Draft ICCVAM Summary Review Document: The Low Volume Eye Test

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

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

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
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Erratum: May 15, 2009

A typographical error in the Executive Summary of the draft ICCVAM LVET Summary Review Document (SRD; April 1, 2009) incorrectly states that there is "*no* information on the performance of known human corrosives in the LVET". Section 5.0 of the SRD recognizes that there are limited data on such substances. Therefore, the Executive Summary should instead indicate that there is "*limited* information on the performance of known human corrosives in the LVET". We apologize for any confusion this oversight has created.

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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 (EPA), U.S. Food and Drug Administration, and U.S. Occupational Health and Safety Administration have testing requirements and guidelines 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 (Draize et al. 1944). 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) as an alternative with the intent to both refine the Draize rabbit eye test and to potentially more closely predict the accidental human response to ocular hazard due to the site of test substance application (corneal surface) and decreased volume of exposure (10µL) used. However, this hypothesis has yet to be clearly demonstrated, and thus the LVET has yet to be adopted as a reference test method by any regulatory agency.

ICCVAM is now reviewing the validity of the LVET because LVET data are used to support the validity of one of the *in vitro* test methods proposed in an *in vitro* testing strategy for antimicrobial cleaning products (see ICCVAM 2009 Summary Review Document, http://iccvam.niehs.nih.gov/methods/ocutox/antimicro/BRD.pdf). The OTWG and NICEATM prepared a draft summary review document (SRD) that summarizes the current validation status of the LVET based on available information and data obtained by NICEATM. This draft ICCVAM SRD forms the basis for draft ICCVAM test method recommendations, which are provided in a separate document.

An international independent scientific peer review panel (Panel) will be convened in public forum on May 19-21, 2009, to develop conclusions and recommendations on the LVET. The panel includes expert scientists nominated by ECVAM and JaCVAM and we anticipate that these organizations will be able to use the independent report of the panel for their deliberations and development of test method recommendations. The Panel will meet to consider this SRD and to evaluate the extent to which the available information supports the draft ICCVAM test method recommendations. ICCVAM will consider the conclusions and recommendations of the Panel, along with comments received from the public and SACATM, and then finalize the SRD 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 contributing to the preparation of this summary review document, including the following staff from the NICEATM Support Contractor, Integrated Laboratory Systems, Inc.: David Allen, Ph.D., Jon Hamm, Ph.D., Nelson Johnson, Elizabeth Lipscomb, Ph.D., Linda Litchfield, Gregory Moyer, M.B.A., Catherine Sprankle, and Jim Truax. We also thank the members of the ICCVAM OTWG, chaired by Karen Hamernik, Ph.D. (EPA) and Jill Merrill, Ph.D. (U.S. Food and Drug Administration), and ICCVAM representatives who subsequently 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 OTWG 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 represents a major cause of visual impairment. U.S. and international regulatory agencies currently use the Draize rabbit eye test (Draize et al. 1944) as the preferred method to identify potential ocular hazards associated with such chemicals. This procedure involves the introduction of 100μL of the test substance 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. One such method, the low volume eye test (LVET), was developed by Griffith et al. (1980) as an alternative with the intent to both refine the rabbit eye test and to potentially more closely predict the accidental human response to ocular hazard due to the site of test substance application (corneal surface) and decreased volume of exposure (10μL) used. However, this hypothesis has yet to be clearly demonstrated, and thus the LVET has yet to be adopted as a reference test method by any regulatory agency. This report provides a summary of the usefulness and limitations of the LVET as an acceptable *in vivo* reference test method by reviewing the currently available scientific literature.

The majority of 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. (1996) evaluated 25 surfactant formulations and their hazard classifications by the EPA and GHS, and reported several incidences of underprediction of an ocular corrosive or severe irritant response in the Draize rabbit eye test by the LVET method. While some have used these data to state that the Draize eye test is excessively overpredictive, there is no information on the performance of known human corrosives in the LVET.

Freeberg et al. (1984) used both LVET and Draize to test 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) used both LVET and Draize to test 14 cleaning products, and compared the responses to human accidental eye exposures. They too concluded that the LVET response more closely relates to the human experience than the Draize rabbit test. Ghassemi et al. (1993) and Roggeband et al. (2000) both concluded

that the smaller volume (10µL) used in the LVET is more appropriate when compared directly with human clinical data. However, the lack of available Draize rabbit eye test data in these studies precludes any direct comparison with LVET.

This review of the validity of the LVET was undertaken because LVET data is used to support the validity of one of the *in vitro* test methods proposed in an *in vitro* testing strategy for antimicrobial cleaning products (see ICCVAM 2009 Summary Review Document, http://iccvam.niehs.nih.gov/methods/ocutox/antimicro/BRD.pdf). Comparative traditional Draize rabbit data with which to evaluate the accuracy of the LVET are only available for limited types and numbers of substances (i.e., surfactant-containing personal and household cleaning products), and comparative human data from clinical studies and accidental exposures proposed to support its accuracy are largely with substances that are mild or non-irritating. 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 irritant 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 in humans.

Thus, while the LVET is proposed as more likely to approximate the volume of a substance that could enter the human eye experimentally, 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 where 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.

1.0 Background on Ocular Safety Testing

Accidental eye injury is a leading cause of visual impairment in the U.S., and 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 2000 U.S. workers have a job-related eye injury that requires medical treatment. Even more 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 requirements for assessing the hazard potential of substances that may come in contact with human eyes. These testing requirements have effectively protected consumers and workers. 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]), OECD 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 (see **Table 1-1**).

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; OSHA = Occupational Safety and Health Administration; TSCA = Toxic Substances Control Act.

2.0 Regulatory Testing Requirements for Ocular Hazards

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 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 the severity of the effects. The EPA classifies substances into four ocular irritant categories, ranging from I to 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, 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 number 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) 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.

For the purpose of harmonizing the classification of ocular irritants internationally, the United Nations (UN) Globally Harmonized System of Classification and Labeling of Chemicals (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 persistence, as Category 2A ("irritating to eyes"; reverses within 21 days) and Category 2B ("mildly irritating to eyes"; reverses within seven days). The GHS categories are based on severity of the lesions and/or the duration of persistence.

375 **Table 2-1 Ocular Toxicity Classification Systems**

Regulatory Agency (Authorizing Act)	Number of Animals	Observation Days (after treatment)	Mean score taken?	Positive response	Irritant/Non-irritant Classification
EPA (FIFRA, The Federal Environmental Pesticide Control Act, and TSCA)	At least 3	1 hr, 1, 2, 3, 7, 14, 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
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 averaged over all animals in study 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** (1) 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** (1) 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

Regulatory Agency (Authorizing Act)	Number of Animals	Observation Days (after treatment)	Mean score taken?	Positive response	Irritant/Non-irritant Classification
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 at least one of the following scores on Day 21 = Eye Irritant Category 1: Cornea ≥ 1 Iritis ≥ 1 Redness ≥ 2 Chemosis ≥ 2
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: Cornea and Iritis scores < 1 and Redness and Chemosis scores < 2
CPSC (Federal Hazardous Substances Act, FDA (Food, Drug, and Cosmetics Act), and 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 3 or more positive animals = Irritant 1-2 positive animals = Go to 3rd Tier

Abbreviations: CPSC = U.S. Consumer Product Safety Commission; FDA = U.S. Food and Drug Administration; EPA = U.S. Environmental Protection Agency; FIFRA = Federal Insecticide, Fungicide, and Rodenticide Act; GHS = United Nations (UN) Globally Harmonized System of Classification and Labeling of Chemicals; OSHA = U.S. Occupational Safety and Health Administration; TSCA = Toxic Substances Control Act

3.0 Principle of the Low Volume Eye Test (LVET)

The LVET is an *in vivo* rabbit eye test developed by Griffith et al. (1980), which, like the Draize test, was designed to determine the extent of potential ocular hazard of a test material by evaluating the ocular irritation response in the rabbit when administered to the eye as a single dose. 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 Draize Eye Test and LVET 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

LVET = low volume eye test

To date, the LVET has not been demonstrated as an adequately valid *in vivo* reference test method, and has yet to be 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. The International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.) submitted a background review document (BRD) in February 2007 to the European Centre for the Validation of Alternative Methods (ECVAM) for an independent peer review by their Scientific Advisory Committee (ESAC) (**Appendix A**). The A.I.S.E. BRD 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 primarily based on the assertion that the LVET is more representative

403 of the human response to a potential ocular hazard than the Draize rabbit eye test, given that
404 the site (corneal surface) and volume of exposure used in the LVET more closely resemble
405 that of accidental human exposure than does the Draize. As a result, a reported advantage of
406 the LVET is that it underpredicts the Draize test and is thereby less overpredictive of the
407 human response than the Draize test. However, definitive data to support this claim are not
408 available.

409 **Table 3-2 Scale of Weighted Scores for Grading the Severity of Ocular Lesions[1]**

Lesion	Score[2]
Cornea	
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	
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	
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	

410 [1] From Draize et al. (1944)

411 [2] The maximum total score is the sum of all scores obtained for the cornea, iris and conjunctiva. Scores of
412 0 are assigned for each parameter if the cornea, iris, or conjunctiva are normal.
413

414

4.0 Performance of the LVET vs. the Draize Rabbit Eye Test

As noted in the ECVAM BRD (**Appendix A**), most of the original data generated with the LVET were from surfactant-based mixtures or surfactant-based products. Most of the products tested produced only mild ocular irritant responses or are not ocular irritants. According to the ECVAM BRD (**Appendix A**), "most of the LVET results do not trigger an eye hazard classification based on European regulatory criteria and this correlates well with the extensive data on human experience with these products from the marketplace." However, 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. This is illustrated by the results of Gettings et al. (1996) in their evaluation of 25 surfactant-containing formulations and the resulting hazard classifications according to the EPA and GHS classification systems (**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		LVET				
		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

EPA = Environmental Protection Agency ocular hazard classification; LVET = low volume eye test

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		LVET				
		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

GHS = United Nations Globally Harmonised System ocular hazard classification; LVET = low volume eye test

Based on the data provided in **Tables 4-1** and **4-2**, it is clear that there are multiple instances of underprediction of an ocular corrosive or severe irritant response in the Draize rabbit eye test by the LVET. When using the EPA hazard classification system, 60% (6/10) of Draize Category I substances were underpredicted as Category III (i.e., mild irritant) in the LVET (**Table 4-3**). When using the GHS hazard classification system, all eight of the Draize Category 1 substances were underpredicted; 50% (4/8) as Category 2B (i.e., mild irritant) and 50% (4/8) as Not Labeled (i.e., nonirritant) (**Table 4-4**). These data raise concern about the capability of the LVET to reliably detect ocular corrosives or severe irritants (i.e., EPA Category I, EU Category R41, or GHS Category 1).

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

Draize Category	LVET Category	Product
Category I	Category II	HZY (Anti-dandruff 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

453 **Table 4-4 Extent of Underprediction of LVET vs. Draize Rabbit Eye Test**
454 **Results According to the GHS Classification System**

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 (Anti-dandruff 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)

455 Abbreviations: GHS = United Nations Globally Harmonised System ocular hazard classification;
456 LVET = low volume eye test
457

5.0 Performance of the LVET 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 since such exposures are likely immediately followed by flushing the eyes with large volumes of water, and 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 0.01 or 0.1 mL of substances "recognized as slightly irritating, moderately irritating, or severely irritating/corrosive to humans". Among the ocular corrosive or severe irritant substances were:

- 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 1974)
- 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 1974)

Although detailed animal data are not available, the summary data provided by Griffith et al. (1980) indicate that the lesions induced by 0.01 or 0.1 mL 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 irritant 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 in the evaluation described above could be seen as providing support for 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 are cited in the ECVAM BRD (**Appendix A**) as supporting data for the demonstrated usefulness of the LVET (Freeberg et al. 1984, 1986a, 1986b; Ghassemi et al. 1993; Roggeband et al. 2000).

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: non-ionic surfactant, amphoteric surfactant, hydrotrope, solvent, and water. This study is a direct comparison of LVET results to human clinical data (using either 10 μL or 100 μL doses) for the same test substance. There are no Draize rabbit eye test data reported, and therefore no comparison of the LVET to the standard eye test is possible. The ocular lesions that were produced in this study and their subsequent time to clear would 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 μL 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 (Ghassemi et al. 1993)

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 (10μL)	Conj	0/10	0/10	10/10	0.1	1/1hr; 4/2hr; 6/4hr; 10/24hr	48 hr
Human (100μL)	Conj	0/10	0/10	10/10	0.2	1/1hr; 2/2hr; 9/24hr; 2/46hr	70hr

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

5.2 Roggeband et al. (2000)

Similarly, Roggeband et al. (2000) provides an evaluation of *two products*, a dishwashing liquid (pH 8, contains anionic surfactant, non-ionic surfactant, soap, ethanol, water) and a liquid laundry detergent (pH 7, contains anionic surfactant, non-ionic surfactant, ethanol, water). This study is a direct comparison of 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 would suggest that these products are mild ocular irritants (**Table 5-2**). The authors conclude that these data support the notion that an accidental exposure would be approximately 10 μL or less, and that a volume of 10 μL would provide 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 3μL of liquid laundry detergent before pre-determined 'cut-off' ocular responses were observed above which it would have been ethically unacceptable to proceed."

529 **Table 5-2 Human and Rabbit Eye Responses to a Liquid Laundry Detergent (1 µL, Roggeband et al. 2000)**

Human Volunteer	Human				Animal Number	Rabbit LVET[1]			
	1 hour		24 hour			1 hour		24 hour	
	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					

530 (c) = test substance dosed on the central cornea; Conj = conjunctiva; LVET = low volume eye test; (scs) = test substance dosed on the superior conjunctival sac
531 [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) provides comparisons of data from LVET, Draize rabbit eye test, and human studies or experience. Freeberg et al. presents LVET and Draize rabbit eye test data for 29 cleaning products (laundry products, household cleaning products, and dishwashing products) as compared to human experience data. The ocular lesions that were produced in this study and their subsequent time to clear would suggest that these products are either mild ocular irritants or nonirritants (**Table 5-3**). The human data were obtained from medical records of factory and consumer accidental eye exposures (515 reports over a two-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 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 (Days) (Range)	Average ± SD Median Time to Clear (Days) (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)

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) compares 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 in rabbits overpredicted human response to 10 µL or 100 µL of the same product. The ocular lesions (both type and longevity) in the rabbit Draize (100

μL) were more severe than in the human test using the same volume as the rabbit Draize (100 μL). 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 would suggest that these products are would be classified as mild ocular irritants based on the Draize eye test results, the LVET, or human results (**Table 5-4**).

Table 5-4 Human Clinical Study and Rabbit Data from Freeberg et al. (1986a)

Test Product	Concentration (% in water)	Time-to-Clear (hr)			
		Dosing Procedure			
		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

Abbreviations: NT = Not tested

5.5 Freeberg et al. (1986b)

Freeberg et al. (1986b) presents 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) compared to human experience data. The ocular lesions that were produced in this study and their subsequent

571 time to clear would suggest that these products would be classified as moderate to severe
572 ocular irritants based on the Draize eye test results, while most would be classified as mild
573 ocular irritants by the LVET (**Table 5-5**). The human data were obtained from medical
574 records of factory and consumer accidental eye exposures (218 reports over an 18-month
575 period). Similar to Freeberg et al. (1986a), rabbit LVET and Draize tests both overpredicted
576 the human response due to accidental eye exposure (based on time-to-clear). Because the
577 time-to-clear was longer for substances tested in the Draize rabbit eye test than in the
578 LVET, the authors concluded that the LVET outcome more closely relates to the human
579 experience than the Draize rabbit eye test.

580 **Table 5-5 Human Accidental Exposure and Rabbit Data from Freeberg et al.**
581 **(1986b)**

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

582 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 only these product types. Although LVET data have been used by at least one personal care products company to support submission of data to the EPA for registration of an antimicrobial cleaning product, these results were reportedly used by EPA reviewers in a weight-of-evidence approach, with supporting Draize rabbit eye test data and human post-marketing surveillance data (i.e., commercial products for which there is an opportunity for adverse events to be reported by the consumer).

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 since 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 with mild irritant or nonirritant substances. 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 experimentally, 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 where 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

7.0 References

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

CPSC. 1995. Test for eye irritants. 16CFR1500.42.

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.

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

EPA. 2003. Label Review Manual: 3rd Ed. EPA735-B-03-001. Office of Prevention, Pesticides and Toxic Substances. Washington: 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.

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

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

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

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

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, 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.

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.

Gettings S, Lordo R, Hintze K, Bagley D, Casterton P, Chudkowski M, et al. 1996. 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.

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 meeting.

Grant W. 1974. Toxicology of the Eye, 2nd ed. Charles C. Thomas, Springfield, IL.

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.

650 McLaughlin R. 1946. Chemical burns of the human cornea. Am J Ophthamol 29:1355-
651 1362.

652 OECD. 2002. Test guideline 405, Acute eye irritation/corrosion, adopted February 24,
653 1987. In OECD Guidelines for Testing of Chemicals. OECD, Paris.

654 Occupational Safety and Health Act of 1970 (OSHA). 1970. Public Law 91-596

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

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

659 UN. 2002. OECD Guideline for the Testing of Chemicals. Acute Eye Irritation/Corrosion.
660 Adopted: 24th April 2002. New York, Geneva:United Nations Publications.

661 UN. 2003. Globally Harmonised System of Classification and Labelling of Chemicals
662 (GHS). New York, Geneva:United Nations Publications.

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