxyethylene amine, 5%	-	-	40.0	Moderate	-	-
Alkylbenyldimethylammonium chloride, 10%		-	70.6	Severe	-	-
Acid	-	-	-	-	-	-
3% Acetic Acid	-	-	5.0	Minim	-	-
10% Acetic Acid	-	-	9.5	Minim	68	1/I/R41
Base	-	-	-	-	-	-
2% Sodium Hydroxide	-	-	5.0	Minim	-	-
8% Sodium Hydroxide	-	-	50.8	Severe	-	-

continued

Table 4-8 Summary of Available Rabbit LVET Data (continued)

Chemical Class	Eye Data					
	Human[2]		Rabbit LVET		Rabbit Draize[1]	
			MAS	Category[3]	MMAS	Category[4]
Aldehyde	-	-	-	-	-	-
Acetone	-	-	3.8	Minim	65.8	2A/II/R36
Formaldehyde, 37%	-	-	79.7	Severe	-	-
Alcohol	-	-	-	-	-	-
Parafluoroaniline	-	-	43.3	Moderate	-	-
Cyclohexanol	-	-	45.8	Moderate	79.8	1/I/R41
Bleach	-	-	-	-	-	-
Sodium Perborate Monohydrate	-	-	8.3	Minim	-	-
Sodium Hypochlorite (2.4%)	Severe[5]	-	11.8	Minim	-	-
10% Hydrogen Peroxide	-	-	30.3	Moderate	-	-
15% Hydrogen Peroxide	-	-	68.3	Severe	-	-

Data from Jester (2006).

Abbreviations: ATSDR = Agency for Toxic Substances and Disease Registry; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; LVET = low volume eye test; MAS = maximum average score; MMAS = mean maximum average score; MSDS = material safety data sheet.

[1] Data obtained from ECETOC database (ECETOC 1998). Hazard classifications based on EPA (EPA 2003), Globally Harmonized System (UN 2007), and European Union (EU 2001) were determined by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods based on ECETOC Draize data.

[2] Data compiled from accidental exposures (ATSDR database).

[3] MAS categorization data compiled from classification table of Kay and Calandra, (1962) (see Table 4-8). Eye irritancy classification scores based on *in vivo* confocal microscopy and light microscopy also available in Jester (2006).

[4] Category classification– EPA/GHS/EU.

[5] Labeled as moderately irritating or severe irritant/corrosive in humans at or above 5.25% based on some MSDS reports, while labeled as corrosive in humans above 14%.

5.0 Performance of the Low Volume Eye Test vs. the Draize Rabbit Eye Test Considering Human Study Data and Experience

Human data on potential ocular hazards are available either from accidental exposures or from clinical studies. Accidental exposures are not generally considered to be a reliable source of the true ocular hazard potential because such exposures are likely immediately followed by flushing the eyes with large volumes of water. Thus they may not represent the most severe lesion that might be produced by such an exposure. Griffith et al. (1980) conducted a series of rabbit eye test studies using either 10 or 100 μL of substances "recognized as slightly irritating, moderately irritating, or severely irritating/corrosive to humans."

The ocular corrosive or severe irritant substances included the following:

- Acetic acid (10%), which is referenced as a severe irritant based on splashes of vinegar (containing 4% to 10% acetic acid) reported to cause pain, conjunctival hyperemia, and occasionally permanent opacity of the human cornea
- Calcium hydroxide (hydrated lime), which is referenced as one of the most common causes of severe chemical burns of the eye (McLaughlin 1946; Grant and Schuman 1993)
- Formaldehyde (38%), which is referenced for the range of injuries caused by splashes in the human eye from minor transient discomfort to severe, permanent corneal opacities (Grant and Schuman 1993)

Although detailed animal data are not available, the summary data provided by Griffith et al. (1980) indicate that the lesions induced by either 10 or 100 μL of these substances were not reversible within 21 days. However, such accidental exposures as human reference data make definitive quantitative measures of amount and time of exposure impossible to obtain. Ethical considerations and results based largely on the Draize rabbit eye test have limited the severity of substances that can be tested in human clinical studies. As a result, comparisons to human data are based on clinical study tests with mild irritants or substances not labeled as irritants. Such data provide little assurance to the regulatory agencies charged with protecting public health that the LVET can provide adequate protection from substances that may cause moderate or severe ocular injuries.

The fact that seemingly innocuous commercial consumer products were identified as ocular corrosives or severe irritants by the Draize eye test could be seen as supporting the contention that the Draize eye test is excessively overpredictive of the actual hazard to humans. However, because of the paucity of information on the performance of known human corrosives in the LVET, these data cannot simply be dismissed.

Several studies have published supporting data for the demonstrated usefulness of the LVET (Ghassemi et al. 1993; Roggeband et al. 2000; Freeberg et al. 1984, 1986a, 1986b).

5.1 Ghassemi et al. (1993)

Ghassemi et al. (1993) provides an evaluation of *a single product*, a liquid household cleaner (pH 3) reportedly containing the following qualitative formula: nonionic surfactant, amphoteric surfactant, hydrotrope, solvent, and water. This study directly compares LVET results to human clinical data (using either 10 or 100 μL doses) for the same test substance. No Draize rabbit eye test data had been reported; therefore, LVET results could not be compared to those of the standard eye test. The ocular lesions that were produced in this study and their subsequent time to clear suggest that this product is a mild ocular irritant (**Table 5-1**). The authors conclude that because the direct application to the human eye using either 10 or 100 μL doses produced similar results, the smaller volume for testing is more appropriate anatomically and physiologically based on eye volume capacity and subsequent tear volume.

Table 5-1 Summary of Rabbit and Human Responses to an Undiluted Liquid Household Cleaner

Species	Ocular Tissues Involved	Number of Eyes Affected			Mean CR at 24 hr	Eyes Cleared/ Time to Clear	Max Time to Clear
		Cornea	Iris	Conj			
Rabbit LVET	Cornea Iris Conj	3/3	2/3	3/3	2	2/4 days 1/7 days	7 days
Human (10 μL)	Conj	0/10	0/10	10/10	0.1	1/1hr; 4/2hr; 6/4hr; 10/24hr	48 hr
Human (100 μL)	Conj	0/10	0/10	10/10	0.2	1/1hr; 2/2hr; 9/24hr; 2/46hr	70hr

Data from Ghassemi et al. (1993).

Abbreviations: Conj = conjunctiva; CR = conjunctival redness; hr = hour; LVET = low volume eye test (10 μL dose volume).

5.2 Roggeband et al. (2000)

Roggeband et al. (2000) evaluates two products, a dishwashing liquid (pH 8, contains anionic surfactant, nonionic surfactant, soap, ethanol, water) and a liquid laundry detergent (pH 7, contains anionic surfactant, nonionic surfactant, ethanol, water). This study directly compares modified LVET results to those of a human clinical study. Both rabbits and humans were dosed with either 3 μL (dishwashing detergent) or 1 μL (liquid laundry detergent) of the test products. There are no corresponding Draize rabbit eye test data. The ocular lesions that were produced in this study and their subsequent time to clear suggest that these products are mild ocular irritants (**Table 5-2**). The authors conclude that these data support the notion that (1) an accidental exposure would be approximately 10 μL or less and (2) a volume of 10 μL provides a suitable margin of safety. This is based on (1) knowledge of the anatomical and physiological characteristics of the eye and (2) the fact that study participants in Roggeband et al. (2000) could "only be exposed to 1 μL of dishwashing liquid and 3 μL of liquid laundry detergent before predetermined 'cut-off' ocular responses were observed above which it would have been ethically unacceptable to proceed" (Roggeband et al. 2000).

Table 5-2 Human and Rabbit Eye Responses to a Liquid Laundry Detergent (1 µL)

Human Volunteer	Human				Animal Number	Rabbit LVET[1]			
	1 hour		24 hours			1 hour		24 hours	
	Cornea	Conj	Cornea	Conj		Cornea	Conj	Cornea	Conj
5	0	1/1	0	0/0	28 (c)	0/0	1/1/0	1/2	2/1/1
6	0	1/0	0	0/0	29 (c)	0/0	1/1/0	1/2	2/1/1
21	0	1/0	0	0/0	30 (c)	0/0	1/1/0	0/0	2/1/1
23	1/2	1/0	0	1/0	31 (scs)	0/0	1/1/0	1/4	2/1/0
25	1/1	1/0	0	0/0	32 (scs)	0/0	1/1/0	1/3	2/1/1
27	0	1/0	0	1/0	33 (scs)	0/0	1/1/0	1/4	2/1/1
28	0	1/0	0	0/0					
30	0	0/0	0	0/0					
32	0	1/0	0	0/0					
34	0	1/0	0	0/0					

Data from Roggeband et al. (2000).

Abbreviations: (c) = test substance dosed on the central cornea; Conj = conjunctiva; LVET = low volume eye test; (scs) = test substance dosed on the superior conjunctival sac.

[1]Low volume eye test was modified to use 1 µL instead of 10 µL.

5.3 Freeberg et al. (1984)

A series of studies by Freeberg et al. (1984) compare data from LVET, Draize rabbit eye test, and human studies or experience. Freeberg et al. (1984) compares LVET and Draize rabbit eye test data for 29 cleaning products (laundry products, household cleaning products, and dishwashing products) to human experience data. The ocular lesions that were produced in this study and their subsequent time to clear suggest that these products are either mild ocular irritants or substances not labeled as irritants (**Table 5-3**). The human data were obtained from medical records of factory and consumer accidental eye exposures (515 reports over a 2-year period). The results indicate that both rabbit LVET and Draize eye tests overpredicted (based on time to clear of ocular lesions) the human response based on accidental eye exposure to the cleaning products. The time to clear was longer in the Draize eye test than in the LVET for the same product, forming the basis for the conclusion that the LVET more closely predicts the human response.

Table 5-3 Summary of Rabbit and Human Accidental Exposure Data from Freeberg et al. (1984)

Species	Test Method	Number of Products	Average ± SD Mean Time to Clear (Day Range)	Average ± SD Median Time to Clear) (Day Range)	Average ± SD Number of Incidents (Range)
Rabbit	LVET	17	7.3 ± 7.2 (1.3–28.8)	6.2 ± 8.8 (0.7–35)	Not Applicable
Rabbit	Draize	26	20.4 ± 7.2 (3.1–33.5)	20.2 ± 12.3 (1.4–35)	Not Applicable
Human	Experience data[1]	29	2.4 ± 2.1 (0.2–9.5)	1.5 ± 1.5 (0.1–1.8)	16.2 ± 8.4 (3–68)

Data from Freeberg et al. (1984).

Abbreviations: LVET = low volume eye test; SD = standard deviation.

[1]Experience data = combined manufacturing and consumer accidental exposures.

5.4 Freeberg et al. (1986a)

Freeberg et al. (1986a) compared rabbit eye test results (both LVET and Draize) with those of human studies (both 10 μL and 100 μL dose volumes) for four cleaning products (a liquid fabric softener, liquid shampoo, liquid hand soap, and liquid laundry detergent). The results indicate that the LVET overpredicted the human response to 10 μL and 100 μL of the same product. The ocular lesions in the Draize rabbit eye test (100 μL) were more severe (both type and longevity) than in the human test using the same volume. While the majority of effects in humans were conjunctival, the corneal effects in humans were minimal and transient. The corneal effects in rabbits were more severe and recovered less quickly. The ocular lesions that were produced in this study and their subsequent time to clear suggest that these products would be classified as mild ocular irritants based on the Draize rabbit eye test results, the LVET, and human results (**Table 5-4**).

Table 5-4 Human Clinical Study and Rabbit Data

Test Product	Concentration (% in water)	Time to Clear (hr)			
		Dosing Volume			
		Rabbit	Human		Rabbit
		10 μL	10 μL	100 μL	100 μL
Liquid fabric Softener	60	45	18.9	24.9	45
	80	66	12.6	33.6	93
	100	27	13.2	12.5	84
Liquid shampoo	4	5	1.5	2.5	NT
	16	19.8	1.9	2.6	36.5
	20	33	7.5	7.9	63
Liquid hand soap	8	24	1.5	31.5	63
	10	42	10.5	9.1	66
	12	42	1.7	NT	NT
Liquid laundry detergent	2	8.8	2	24.1	27.8
	3	19.8	4.7	1.8	60
	4	39.8	4.8	19.8	75

Data from Freeberg et al. (1986a).

Abbreviation: NT = not tested.

5.5 Freeberg et al. (1986b)

Freeberg et al. (1986b) compares LVET and Draize rabbit eye test data for 14 cleaning products (liquid and solid laundry products, liquid and solid household cleaning products, liquid and solid dishwashing products, and liquid shampoos) to human experience data. The ocular lesions that were produced in this study and their subsequent time to clear suggest that these products would be classified as moderate to severe ocular irritants based on the Draize rabbit eye test results. Most would be classified as mild ocular irritants by the LVET (**Table 5-5**). The human data were obtained from medical records of factory and consumer accidental eye exposures (218 reports over an 18-month period). Similar to Freeberg et al. (1986a), rabbit LVET and Draize tests both overpredicted the human response due to accidental eye exposure (based on time to clear). Because the time to clear was longer for substances tested in the Draize rabbit eye test than in the LVET, the authors concluded

that the LVET outcome more closely relates to the human experience than the Draize rabbit eye test does.

Table 5-5 Human Accidental Exposure and Rabbit Data

Product	Mean Time to Clear (Days)		
	Human	Rabbit LVET	Rabbit Draize
Liquid Laundry Product #1	1.92	26.6	35.0
Liquid Dishwashing Product #1	0.77	8.2	25.7
Solid Dishwashing Product #1	0.59	4.6	18.3
Liquid Dishwashing Product #2	0.43	7.7	11.7
Liquid Household Cleaning Product #1	0.38	-	11.1
Liquid Dishwashing Product #3	0.30	3.9	22.2
Liquid Household Cleaning Product #2	0.23	4.0	15.2
Solid Household Cleaning Product #1	0.19	1.3	29.2
Solid Dishwashing Product #1	0.08	2.1	13.8
Solid Dishwashing Product #1	0.06	2.9	15.1

Data from Freeberg et al. (1986b).

Abbreviation: LVET = low volume eye test.

6.0 Summary

Because studies conducted with the LVET have been limited to tests of surfactant-containing personal and household cleaning products, the applicability domain for which the LVET can be considered is necessarily restricted to these product types. As summarized in **Table 6-1**, LVET data have previously been used by one personal-care product company to support submission of data to the EPA for the registration of at least five antimicrobial cleaning products. The results were used by EPA reviewers in a weight-of-evidence approach, in conjunction with either consumer incidence data (i.e., commercial products for which there is an opportunity for adverse events to be reported by the consumer) and/or Draize data for similar, structurally related substances. Each study was considered on a case-by-case basis and several submissions were deemed unacceptable by the EPA because either the LVET study was not considered an acceptable fulfillment of the eye irritation data requirement and/or the further confirmatory information provided by the submitter was insufficient (**Table 6-1**). Based on the data provided to NICEATM in the Data Evaluation Reports (DERs), it appears that a final EPA ocular hazard classification was not assigned for any product using LVET data alone.

As indicated in the studies summarized above, human data on potential ocular hazards are available either from accidental exposures or from clinical studies. Accidental exposures are not generally considered to be a reliable source of the true ocular hazard potential because such exposures are likely immediately followed by flushing the eyes with large volumes of water. Such accidents make definitive quantitative measures of amount and time of exposure impossible to obtain. Although the Draize eye test is reported to be excessively overpredictive of the human response, ethical considerations based largely on results from the Draize rabbit eye test are used to limit the types of substances that can be tested in human clinical studies. As a result, comparisons to human clinical study data are based on tests of mild irritants or substances not labeled as irritants. Such data provide little assurance to the regulatory agencies charged with protecting public health that the LVET can provide adequate protection from more severe ocular injuries.

Thus, while the LVET is proposed as more likely to approximate the volume of a substance that could enter the human eye accidently, there are limited data to indicate whether it can accurately identify the ocular hazard of substances known to cause moderate, severe, or permanent human ocular injuries. In contrast, there are no documented instances in which a substance with a hazard category determined in the Draize eye test produced a more severe hazard category response in humans following accidental exposures or ethical human studies.

Table 6-1 Summary of Ocular Hazard Classifications for EPA Registered Antimicrobial Cleaning Products: Consideration of LVET Data and EPA Determinations[1]

EPA Registration Number or Submission Code	Submission Date	Animal Data from LVET Study	EPA Hazard Category Based on LVET Data	Additional Submission Information	Final EPA Classification Provided in DER
3573-AO	Jul 20, 2000	No corneal opacity, iritis, or conjunctival irritation (n=6).	Category IV	Consumer incidence data	Study unacceptable[2]
	Jun 6, 2001	Same as for Jul 20, 2000	Consumer incidence data; LVET and Draize data for similar substances		Category III

continued

Table 6-1 Summary of Ocular Hazard Classifications for EPA Registered Antimicrobial Cleaning Products: Consideration of LVET Data and EPA Determinations[1] (continued)

EPA Registration Number or Submission Code	Submission Date	Animal Data from LVET Study	EPA Hazard Category Based on LVET Data	Additional Submission Information	Final EPA Classification Provided in DER
3573-TE	Aug 9, 2000	No corneal opacity, iritis, redness, or chemosis at day 1 (n=3).	Category IV	None	Study unacceptable[2]
	Feb 7, 2001	Repeat submission from Aug 9, 2000		Animal data for similar substances	Category IV
3573-72	Jun 6, 2001	NP	Category III	Consumer incidence data; LVET and Draize data for similar substances	Category III
3573-AI	Jun 6, 2001	NP	NP	NP	Category II
S596273	Jun 27, 2001	No corneal effects or iritis observed. Conjunctivitis resolved by 72 hr (n=3).	Category III	None	Study unacceptable[2]
3573-TG	Jul 25, 2001	NP	Category III	Consumer incidence data; Animal skin irritation study-Category I (severe irritant)	Study unacceptable[3]

Abbreviations: DER = Data Evaluation Reports; EPA = U.S. Environmental Protection Agency; LVET = low volume eye test; NP = not provided (i.e., information not contained in and/or not provided to NICEATM in DERs).

[1] Data source: Obtained from a Freedom of Information Act request submitted to EPA for LVET data used to support the submission of data for the registration of antimicrobial cleaning products.

[2] "The EPA does not consider the LVET study to be an acceptable fulfillment of the eye irritation data requirement."

[3] "It is now the Product Safety Branch's (PSB) policy to take a weight of the evidence approach to the situation by considering individual LVET studies for possible acceptance on a case by case basis if they are significantly supplemented by further, confirmatory information. In the present case, that confirmatory further information is not sufficient."

7.0 References

Bruner LH, Parker RD, Bruce RD. 1992. Reducing the number of rabbits in the low-volume eye test. Fund Appl Toxicol 19:330-335.

Chiou GCY. 1999. Ophthalmic Toxicology. In: Target Organ Toxicology Series (Hayes AW, Thoams JA, Gardner DG, eds). Philadelphia, PA:CRC Press.

Clean Air Act Amendments of 1990. 1990. Public Law 101-549. Available: http://www.ehso.com/caa.htm.

CPSC. 1995. Test for eye irritants. 16 CFR 1500.42.

Draize J, Woodard G, Calvery H. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharm Exp Ther 82:377–390.

ECETOC. 1998. Eye Irritation Reference Chemicals Data Bank. 2d ed. Technical Report No. 48 (2). European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). Brussels, Belgium:ECETOC.

EPA. 1998. Health Effects Test Guideline. OPPTS 870.2400 Acute Eye Irritation. EPA 712-C-98-195. Washington, DC:U.S. Environmental Protection Agency.

EPA. 2003. Label Review Manual. 3rd ed. EPA735-B-03-001. Office of Prevention, Pesticides and Toxic Substances. Washington, DC:U.S. Environmental Protection Agency.

EU. 2001. Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L255:1–333.

Federal Environmental Pesticide Control Act. 1972. Public Law 92-516.

Federal Hazardous Substances Act. 1964. Public Law 86-613.

Federal Insecticide, Fungicide and Rodenticide Act. 1947. Public Law 80-102.

Food, Drug and Cosmetic Act of 1938. 1938. Public Law 75-717.

Freeberg FE, Griffith JF, Bruce RD, Bay PHS. 1984. Correlation of animal test methods with human experience for household products. J Toxic Cut & Ocular Toxic 1:53–64.

Freeberg FE, Hooker DT, Griffith JF. 1986b. Correlation of animal test methods with human experience for household products: an update. J Toxic Cut & Ocular Toxic 5:115–123.

Freeberg FE, Nixon GA, Reer PJ, Weaver JE, Bruce RD, Griffith JF, et al. 1986a. Human and rabbit eye responses to chemical insult. Fundam Appl Toxicol 7:626–634.

Gettings S, Lordo R, Hintze K, Bagley D, Casterton P, Chudkowski M, et al. 1996a. The CTFA evaluation of alternatives program: an evaluation of *in vitro* alternatives to the Draize primary eye irritation test. (Phase III) surfactant-based formulations. Food Chem Toxic 34:79–117.

Gettings SD, Lordo RA, Demetrulias J, Feder PI, Hintze KL. 1996b. Comparison of low-volume, Draize and *in vitro* eye irritation test data. I. Hydroalcoholic formulations. Food Chem Toxicol 34:737–749.

Gettings SD, Lordo RA, Feder PI, Hintze KL. 1998. A comparison of low volume, Draize and *in vitro* eye irritation test data. III. Surfactant-based formulations. Food Chem Toxicol 36:209–231.

Ghassemi A, Sauers LH, Bruner LH, Reer PJ, Hall RH. 1993. Demonstrating the human safety of a new household cleaning (HSC) product using alternatives to the Draize eye irritation test. Presentation made at the U.S. Society of Toxicology New Orleans, Louisiana.

Grant WM, Schuman JS. 1993. Toxicology of the Eye: Effects on the Eyes and Visual System from Chemicals, Drugs, Metals and Minerals, Plants, Toxins, and Venoms; Also Systemic Side Effects from Eye Medications. 4th ed. Springfield, IL:Charles C. Thomas.

Griffith J, Nixon G, Bruce R, Reer P, Bannan E. 1980. Dose-response studies with chemical irritants in the albino rabbit eye as a basis for selecting optimum testing conditions for predicting hazard to the human eye. Toxic Appl Pharmacol 55:501–513.

ICCVAM. 2003. ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods. NIH Publication No. 03-4508. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

ICCVAM. 2010. ICCVAM Test Method Evaluation Report: Current Validation Status of a Proposed *In Vitro* Testing Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products. NIH Publication No. 10-7513. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

Jester JV. 2006. Extent of corneal injury as biomarker for hazard assessment and the development of alternative models to the Draize rabbit eye test. Cutan Ocul Toxicol 25:41–54.

Kay JH, Calandra JC. 1962. Interpretation of eye irritation tests. J Soc Cosmet Chem 13:281–289.

Maurer JK, Molai A, Parker RD, Li L, Carr GJ, Petroll WM, Cavanagh HD, Jester JV. 2001a. Pathology of ocular irritation with bleaching agents in the rabbit low-volume eye test Toxicologic Pathology 29:308–319.

Maurer JK, Molai A, Parker RD, Li L, Carr GJ, Petroll WM, Cavanagh HD, Jester JV. 2001b. Pathology of ocular irritation with acetone, cyclohexanol, parafluoroaniline, and formaldehyde in the rabbit low-volume eye test. Toxicologic Pathology 29:187–199.

McGwin G. Hall TA, Xie A, Owsley C. 2006. Trends in eye injury in the United States, 1992-2001. Inv Ophthalmol Vis Science 47:521–527.

McLaughlin R. 1946. Chemical burns of the human cornea. Am J Ophthamol 29:1355–1362.

Occupational Safety and Health Act of 1970. 1970. Public Law 91-596

Organisation for Economic Co-operation and Development. 2002. Test guideline 405, Acute eye irritation/corrosion, adopted February 24, 1987. In: OECD Guidelines for Testing of Chemicals. Paris:OECD.

Roggeband R, York M, Pericoi M, Braun W. 2000. Eye irritation responses in rabbit and man after single applications of equal volumes of undiluted model liquid detergent products. Food Chem Toxic 38:727–734.

Toxic Substances Control Act of 1976. 1976. Public Law 94-469.

United Nations. 2007. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). New York, Geneva:United Nations Publications.

Wagoner MD. 1997. Chemical injuries of the eye: Current concepts in pathophysiology and therapy. Surv Ophthalmol 41:275–313.

Wilhelmus K. 2001. The Draize eye test. Survey of Ophthalmology 45:493–515.

8.0 Glossary[1]

Assay:[2] The experimental system used. Often used interchangeably with *test* and *test method.*

Canthus: The angle formed by the meeting of the upper and lower eyelids at either side of the eye.

Chemosis: A form of eye irritation in which the membranes that line the eyelids and surface of the eye (*conjunctiva*) become swollen.

Classification system: An arrangement of quantified results or data into groups or categories according to previously established criteria.

Confocal microscopy: An optical imaging technique that increases the contrast of micrographs. It can used to reconstruct three-dimensional images by use of a spatial pinhole to eliminate out-of-focus light or flare in specimens that are thicker than the focal plane.

Conjunctiva: The mucous membrane that lines the inner surfaces of the eyelids and folds back to cover the front surface of the eyeball, except for the central clear portion of the outer eye (the cornea). The conjunctiva is composed of three sections: palpebral conjunctiva, bulbar conjunctiva, and fornix.

Conjunctival sac: The space located between the eyelid and the conjunctiva-covered eyeball. Substances are instilled into the sac to conduct an *in vivo* eye test.

Cornea: The transparent part of the coat of the eyeball that covers the iris and pupil and admits light to the interior.

Corneal opacity: A subjective measurement of the extent of opaqueness of the cornea following exposure to a test substance. Increased corneal opacity is indicative of damage to the cornea.

Corneal stroma: The substantia propia: a tough, fibrous, transparent layer consisting of plates of collagen fibrils (lamellae) produced by keratocytes that make up 10% of the stroma. The fibrils run parallel to each other, but are positioned at right angles to adjacent lamellae.

Corrosion: Destruction of tissue at the site of contact with a substance.

Corrosive: A substance that causes irreversible tissue damage at the site of contact.

Distress: To cause pain, or stress, or suffering to.

Endpoint:[2] The biological process, response, or effect assessed by a test method.

Globally Harmonized System (GHS): A classification system presented by the United Nations that provides (a) a harmonized criteria for classifying substances and mixtures according to their health, environmental and physical hazards, and (b) harmonized hazard communication elements, including requirements for labeling and safety data sheets.

Hazard:[2] The potential for an adverse health or ecological effect. A hazard potential results only if an exposure occurs that leads to the possibility of an adverse effect being manifested.

Hyperemia: An increase in blood flow to a tissue (e.g., cornea).

In vitro: In glass. Refers to assays that are carried out in an artificial system (e.g., in a test tube or petri dish) and typically use single-cell organisms, cultured cells, cell-free extracts, or purified cellular components.

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[1] The definitions in this Glossary are restricted to their uses with respect to the Draize rabbit eye test method and in the assessment or treatment of pain and distress.

[2] Definition used by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM 2003)

In vivo: In the living organism. Refers to assays performed in multicellular organisms.

Iris: The contractile diaphragm perforated by the pupil and forming the colored portion of the eye.

Not Labeled: (a) A substance the produces no changes in the eye following application to the anterior surface of the eye. (b) Substances that are not classified as GHS Category 1, 2A, or 2B; or EU R41 or R36 ocular irritants.

Ocular: Of or relating to the eye.

Ocular corrosive: A substance that causes irreversible tissue damage in the eye following application to the anterior surface of the eye.

Ocular irritant: A substance that produces a reversible change in the eye following application to the anterior surface of the eye.

Pain: An unpleasant sensation occurring in varying degrees of severity as a consequence of injury, disease, or emotional disorder; suffering or distress.

pH: A measure of the acidity or alkalinity of a solution. A pH of 7.0 is neutral; higher pHs are alkaline, lower pHs are acidic.

Protocol:[2] The precise, step-by-step description of a test, including the listing of all necessary reagents, criteria and procedures for the evaluation of the test data.

Severe irritant: (a) A substance that causes tissue damage in the eye following application to the anterior surface of the eye that is not reversible within 21 days of application or causes serious physical decay of vision. (b) Substances that are classified as GHS Category 1, EPA Category I, or EU R41 ocular irritants.

Test:[2] The experimental system used; used interchangeably with *test method* and *assay*.

Test method:[2] A process or procedure used to obtain information on the characteristics of a substance or agent. Toxicological test methods generate information regarding the ability of a substance or agent to produce a specified biological effect under specified conditions. Used interchangeably with *test* and *assay*. See also *validated test method* and *reference test*.

Validated test method:[2] An accepted test method for which validation studies have been completed to determine the relevance and reliability of this method for a specific proposed use.

Validation:[2] The process by which the reliability and relevance of a procedure are established for a specific purpose.

Weight of evidence (process): The strengths and weaknesses of a collection of information are used as the basis for a conclusion that may not be evident from the individual data.