ICCVAM Test Method Evaluation Report: Current Validation Status of *In Vitro* Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products (Volume 2)

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

NIH Publication Number 10-7553

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

This document is available electronically at:
https://ntp.niehs.nih.gov/iccvam/docs/ocutox_docs/invitro-2010/tmer-vol2.pdf

When referencing this document, please cite as follows:
ICCVAM. 2010. ICCVAM Test Method Evaluation Report: Current Validation Status of *In Vitro* Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products. NIH Publication No. 10-7553. Research Triangle Park, NC: National Institute of Environmental Health Sciences.

Table of Contents
Volume 1 of 2

Table of Contents
Volume 2 of 2

List of Tables

List of Abbreviations and Acronyms

AMCP	Antimicrobial cleaning product
BCOP	Bovine corneal opacity and permeability
BRD	Background review document
CAM	Chorioallantoic membrane
CEC	Commission of European Communities
CM	Cytosensor® Microphysiometer
CV	Coefficient of variation
ºC	Degrees centigrade
ECVAM	European Centre for the Validation of Alternative Methods
EPA	U.S. Environmental Protection Agency
ESAC	ECVAM Scientific Advisory Committee
EU	European Union
FHSA	Federal Hazardous Substances Act
FR	*Federal Register*
g	Gram
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
HET-CAM	Hen's egg test–chorioallantoic membrane
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Isolated chicken eye
IRE	Isolated rabbit eye
IS	Irritation score
MeSH	Medical Subject Headings
mL	Milliliter
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NTP	U.S. National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OTWG	ICCVAM Ocular Toxicity Working Group
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
UN	United Nations
UV/VIS	Ultraviolet/Visible

Interagency Coordinating Committee on the Validation of Alternative Methods: Agency Representatives

Agency for Toxic Substances and Disease Registry
* Moiz Mumtaz, Ph.D.
Bruce Fowler, Ph.D.
Edward Murray, Ph.D.
Eric Sampson, Ph.D.

Consumer Product Safety Commission
* Marilyn L. Wind, Ph.D. (Chair)
+Kristina Hatlelid, Ph.D.
Joanna Matheson, Ph.D.

Department of Agriculture
* Jodie Kulpa-Eddy, D.V.M. (Vice-Chair)
+Elizabeth Goldentyer, D.V.M.

Department of Defense
* Robert E. Foster, Ph.D.
+Patty Decot
Harry Salem, Ph.D.
Peter J. Schultheiss, D.V.M., DACLAM

Department of Energy
* Michael Kuperberg, Ph.D.
+Marvin Stodolsky, Ph.D.

Department of the Interior
* Barnett A. Rattner, Ph.D.
+Sarah Gerould, Ph.D. (to Feb. 2009)

Department of Transportation
* George Cushmac, Ph.D.
+Steve Hwang, Ph.D.

Environmental Protection Agency
Office of Pesticide Programs
* John R. "Jack" Fowle III, Ph.D., DABT
+Vicki Dellarco, Ph.D.
+Tina Levine, Ph.D.
Deborah McCall
Christine Augustyniak, Ph.D. *(U.S. Coordinator, OECD Test Guidelines Program)*
Office of Pollution Prevention and Toxics
Jerry Smrchek, Ph.D. *(U.S. Coordinator, OECD Test Guidelines Program,* to July 2009*)*
Office of Research and Development
Suzanne McMaster, Ph.D. (to Dec. 2008)
Julian Preston, Ph.D. (to July 2009)
Stephanie Padilla, Ph.D. (to July 2009)
Office of Science Coordination and Policy
Karen Hamernik, Ph.D. (to July 2009)

Food and Drug Administration
Office of the Commissioner
* Suzanne Fitzpatrick, Ph.D., DABT
Center for Biologics Evaluation and Research
Richard McFarland, Ph.D., M.D.
Ying Huang, Ph.D.
Center for Devices and Radiological Health
Melvin E. Stratmeyer, Ph.D.
Vasant G. Malshet, Ph.D., DABT
Center for Drug Evaluation and Research
+Abigail C. Jacobs, Ph.D.
Paul C. Brown, Ph.D.
Center for Food Safety and Applied Nutrition
David G. Hattan, Ph.D.
Robert L. Bronaugh, Ph.D.
Center for Veterinary Medicine
Devaraya Jagannath, Ph.D.
M. Cecilia Aguila, D.V.M.
National Center for Toxicological Research
Paul Howard, Ph.D.
Donna Mendrick, Ph.D.
William T. Allaben, Ph.D. (to Jan. 2009)
Office of Regulatory Affairs
Lawrence D'Hoostelaere, Ph.D.

National Cancer Institute
* T. Kevin Howcroft, Ph.D.
Chand Khanna, D.V.M., Ph.D.
Alan Poland, M.D. (to Oct. 2008)

National Institute of Environmental Health Sciences
* William S. Stokes, D.V.M., DACLAM
+Raymond R. Tice, Ph.D.
Rajendra S. Chhabra, Ph.D., DABT
Jerrold J. Heindel, Ph.D.

National Institute for Occupational Safety and Health
* Paul Nicolaysen, V.M.D.
+K. Murali Rao, M.D., Ph.D.

National Institutes of Health
* Margaret D. Snyder, Ph.D.

National Library of Medicine
* Pertti (Bert) Hakkinen, Ph.D.
+Jeanne Goshorn, M.S.

Occupational Safety and Health Administration
* Surender Ahir, Ph.D.

* Principal agency representative
+ Alternate principal agency representative

Acknowledgements

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Ocular Toxicity Working Group (OTWG)

U.S. Consumer Product Safety Commission
Marilyn L. Wind, Ph.D.
Adrienne Layton, Ph.D.

U.S. Department of Defense
Harry Salem, Ph.D.

U.S. Department of Transportation
Steve Hwang, Ph.D.

U.S. Environmental Protection Agency
Office of Pesticide Programs
Meta Bonner, Ph.D.
Jonathan Chen, Ph.D.
John R. "Jack" Fowle III, Ph.D., DABT
Masih Hashim, D.V.M., Ph.D.
Karen Hicks
Marianne Lewis
Debbie McCall
Timothy McMahon, Ph.D.
Mark Perry
John Redden
Jenny Tao, Ph.D.
Office of Research and Development
Andrew Geller, Ph.D.
Office of Science Coordination and Policy
Karen Hamernik, Ph.D.

U.S. Food and Drug Administration
Center for Drug Evaluation and Research
Paul Brown, Ph.D.
Wiley Chambers, M.D.
Abigail (Abby) Jacobs, Ph.D.
Jill Merrill, Ph.D., DABT (OTWG Chair)
Center for Food Safety and Applied Nutrition
Robert Bronaugh, Ph.D.
Donnie Lowther
Office of the Commissioner
Suzanne Fitzpatrick, Ph.D., DABT

National Institute of Environmental Health Sciences
Warren Casey, Ph.D., DABT
Mark F. Cesta, D.V.M, DACVP
Raymond (Buck) Grissom, Ph.D.
William Stokes, D.V.M., DACLAM

Occupational Safety and Health Administration
Surender Ahir, Ph.D.

European Centre for the Validation of Alternative Methods – Liaison
João Barroso, Ph.D.
Thomas Cole, Ph.D.
Valerie Zuang, Ph.D.

Japanese Center for the Validation of Alternative Methods – Liaison
Hajime Kojima, Ph.D.

Alternative Ocular Safety Testing Methods and Approaches Independent Scientific Peer Review Panel (May 19–21, 2009)

Hongshik Ahn, Ph.D.
Professor
Stony Brook University
Stony Brook, NY

Paul T. Bailey, Ph.D.
Bailey & Associates Consulting
Neshanic Station, NJ

Richard Dubielzig, D.V.M.
Professor
School of Veterinary Medicine
University of Wisconsin-Madison
Madison, WI

Henry Edelhauser, Ph.D.[1]
Professor of Ophthalmology and Director of Ophthalmic Research
Emory University School of Medicine
Atlanta, GA

Mark Evans, D.V.M., Ph.D., DACVP
Pathology Lead for Ophthalmology Therapeutic Area
Pfizer Global Research and Development
La Jolla Drug Safety Research and Development
San Diego, CA

A. Wallace Hayes, Ph.D., DABT, FATS, ERT
Visiting Scientist (Harvard)
Principal Advisor
Spherix Incorporated
Bethesda, MD
Harvard School of Public Health
Andover, MA

James V. Jester, Ph.D.
Professor of Ophthalmology and Biomedical Engineering
Endowed Chair
University of California-Irvine
Orange, CA

Tadashi Kosaka, D.V.M., Ph.D.
Associate Director
Chief, Laboratory of Immunotoxicology and Acute Toxicology
Toxicology Division
The Institute of Environmental Toxicology
Ibaraki, Japan

Alison McLaughlin, M.Sc., DABT
Health Canada
Environmental Impact Initiative
Office of Science and Risk Management
Health Products and Food Branch
Ottawa, Ontario, Canada

J. Lynn Palmer, Ph.D.
Associate Professor
Dept. of Palliative Care & Rehabilitation Medicine
University of Texas
MD Anderson Cancer Center
Houston, TX

Robert Peiffer, Jr., D.V.M., Ph.D., DACVO
Senior Investigator
Safety Assessment Toxicology
Merck Research Laboratories
Doylestown, PA

Denise Rodeheaver, Ph.D., DABT
Assistant Director
Dept. of Toxicology
Alcon Research Ltd.
Fort Worth, TX

Donald Sawyer, D.V.M., Ph.D., DACVA
Professor Emeritus
Retired, Michigan State University
(Summer Residence)
Okemos, MI
(Winter Residence)
Tucson, AZ

[1] Drs. Edelhauser, Thake, and Tseng were unable to attend the public meeting on May 19–21, 2009. However, they were involved in the peer review of the background review documents and concur with the conclusions and recommendations included in the *Independent Scientific Peer Review Panel Report – Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Strategies.*

Kirk Tarlo, Ph.D., DABT
Scientific Director
Comparative Biology and Safety Sciences
Amgen, Inc.
Thousand Oaks, CA

Daryl C. Thake, D.V.M., DACVP[1]
Midwest ToxPath Sciences Inc.
Chesterfield, MO

Scheffer Tseng, M.D., Ph.D.[1]
Director, Ocular Surface (OS) Center
Medical Director OS Research & Education Foundation
Director R&D Department
Tissue Tech, Inc.
Ocular Surface Center, P.A.
Miami, FL

Jan van der Valk, Ph.D.
Senior Scientist
Department of Animals in Science and Society
Faculty of Veterinary Medicine
Utrecht University
Netherlands Centre Alternatives to Animal Use
Utrecht, Netherlands

Philippe Vanparys, Ph.D., DABT
Managing Director
CARDAM: Centre for Advanced Research & Development
Mol, Belgium

Maria Pilar Vinardell, Ph.D.
Director, Department of Physiology
Professor of Physiology and Pathology
Department Fisologia
Facultat de Farmacia
Universitat de Barcelona
Barcelona, Spain

Fu-Shin Yu, Ph.D.
Director of Research
Department of Ophthalmology & Anatomy
School of Medicine
Wayne State University
Detroit, MI

Sherry Ward, Ph.D., MBA
In Vitro Toxicology Consultant
BioTred Solutions
Science Advisor
International Foundation for Ethical Research
New Market, MD

Daniel Wilson, Ph.D., DABT
Mammalian Toxicology Consultant, Toxicology and Environmental Research Consulting
The Dow Chemical Co.
Midland, MI

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

National Institute of Environmental Health Sciences

William Stokes, D.V.M., DACLAM
Director; Project Officer

Warren Casey, Ph.D., DABT
Deputy Director

Deborah McCarley
Special Assistant; Assistant Project Officer

NICEATM Support Contract Staff (Integrated Laboratory Systems [ILS], Inc.)

David Allen, Ph.D.
Jonathan Hamm, Ph.D.
Nelson Johnson
Brett Jones, Ph.D.
Elizabeth Lipscomb, Ph.D.
Linda Litchfield
Steven Morefield, M.D.
Catherine Sprankle
James Truax, M.A.
Linda Wilson

Statistical Consultant for ILS, Inc.

Joseph Haseman, Ph.D.

Other Acknowledgements

ICCVAM and NICEATM gratefully acknowledge the following individuals and institutions that submitted data to NICEATM for the evaluation of alternative ocular safety testing methods and approaches.

Access Business Group
Luann Potts
Tom Truszkowski
Washington, DC

Clorox
Pleasanton, CA

Colgate-Palmolive Company
Piscataway, NJ

Cosmetics, Toiletry, and Fragrance Association
Carol Eisenmann, Ph.D.
Washington, DC

The Dial Corporation
Scottsdale, AZ

ECOLabs
St. Paul, MN

ECVAM
Chantra Eskes, Ph.D.
Ispra, Italy

ExxonMobil Biomedical Sciences, Inc.
James Freeman, Ph.D.
Annandale, NJ

Institute for In Vitro Sciences, Inc.
Rodger Curren, Ph.D.
John Harbell Ph.D. (until March 2006)
Jennifer Nash, M.S.
Angela Sizemore, B.S.
Gaithersburg, MD

JohnsonDiversey, Inc.
John Hamilton, Ph.D.
Sarah Willems, B.S.
Sturdivant, WI

Johnson & Johnson Pharmaceutical R&D
Phillipe Vanparys, Ph.D.
Freddy van Goethem, Ph.D.
Beerse, Belgium

L'Oreal
Christine Van den Berghe, Ph.D.
Paris, France

MatTek Corporation
Patrick Hayden, Ph.D.
Ashland. MA

Merck
Joseph Sina, Ph.D.
West Point, PA

National Institute of Health Sciences
Yasuo Ohno, Ph.D.
Tokyo, Japan

S.C. Johnson & Son
Nicole Cuellar, M.S.
Judith Swanson, B.S./B.A.
Racine, WI

The Procter & Gamble Company
Dan Marsman, D.V.M., Ph.D., DABT
Len Sauers, Ph.D.
Cincinnati, OH

TNO Nutrition and Food Research Institute
Menk Prinsen, Ph.D.
Zeist, The Netherlands

U.S. Food and Drug Administration
Donnie Lowther
College Park, MD

ZEBET
Manfred Liebsch, Ph.D.
Horst Spielmann, Dr. med.
Berlin, Germany

This page intentionally left blank

Preface

Eye injury is a leading cause of visual impairment in the United States with 40,000 to 50,000 new cases of impaired vision reported each year.[1] Many eye injuries occur due to contact with workplace or household products or chemicals. Accidents involving common household products (e.g., oven cleaner and bleach) cause about 125,000 eye injuries each year.[2] These products often cause chemical burns and emergency room visits.[3] Each day about 2,000 U.S. workers have a job-related eye injury that requires medical treatment. Although the majority of these eye injuries result from mechanical sources, chemical burns from industrial chemicals or cleaning products are common.[4]

To prevent eye injuries, regulatory agencies require testing to determine if chemicals and products may cause eye damage. This testing information is used to classify the ocular hazard and determine appropriate labeling to warn consumers and workers of the potential hazard. Appropriate labeling tells users how to avoid exposure that could damage the eye and what emergency procedures should be followed if there is accidental exposure. Nearly all ocular safety testing has been conducted using the Draize rabbit eye test (Draize et al. 1944), although *in vitro* methods can now be used to identify whether substances cause severe irritation or permanent eye damage. The Draize rabbit eye test involves instillation of 0.1 mL of the test substance into the conjunctival sac of one eye. The other eye serves as the untreated control. The eye is examined at least daily for up to 21 days. The presence and severity of any injuries to the cornea, conjunctiva, and the iris (tissues inside the eye) are scored, and the duration that the injuries persist is recorded.

In 2006, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) evaluated the validation status of the bovine corneal opacity and permeability (BCOP), hen's egg test–chorioallantoic membrane (HET-CAM), isolated chicken eye (ICE), and isolated rabbit eye (IRE) test methods for their ability to identify ocular corrosives and severe irritants. Based on the validation database and performance, ICCVAM recommended that positive results in the BCOP and ICE test methods could be used to identify ocular corrosives and severe irritants without the need for animal testing. These test methods should always be considered before using animals and should be used where determined appropriate. Following their acceptance by U.S. Federal regulatory agencies in 2008, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and ICCVAM developed Organisation for Economic Co-operation and Development (OECD) international test guidelines for the BCOP and ICE test methods. The OECD adopted the guidelines in 2009.[5] As a result, substances that may cause severe irritation or permanent damage to eyes can now be identified using these methods without the use of live animals in the 31 member countries of the OECD.

This test method evaluation report provides ICCVAM's recommendations regarding the BCOP, HET-CAM, ICE, and IRE test methods for identifying nonsevere ocular irritants and substances not labeled as irritants. The report also includes recommendations on the Cytosensor® Microphysiometer (CM) test method, which was not part of the 2006 evaluation. The report summarizes the validation status of each test method and provides the ICCVAM-recommended BCOP, CM, HET-CAM, ICE, and IRE test method protocols.

---

[1] Available at: http://www.preventblindness.org/resources/factsheets/Eye_Injuries_FS93.PDF
[2] Available at: http://www.geteyesmart.org/eyesmart/injuries/home.cfm
[3] From the CPSC NEISS Database, 2007
[4] Available at: http://www.cdc.gov/niosh/topics/eye/
[5] Test Guideline 437. Bovine corneal opacity and permeability test method for identifying ocular corrosives and severe irritants; Test Guideline 438. Isolated chicken eye test method for identifying ocular corrosives and severe irritants. Both In: OECD Guidelines for Testing of Chemicals. Paris:Organisation for Economic Co-operation and Development

As part of ICCVAM's ongoing international collaborations, scientists from the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM) served as liaisons to the ICCVAM Ocular Toxicity Working Group (OTWG). ICCVAM, NICEATM, and the OTWG prepared (1) draft background review documents (BRDs) describing the validation status of each test method, including reliability and accuracy, and (2) draft test method recommendations for their usefulness and limitations.

ICCVAM released these documents to the public for comment prior to a meeting of an independent international scientific peer review panel (Panel). The Panel met in public session on May 19–21, 2009, and prepared a report summarizing its conclusions and recommendations. The Panel report was provided to the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) along with the draft BRDs, draft test method recommendations, and all public comments. A detailed timeline of the evaluation is included with this report.

ICCVAM solicited and considered public comments and stakeholder involvement throughout the test method evaluation process. ICCVAM considered the SACATM comments, the conclusions of the Panel, and all public comments before finalizing the ICCVAM test method recommendations for each test method. The recommendations and the BRDs, which are provided as appendices, are incorporated in this ICCVAM test method evaluation report. As required by the ICCVAM Authorization Act, ICCVAM will forward its recommendations to U.S. Federal agencies for consideration. Federal agencies must respond to ICCVAM within 180 days after receiving the ICCVAM test method recommendations. ICCVAM recommendations are available to the public on the NICEATM–ICCVAM website, and agency responses will also be made available on the website as they are received.

We gratefully acknowledge the many individuals who contributed to the preparation, review, and revision of this report. We especially recognize the Panel members for their thoughtful evaluations and generous contributions of time and effort. Special thanks are extended to Dr. A. Wallace Hayes for serving as the Panel Chair and to Dr. Paul Bailey, Dr. Donald Sawyer, Dr. Kirk Tarlo, and Dr. Daniel Wilson for their service as Evaluation Group Chairs. We thank the OTWG for assuring a meaningful and comprehensive review. We especially thank Dr. Jill Merrill (U.S. Food and Drug Administration Center for Drug Evaluation and Research) and Dr. Karen Hamernik (EPA, until April 2009) for serving as Co-Chairs of the OTWG. Integrated Laboratory Systems, Inc., the NICEATM support contractor, provided excellent scientific support, for which we thank Dr. David Allen, Dr. Jonathan Hamm, Nelson Johnson, Dr. Brett Jones, Dr. Elizabeth Lipscomb, and James Truax. Finally, we thank the ECVAM liaisons Drs. João Barroso, Thomas Cole, and Valerie Zuang and the JaCVAM liaison Dr. Hajime Kojima for their participation and contributions.

Marilyn Wind, Ph.D.
Deputy Associate Executive Director
Directorate for Health Sciences
U.S. Consumer Product Safety Commission
Chair, ICCVAM

William S. Stokes, D.V.M., DACLAM
Rear Admiral/Assistant Surgeon General, U.S. Public Health Service
Director, NICEATM
Executive Director, ICCVAM

Executive Summary

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently evaluated the validation status of test methods to identify substances that cause reversible eye injuries or do not cause sufficient eye damage to require hazard labeling: the bovine corneal opacity and permeability (BCOP), Cytosensor® Microphysiometer (CM), hen's egg test–chorioallantoic membrane (HET-CAM), isolated chicken eye (ICE), and isolated rabbit eye (IRE) test methods. Nearly all ocular safety testing has been conducted using the *in vivo* Draize rabbit eye test (Draize et al. 1944) to evaluate the potential for substances to cause ocular irritation and other ocular injuries, an acute reaction that may involve corneal cloudiness and ulceration, swelling and redness of the conjunctiva, and/or visible damage to the inside of the eye (iritis). The BCOP, CM, HET-CAM, ICE, and IRE methods are *in vitro* test methods that predict the extent of ocular damage that might occur *in vivo* without requiring the use of live animals. This test method evaluation report provides ICCVAM's recommendations for each *in vitro* test method as an alternative to the Draize rabbit eye test, based on demonstrated validity (usefulness and limitations). This report includes (1) protocols recommended by ICCVAM for future data collection and evaluation for the BCOP, CM, HET-CAM, ICE, and IRE test methods, (2) final background review documents (BRDs) describing the validation status of these test methods, and (3) recommendations for future studies.

Following a nomination by the U.S. Environmental Protection Agency (EPA) requesting an evaluation of several alternative methods and approaches for reducing, replacing, and refining the use of rabbits in the current *in vivo* eye irritation test method, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), ICCVAM, and the ICCVAM Ocular Toxicology Working Group prepared draft BRDs and draft test method recommendations. The drafts were provided to an independent international scientific peer review panel (hereafter "Panel") and to the public for comment. The Panel met in public session on May 19-21, 2009, to discuss its peer review of the ICCVAM draft BRDs and to provide conclusions and recommendations regarding the validation status of the BCOP, CM, HET-CAM, ICE, and IRE test methods. The Panel also reviewed how well the information contained in the draft BRDs supported ICCVAM's draft test method recommendations.

In finalizing this test method evaluation report and the BRDs, which are included here as appendices, ICCVAM considered (1) the conclusions and recommendations of the Panel, (2) comments from ICCVAM's Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), and (3) public comments.

The Bovine Corneal Opacity and Permeability (BCOP) Test Method

ICCVAM Recommendations: BCOP Test Method Usefulness and Limitations
ICCVAM concludes that the accuracy and reliability of the BCOP test method does **not** support its use as a screening test to distinguish substances not labeled as irritants (EPA Category IV, European Union [EU] Not Labeled, Federal Hazardous Substances Act [FHSA] Not Labeled, United Nations Globally Harmonized System of Classification and Labelling of Chemicals [GHS] Not Classified) from all other hazard categories (EPA Category I, II, or III; EU R41 or R36; FHSA Irritant; GHS Category 1, 2A, or 2B) when results are to be used specifically to classify and label substances under the EPA, EU, FHSA, or GHS classification systems. For the BCOP validation database of 211 substances, false positive rates were high, ranging from 53% (24/45) to 70% (63/90), depending on the hazard classification system used. Therefore, all positive results from these tests would require additional testing in a valid test system that can accurately characterize whether such substances require hazard labeling. False negative rates were 0% for the EU (0/54) and GHS (0/97) classification systems, 5% (6/132) for the FHSA classification system, and 6% (8/142) for the EPA classification system.

Among the eight EPA false negatives were three substances (3/8 [38%]) that were classified as EPA eye irritants based on at least one rabbit with corneal injuries and opacity that did not resolve until day 3 of the study. A fourth substance was classified as an EPA eye irritant based on all six rabbits with a conjunctival redness score of 2 (n = 4; *diffuse, crimson color of the conjunctiva, individual blood vessels not easily discernable*) or 3 (n = 2; *diffuse beefy red*). The conjunctival redness scores for two of these animals did not recover to a score of 1 (*some blood vessels definitely hyperemic*) until day 6 of the study. The conjunctival redness scores for the remaining four rabbits recovered to a score of 1 on day 2 of the study. These four EPA false negative substances were also false negatives for the FHSA classification system. Given the significant lesions associated with these false negative substances, the BCOP test method cannot be recommended as a screening test to identify substances not labeled as irritants (i.e., EPA Category IV, FHSA Not Labeled) for the EPA or FHSA classification systems.

Furthermore, although the false negative rate was 0% (0/97) for the GHS classification scheme, the GHS does not classify substances as eye hazards that produce the corneal and conjunctival injuries described above, which are required to be labeled as eye hazards according to the EPA and FHSA classification systems. These findings led NICEATM-ICCVAM to look more closely at the GHS eye hazard classification criteria. NICEATM evaluated results from rabbit eye test studies from two independent databases: (1) 149 studies obtained from a publicly available database (ECETOC 1998) and (2) 144 studies included in the Organization for Economic Cooperation and Development (OECD) Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries (OECD 1999). These data, which are included here as an appendix, confirmed that approximately 30% of the substances requiring labeling for eye irritation hazard based on current U.S. hazard classification requirements (EPA and FHSA) are not labeled as eye irritation hazards by the GHS system. This includes at least 70% of currently labeled EPA Category III irritants (those causing eye injuries persisting for 24 hours to 7 days) that would not require hazard labeling using the GHS system. The nature, severity, and duration of these eye injuries suggest the potential to cause human injury. The purpose of ocular toxicity labeling is to communicate potential hazards of chemicals and products to workers and consumers so that appropriate measures can be taken to avoid accidental or inadvertent contact with the eye. In addition, ocular safety labels provide the necessary first aid measures that should be taken in the event of accidental exposures.

The GHS was established based on principles agreed to by participants, which included assuring that "the level of protection offered to workers, consumers, the general public and the environment should not be reduced as a result of harmonizing the classification and labeling systems" (UN 2007). ICCVAM has conducted technical analyses to support the development of appropriate recommendations for GHS options that would continue to provide protection that is at least equivalent to current U.S. eye irritation hazard classification and labeling requirements. ICCVAM recommends that U.S. agencies consider the GHS eye irritation hazard classification criteria and hazard categories and the level of protection they provide compared to current U.S. hazard classification systems.

Federal law requires agencies to determine that new test methods recommended by ICCVAM generate data that are at least equivalent to data generated by current test methods required or recommended by each agency for hazard identification purposes. Until the issues associated with the GHS system as outlined above are further discussed, ICCVAM is deferring final recommendations on the usefulness and limitations of using the BCOP test method as a screening test to identify substances not labeled as irritants according to the GHS classification system.

ICCVAM Recommendations: BCOP Test Method Protocol

For use of the BCOP test method as a screening test to identify substances as ocular corrosives and severe irritants (EPA Category I, EU R41, GHS Category 1), ICCVAM recommends using the

updated ICCVAM BCOP test method protocol included as an appendix to this report. All future studies intended to further characterize the usefulness and limitations of the BCOP test method should be conducted using this protocol.

ICCVAM Recommendations: BCOP Future Studies
ICCVAM recommends additional studies to further characterize and potentially improve the usefulness and applicability of the BCOP test method to distinguish ocular irritants from all hazard categories:

- Additional optimization studies/evaluations should be conducted to improve the correct classification of mild and moderate ocular irritants and substances not labeled as irritants. After optimization, additional studies to further assess the reliability and accuracy of the test method are recommended.
- Histopathological evaluation of the corneal tissue, using standardized procedures, should be included when the BCOP test method is used. Such data will help develop decision criteria and future assessments on the usefulness of this endpoint for classifying and labeling substances, especially those that may otherwise produce borderline or false negative results.
- Users of the BCOP test method should provide all data that are generated from future studies, because they could help to further characterize the usefulness and limitations of the BCOP test method to identify all ocular hazard categories.

ICCVAM Recommendations: BCOP Performance Standards
Based on the available data and associated performance described above, ICCVAM recommends that the development of performance standards for the BCOP test method is not warranted at this time.

Validation Status of the BCOP Test Method
The BCOP test method is an *in vitro* method that provides short-term maintenance of physiological and biochemical function of the bovine cornea. Quantitative changes in opacity and fluorescein permeability are assessed as indicators of potential ocular irritation.

The accuracy of the BCOP test method was compared to hazard categories based on *in vivo* Draize rabbit eye test data according to the EPA, EU, FHSA, or GHS systems using the current BCOP validation database of 211 substances. When the BCOP test method was used to distinguish substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other categories, accuracy ranged from 64% (76/118) to 83% (161/194), depending on the hazard classification system used. While false positive rates were high (53% [24/45] to 70% [63/90], depending on the hazard classification system used), the false negative rates were low (5% [6/132] for the FHSA system, 6% [8/141] for EPA the system, and 0% [0/54 or 0/97] for the EU and GHS systems, respectively).

Qualitative analyses of interlaboratory reproducibility were conducted to evaluate how well the BCOP hazard classifications agreed among the participating laboratories from the three different interlaboratory validation studies (Balls et al. 1995; Gautheron et al. 1994; and Southee 1998). These evaluations were based on the use of the BCOP test method (1) to identify all ocular hazard categories according to the EPA, EU, or GHS systems, and (2) to distinguish substances not labeled as irritants from all other ocular hazard categories. For both approaches, there was 100% agreement among the multiple laboratories in each study for a majority of the correctly identified ocular irritant hazard categories. Because the performance of the BCOP test method was similar for the EPA and FHSA hazard classification systems, additional reliability analyses were not conducted for the FHSA hazard classification system.

The Cytosensor Microphysiometer (CM) Test Method

ICCVAM Recommendations: CM Test Method Usefulness and Limitations

ICCVAM concludes that the accuracy and reliability of the CM test method support its use as a screening test to identify water-soluble substances (water-soluble surfactants, surfactant-containing formulations, and nonsurfactants) as ocular corrosives and severe irritants (EPA Category I, EU R41, GHS Category 1) in a tiered-testing strategy, as part of a weight-of-evidence approach. False positive rates ranged from 0% (0/17 or 0/18) to 10% (3/29), and false negative rates ranged from 9% (2/23) to 50% (6/12), depending on the classification system used and the type of substance tested. A substance that tests negative with the CM test method would need to be tested in another test method that can identify possible *in vitro* false negative ocular corrosives and severe irritants and distinguish between moderate and mild ocular irritants. Currently, the Draize rabbit eye test is the only test method that can make such a distinction.

ICCVAM further concludes that the accuracy and reliability of the CM test method are sufficient to support its use as a screening test to distinguish water-soluble surfactant chemicals and certain types of surfactant-containing formulations (e.g., cosmetics and personal care product formulations, but not pesticide formulations) as substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled) from all other hazard categories (EPA Category I, II, III; EU R41, R36; FHSA Irritant) when results are to be used specifically to classify and label substances under the EPA, EU, and FHSA classification systems. As noted above, until the issues associated with the GHS classification system are further discussed (see "BCOP Test Method Usefulness and Limitations"), ICCVAM is deferring final recommendations on the usefulness and limitations of using the CM test method as a screening test to identify substances not labeled as irritants according to the GHS classification system.

When the CM test method was used to distinguish substances not listed as irritants from all other hazard categories the validation database of 53 water-soluble surfactants and surfactant-containing formulations, false positive rates were high, ranging from 50% (3/6) to 69% (18/26), depending on the hazard classification system used. However, such positive results would require additional testing in a valid test system that can accurately characterize whether such substances require hazard labeling. Positive results would also need to be additionally tested with methods that can correctly identify moderate and mild ocular irritants. False negative rates ranged from 0% (0/27, 0/28, or 0/40) to 2% (1/42 or 1/47) compared to results from the Draize rabbit eye test. The one false negative substance was EPA Category III or FHSA Irritant based on *in vivo* data. For this substance, six test animals were included in the *in vivo* test. One test animal had no observable effects, three test animals had conjunctival redness (score = 1), and two test animals had corneal opacity (score = 1) that cleared after one day.

Because of the high false negative rates (24% [5/21] to 40% [8/20]for the CM test method when testing water-soluble nonsurfactant substances and formulations, the CM test method is **not** recommended as a screening test to identify substances not labeled as irritants among these types of substances.

Given that the CM test method (INVITTOX Protocol 102) is proposed for use as a screening test to identify ocular corrosives and severe irritants and substances not labeled as irritants, users may want to consider using the CM test method before using another *in vitro* ocular test method for testing these types of substances. However, water-soluble substances that are not identified as ocular corrosives and severe irritants or water-soluble surfactant chemicals and specific types of surfactant-containing formulations that are not identified as substances not labeled as irritants with the CM test method would need to be tested in another test method able to correctly classify substances into each of the four EPA or GHS hazard classification categories. Currently, the only test method accepted for these purposes is the Draize rabbit eye test. Because the CM test method has a high false positive rate for

substances not labeled as irritants (50% [3/6] to 69% [18/26], depending on the hazard classification system used), users may not want to use it if the intended use is to start with identifying substances not labeled as irritants.

ICCVAM Recommendations: CM Test Method Protocol

For use of the CM test method as a screening test to identify water-soluble substances as ocular corrosives and severe irritants (EPA Category I, EU R41, GHS Category 1) or to identify substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled), ICCVAM recommends using the updated ICCVAM CM INVITTOX Protocol 102[6] that is included as an appendix to this report. All future studies intended to further characterize the usefulness and limitations of the CM test method should be conducted using this protocol.

ICCVAM Recommendations: CM Future Studies

ICCVAM recommends that additional studies be conducted to further characterize the usefulness and limitations of the CM test method for use as a screening test to identify ocular corrosives and severe irritants (EPA Category I, GHS Category 1, EU R41) and substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified). This includes additional testing using a broader range of materials to expand the recommended types of substances appropriate for testing.

ICCVAM recommends that a subset of the ICCVAM-recommended reference substances for validation of *in vitro* ocular toxicity test methods for the evaluation of ocular corrosives and severe irritants[7] be tested in the CM test method in order to provide for more direct assessment of the CM test method's utility as a screening test for identifying ocular corrosives and severe irritants. Similarly, a reference set could also be selected from this list for the purposes of assessing the utility of the CM test method as a screening test for identifying substances not labeled as irritants.

Finally, ICCVAM recommends future optimization studies to increase the ability of the CM test method to identify all categories of ocular irritancy hazard classification according to the EPA, EU, or GHS hazard classification systems. This will require more substances in the moderate and mild ocular irritant categories (EPA Category II and III, EU Category R36, or GHS Category 2A and 2B, respectively) be identified and tested.

ICCVAM Recommendations: CM Performance Standards

Based on the available data and associated performance described above, ICCVAM recommends that the development of performance standards for the CM test method is not warranted at this time.

Validation Status of the CM Test Method

The CM test method exposes a population of cells to increasing concentrations of a test substance. The concentration that leads to a 50% decline in the metabolic rate of the cells (the MRD_{50}) is used as an indicator of ocular irritancy potential. An abbreviated version of the European Centre for the Validation of Alternative Methods (ECVAM) CM BRD that does not include confidential business information describes the current validation status of the CM test method, including what is known about its reliability and accuracy, the scope of substances tested, and standardized protocols for the validation study. The following is a synopsis of the information contained within three peer-reviewed publications (Balls et al. 1995; Gettings et al. 1996; Brantom et al. 1997) described in the ECVAM CM BRD and used in the ICCVAM review.

[6] Available at http://ecvam-dbalm.jrc.ec.europa.eu/

[7] http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_tmer.htm

The database of 53 water-soluble surfactants tested in the CM test method included 21 surfactant chemicals and 32 surfactant-containing formulations tested across seven different laboratories. Using INVITTOX Protocol 102 to identify ocular corrosives and severe irritants among the water-soluble surfactants and surfactant-containing formulations, the false positive rate ranged from 3% (1/30) to 10% (3/29), depending on the hazard classification system used, compared to *in vivo* results. The three false positives when using the EPA classification system are classified as Category II (n = 2) or III (n = 1) based on *in vivo* data. The one false positive when using the GHS and EU classification systems is classified as Not Classified and Not Labeled, respectively, based on *in vivo* data. The false negative rate ranged from 9% (2/23) to 22% (5/23), depending on the hazard classification system used, compared to *in vivo* results. In each case, these substances were classified as moderate or mild irritants *in vitro* based on the EPA, EU, and GHS classification systems (i.e., EPA Category II or III; EU R36; or GHS Category 2A or 2B).

The nonsurfactant substances database (n = 29) consisted of 27 water-soluble nonsurfactant chemicals, which included a range of chemical classes (e.g., acids, alcohols, alkalis, and ketones), and water-soluble nonsurfactant formulations (n = 2) tested in seven laboratories. Using INVITTOX Protocol 102 to identify ocular corrosives and severe irritants among the nonsurfactant substances, the false positive rate was 0% (0/17 or 0/18) for all hazard classification systems compared to *in vivo* results. The false negative rate ranged from 29% (2/7) to 50% (6/12), depending on the hazard classification system used, compared to *in vivo* results. Two substances were false negatives when using the EPA classification system and were classified *in vitro* as either Category II/III (n = 1) or IV (n = 1). Five substances were false negatives when using the GHS classification system and were classified *in vitro* as either Category 2A/2B (n = 4) or Not Labeled (n = 1). Six substances were false negatives when using the EU classification system and were classified *in vitro* as either R36 (n = 5) or Not Labeled (n = 1).

Using INVITTOX Protocol 102 to identify substances not labeled as irritants among the database of 53 water-soluble surfactants and surfactant-containing formulations, the false negative rate ranged from 0% (0/27 or 0/28, or 0/40) to 2% (1/46 or 1/47), depending on the hazard classification system used, compared to *in vivo* results. The one substance that was a false negative is classified as EPA Category III based on *in vivo* data from a six-rabbit *in vivo* test. One rabbit had no observable effects, three rabbits had conjunctival redness (score = 1), and two rabbits had corneal opacity (score = 1) that cleared after one day. The false positive rate ranged from 50% (3/6) to 69% (18/ 26), depending on the hazard classification system used, compared to *in vivo* results. Three substances were false positives when using the EPA and FHSA classification systems and were classified *in vitro* as Category II/III or Irritant, respectively. Seventeen substances were false positives when using the GHS classification system and were classified *in vitro* as Category 2A/2B (n = 16) or Category 1 (n = 1). Eighteen substances were false positives when using the EU classification system and were classified *in vitro* as R36 (n = 17) or R41 (n = 1).

Using INVITTOX Protocol 102 to identify substances not labeled as irritants among the database of 29 nonsurfactant substances, the false negative rate ranged from 24% (5/21) to 40% (8/20), and the false positive rate ranged from 25% (1/4 or 2/8) to 40% (2/5), depending on the hazard classification system used, compared to *in vivo* results.

Intralaboratory reproducibility was assessed based on calculated coefficients of variation (CVs) for MRD_{50} values for two different studies. Mean CVs ranged from 10% to 24% and tended to be slightly higher for surfactant substances than for nonsurfactant substances.

Interlaboratory reproducibility of the CM test method was also assessed using the data from validation studies by the European Commission/Home Office (EC/HO; Balls et al. 1995) and European Cosmetic, Toiletry and Perfumery Association (COLIPA; Brantom et al. 1997), which included four laboratories and two laboratories, respectively. Mean CVs in the EC/HO study ranged

from 16% to 37% for surfactant substances and up to 51% for nonsurfactant substances. For surfactant materials, all four laboratories using the CM test method had 100% agreement for 55% (6/11) of the test substances; 75% of the laboratories had identical results for 27% (3/11) of the test substances; and 50% of the laboratories had agreement for 18% (2/11) of the test substances. For nonsurfactant substances, agreement among the laboratories was 100% for 48% (11/23) of the test substances, 75% for 22% (5/23) of the test substances, 67% for 4% (1/23) of the test substances, and 50% for 13% (3/23) of the test substances.

For the COLIPA study, substances were divided into surfactant materials, surfactant-based formulations and mixtures, and nonsurfactant substances. Two laboratories had mean between-laboratory CVs ranging from 16% to 23% for surfactant materials, approximately 16% for surfactant-based formulations and mixtures, and 32% to 51% for nonsurfactant substances. For surfactant materials, the laboratories had 100% agreement for 90% (9/10) of the test substances and 0% agreement for 10% (1/10) of the test substances. For surfactant-based formulations and mixtures, the laboratories had 100% agreement for 100% (7/7) of the test substances. For nonsurfactant substances, the laboratories had 100% agreement for 78% (7/9) of the test substances and 0% agreement for 22% (2/9) of the test substances.

The Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) Test Method

ICCVAM Recommendations: HET-CAM Test Method Usefulness and Limitations

ICCVAM concludes that the accuracy and reliability of the HET-CAM test method does **not** support its use as a screening test to distinguish substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled) from all other hazard categories (EPA Category I, II, or III; EU R41 or R36; FHSA Irritant) when results are to be used specifically to classify and label substances under the EPA, EU, or FHSA classification systems.

The available validation database for the HET-CAM test method has remained unchanged since the original ICCVAM evaluation (ICCVAM 2006b). For the HET-CAM validation database of 60 surfactants and oil/water emulsions, false positive rates were 60% (9/15) to 69% (22/32) and false negative rates were 0% (0/26) to 9% (4/45). Among the four false negatives, 100% (4/4) were EPA Category III substances based on conjunctival redness scores of 2 that required at least three days to resolve. For one of the substances, one of the six rabbits tested had a conjunctival redness score of 2 that required 14 days to resolve. Four of the remaining five rabbits in this study had conjunctival redness scores of 2 that resolved within three days; the last rabbit did not have this lesion. However, there were too few substances in the moderate irritant categories to have sufficient confidence in the ability of HET-CAM to distinguish them from the substances not labeled as irritants category (there were only 2 EPA Category II substances).

ICCVAM Recommendations: HET-CAM Test Method Protocol

The updated ICCVAM-recommended HET-CAM test method protocol is included as an appendix to this report. The protocol has been modified from a generic description of the Irritation Score (IS) analysis method to include a more detailed IS(A) analysis method to be used for prospective studies. However, a description of the IS(B) method is included for retrospective analyses, where IS(B) analysis method data could be converted to fixed time points similar to those used for the IS(A) analysis method. All future studies intended to further characterize the usefulness and limitations of the HET-CAM test method should be conducted using this protocol.

ICCVAM Recommendations: HET-CAM Future Studies
ICCVAM recommends additional studies to further characterize and potentially improve the usefulness and applicability of the HET-CAM test method to distinguish ocular irritants from all hazard categories:

- Additional studies should be conducted to further optimize the HET-CAM test method decision criteria that would be used to identify ocular corrosives and severe irritants (EPA Category I, EU R41, GHS Category 1), as well as moderate irritants (EPA Category II, EU R36, GHS Category 2A) and mild irritants (EPA Category III, GHS Category 2B), as defined by the EPA, GHS, or EU classification systems. Such studies could potentially improve the usefulness of the HET-CAM test method for identifying these types of substances.
- The types of substances appropriate for testing should be expanded to include a broader range of chemical and product classes.
- Users of the HET-CAM test method should provide all data that are generated from future studies, because they could help to further characterize the usefulness and limitations of the HET-CAM test method to identify all ocular hazard categories.

ICCVAM Recommendations: HET-CAM Performance Standards
Based on the available data and associated performance described above, ICCVAM recommends that the development of performance standards for the HET-CAM test method is not warranted at this time.

Validation Status of the HET-CAM Test Method
ICCVAM reviewed HET-CAM performance compared to the Draize rabbit eye test for each classification system (EPA, EU, and GHS) using each of the six HET-CAM protocols (IS[A], IS[B], Q-Score, S-Score, IS, and ITC protocols). With the exception of the IS(A) and IS(B) protocols, all protocols classified at least one *in vivo* moderate or severe irritant substance as a substance not labeled as an irritant (EPA Category IV, EU Not Labeled, GHS Not Classified). The IS(B) overpredicted more than 90% (39/42) of the GHS Not Classified substances. Therefore, more extensive analyses of HET-CAM were restricted to the IS(A) protocol.

No new HET-CAM data have been obtained since the ICCVAM evaluation of the HET-CAM test method for identifying ocular corrosives and severe irritants (ICCVAM 2006b). Overall accuracy in distinguishing substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other categories ranged from 62% (36/58) to 80% (44/55), depending on the hazard classification system used. False positive rates were 60% (9/15) to 69% (22/32) and false negative rates were 0% (0/26) to 9% (4/45). Among the four false negatives, 100% (4/4, all oil/water emulsion cosmetic formulations) were EPA Category III substances based on conjunctival redness scores of 2 that required at least three days to resolve. For one of the substances, one out of the six rabbits tested had a conjunctival redness score of 2 that required 14 days to resolve. Four of the remaining five rabbits in this study had conjunctival redness scores of 2 that resolved within three days; the last rabbit did not have this lesion.

Quantitative and qualitative evaluations of HET-CAM test method reliability have been conducted previously (ICCVAM 2006b). Because the database used for the current evaluation of the HET-CAM test method has not changed, the quantitative evaluation of test method reliability remains unchanged. Additional qualitative analyses of interlaboratory reproducibility were conducted to evaluate how well the HET-CAM hazard classifications agreed among the five laboratories that participated in the interlaboratory validation study (Hagino et al. 1999). These evaluations were based on the use of the HET-CAM test method (1) to identify all ocular hazard categories according to the EPA, EU, or GHS systems, and (2) to distinguish substances not labeled as irritants from all other ocular hazard

categories. For both approaches, there was 100% agreement among the multiple laboratories in each study for a majority of the correctly identified ocular irritant hazard categories. Because the performance of the HET-CAM test method was similar for the EPA and FHSA hazard classification systems, additional reliability analyses were not conducted for the FHSA hazard classification system.

The Isolated Chicken Eye (ICE) Test Method

ICCVAM Recommendations: ICE Test Method Usefulness and Limitations
ICCVAM concludes that the accuracy and reliability of the ICE test method does **not** support its use as a screening test to distinguish substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled) from all other hazard categories (EPA Category I, II, or III; EU R41 or R36; FHSA Irritant) when results are to be used specifically to classify and label substances under the EPA, EU, or FHSA classification systems.

The available validation database for the ICE test method has remained unchanged since the original ICCVAM evaluation (ICCVAM 2006c). For the ICE validation database of 175 substances, false positive rates were 11% (10/93) to 34% (27/79) and false negatives rates were 6% (4/62) to 22% (13/60). Among the false negatives, at least one substance was classified as an ocular corrosive/severe irritant based on Draize rabbit eye test data (n = 1 each for the EPA and GHS systems, and n = 6 for the EU system). Considering the public health impact of misclassifying a corrosive substance as Not Labeled, these false negative results cannot be minimized.

ICCVAM Recommendations: ICE Test Method Protocol
For use of the ICE test method as a screening test to identify substances as ocular corrosives and severe irritants (EPA Category I, GHS Category 1, EU R41), ICCVAM recommends using the updated ICCVAM ICE test method protocol that is included as an appendix to this report. All future studies intended to further characterize the usefulness and limitations of the ICE test method should be conducted using this protocol.

ICCVAM Recommendations: ICE Future Studies
ICCVAM recommends additional studies to further characterize and potentially improve the usefulness and applicability of the ICE test method to distinguish ocular irritants from all hazard categories:

- Additional optimization studies should be conducted to improve the correct classification of mild and moderate ocular irritants and substances not labeled as irritants. After optimization, additional studies to further assess the reliability and accuracy of the test method are recommended.
- Histopathological evaluation of the corneal tissue, using standardized procedures, should be included when the ICE test method is used. Such data will help develop decision criteria and future assessments on the usefulness of this endpoint for classifying and labeling substances, especially those that may otherwise produce borderline or false negative results.
- Users of the ICE test method should provide all data that are generated from future studies, because they could help to further characterize the usefulness and limitations of the ICE test method to identify all ocular hazard categories.

ICCVAM Recommendations: ICE Performance Standards
Based on the available data and associated performance described above, ICCVAM recommends that the development of performance standards for the ICE test method is not warranted at this time.

Validation Status of the ICE Test Method

No new ICE data have been obtained since the ICCVAM evaluation of the ICE test method for identifying ocular corrosives and severe irritants (ICCVAM 2006c). Overall accuracy in distinguishing substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other categories ranged from 78% (110/141) to 85% (130/153), depending on the hazard classification system used. False positive rates were 11% (10/93) to 34% (27/79) and false negative rates were 6% (4/62) to 22% (13/60). Among these false negatives, at least one substance was classified as an ocular corrosive/severe irritant based on Draize rabbit eye test data (n = 1 each for the EPA and GHS systems, and n = 6 for the EU system). Considering the public health impact of misclassifying a corrosive substance as Not Labeled, these false negative results cannot be minimized.

Quantitative and qualitative evaluations of ICE test method reliability have been conducted previously (ICCVAM 2006c). Because the database used for the current evaluation of the ICE test method has not changed, the quantitative evaluation of test method reliability remains unchanged. Additional qualitative analyses of interlaboratory reproducibility were conducted to evaluate how well the ICE hazard classifications agreed among the four laboratories that participated in the interlaboratory validation study (Balls et al. 1995). These evaluations were based on the use of the ICE test method (1) to identify all ocular hazard categories according to the EPA, EU, or GHS systems, and (2) to distinguish substances not labeled as irritants from all other ocular hazard categories. For both approaches, there was 100% agreement among the multiple laboratories in each study for a majority of the correctly identified ocular irritant hazard categories. Because the performance of the ICE test method was similar for the EPA and FHSA classification systems, additional reliability analyses were not conducted for the FHSA classification system.

The Isolated Rabbit Eye (IRE) Test Method

ICCVAM Recommendations: IRE Test Method Usefulness and Limitations

The available validation database for the IRE test method has remained unchanged since the original ICCVAM evaluation (ICCVAM 2006d). Because of the lack of a standardized protocol and insufficient data using all four recommended IRE endpoints, ICCVAM concludes that additional studies are needed before definitive recommendations on the accuracy and reliability of the IRE test method can be made.

ICCVAM Recommendations: IRE Test Method Protocol

An ICCVAM-recommended test method protocol for the IRE test method that should be used for all future IRE studies is included as an appendix to this report. The recommended protocol remains unchanged from the previous ICCVAM evaluation (ICCVAM 2006e) and includes four endpoints that should be measured: maximal corneal opacity (opacity x area), maximal corneal swelling, fluorescein penetration (intensity x area), and assessment of epithelial integrity (at 0.5, 1, 2, 3, and 4 hours after test substance administration.

ICCVAM Recommendations: IRE Future Studies

ICCVAM recommends additional studies to further characterize and potentially improve the usefulness and applicability of the IRE test method to distinguish ocular irritants from all other hazard categories:

- Additional evaluation studies should be conducted to increase the current IRE database and optimize the IRE test method decision criteria. Once these studies are conducted, ICCVAM recommends that additional validation studies be conducted to further evaluate the relevance and reliability of the IRE test method.
- Histopathological evaluation of the corneal tissue, using standardized procedures, should be included when the IRE test method is used. Such data will help develop decision

criteria and future assessments on the usefulness of this endpoint for classifying and labeling substances, especially those that may otherwise produce borderline or false negative results.

- Users of the IRE test method should provide all data that are generated from future studies, because they could help to further characterize the usefulness and limitations of the IRE test method to identify all ocular hazard categories.

ICCVAM Recommendations: IRE Performance Standards

Based on the available data described above, ICCVAM recommends that the development of performance standards for the IRE test method is not warranted at this time.

Validation Status of the IRE Test Method

The performance section of the IRE BRD (ICCVAM 2006d) uses data from Balls et al. (1995), Gettings et al. (1996), and Guerriero et al. (2004). These references were examined for decision criteria that would help classify moderate and mild irritants. There are insufficient data using all four recommended IRE endpoints (corneal opacity, fluorescein penetration, corneal swelling, and observations of significant effect on corneal epithelium) to assess the accuracy and reliability of the IRE test method when all of these endpoints are evaluated in a single study. Furthermore, among the studies that included each endpoint, decision criteria focused on distinguishing ocular corrosives and severe irritants from all other ocular hazard categories (moderate and mild irritants and substances not labeled as irritants) and did not specify decision criteria for each ocular hazard category. For these reasons, an adequate evaluation of the IRE test method for its ability to distinguish substances not labeled as irritants from all other ocular hazard categories is not feasible at this time.

Because of the lack of quantitative IRE test method data for replicate experiments within an individual laboratory, the intralaboratory repeatability and reproducibility of the IRE test method could not be evaluated. However, multilaboratory qualitative and quantitative IRE test data were available for a collaborative study by the Commission of European Communities (CEC 1991) involving three laboratories and a validation study conducted by Balls et al. (1995) involving four laboratories. In the CEC (1991) study, each substance tested was assigned a EU classification (R41, R36, or nonirritant [EU 2001]) based on Draize rabbit eye test results. However, due to the lack of individual rabbit Draize scores, a reliability assessment for the CEC (1991) study using the GHS (UN 2007) or EPA (EPA 2003) classification criteria was not possible. The Balls et al. (1995) data were used for an evaluation of the interlaboratory reproducibility of the IRE test method according to the GHS (UN 2007), EPA (EPA 2003), and EU (EU 2001) classification systems.

ICCVAM Consideration of Public and SACATM Comments

The ICCVAM evaluation process incorporates a high level of transparency. This process is designed to provide numerous opportunities for stakeholder involvement, including submitting written public comments and providing oral comments at ICCVAM independent peer review panel meetings and SACATM meetings. **Table 7-1** lists the nine different opportunities for public comments that were provided during the ICCVAM evaluation of the validation status of alternative ocular safety testing methods and approaches. A total of 37 public comments were received. Comments received in response to or related to the *Federal Register* notices are also available on the NICEATM-ICCVAM website.[8]

[8] Available at http://ntp-apps.niehs.nih.gov/iccvambp/searchPubCom.cfm

This page intentionally left blank

Appendix E

Background Review Document
Current Status of *In Vitro* Test Methods
for Identifying Mild/Moderate Ocular Irritants:
The Hen's Egg Test–Chorioallantoic Membrane (HET-CAM) Test Method

This page intentionally left blank

Background Review Document
Current Status of *In Vitro* Test Methods for Identifying Mild/Moderate Ocular Irritants:
The Hen's Egg Test–Chorioallantoic Membrane (HET-CAM) Test Method

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

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

This page intentionally left blank

Table of Contents

List of Tables

List of Figures

List of Abbreviations and Acronyms

^{o}C	Degrees centigrade
BCOP	Bovine corneal opacity and permeability
BRD	Background review document
CAM	Chorioallantoic membrane
CASRN	Chemical Abstracts Service Registry Number
CEPI	Corneal epithelial cell line
CPSC	U.S. Consumer Product Safety Commission
EC	European Commission
EC/HO	European Commission/British Home Office
ECVAM	European Centre for the Validation of Alternative Methods
EEC	European Economic Council
EPA	U.S. Environmental Protection Agency
EU	European Union
FDA	U.S. Food and Drug Administration
FHSA	U.S. Federal Hazardous Substances Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FR	*Federal Register*
GHS	United Nations Globally Harmonized System for Classification and Labelling of Chemicals
GLP	Good Laboratory Practice
HET-CAM	Hen's egg test–chorioallantoic membrane
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Isolated chicken eye
INVITOX	*In Vitro* Techniques in Toxicology (ERGATT FRAME ECVAM Data bank)
IOMA	Maximal ocular irritation index
IRE	Isolated rabbit eye
IS(A)	Irritation Score (A) Analysis Method
IS(B)	Irritation Score (B) Analysis Method
ITC	Irritation threshold concentration
JaCVAM	Japanese Center for the Evaluation of Alternative Toxicological Methods
MAS	Maximum average score
MCA	Mean chorioallantoic irritation index
MeSH	U.S. National Library of Medicine's Medical Subject Heading
MMTS	Maximum mean total score
mtc	Mean time of coagulation
NC	Not Classified (as irritant)

NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIH	National Institutes of Health
NL	Not Labeled (as irritant)
OD	Optical density
OECD	Organisation for Economic Co-operation and Development
OPPTS	EPA Office of Prevention, Pesticides and Toxic Substances
OSHA	U.S. Occupational Safety and Health Administration
OTWG	Ocular Toxicity Working Group
TNO	TNO Nutrition and Food
UN	United Nations
ZEBET	German Center for Documentation and Evaluation of Alternative Methods to Animal Experiments

Interagency Coordinating Committee on the Validation of Alternative Methods: Agency Representatives

Agency for Toxic Substances and Disease Registry
* Moiz Mumtaz, Ph.D.
Bruce Fowler, Ph.D.
Edward Murray, Ph.D.
Eric Sampson, Ph.D.

Consumer Product Safety Commission
* Marilyn L. Wind, Ph.D. (Chair)
+ Kristina Hatlelid, Ph.D.
Joanna Matheson, Ph.D.

Department of Agriculture
* Jodie Kulpa-Eddy, D.V.M. (Vice-Chair)
+ Elizabeth Goldentyer, D.V.M.

Department of Defense
* Robert E. Foster, Ph.D.
+ Patty Decot
Harry Salem, Ph.D.
Peter J. Schultheiss, D.V.M., DACLAM

Department of Energy
* Michael Kuperberg, Ph.D.
+ Marvin Stodolsky, Ph.D.

Department of the Interior
* Barnett A. Rattner, Ph.D.
+ Sarah Gerould, Ph.D. (to Feb. 2009)

Department of Transportation
* George Cushmac, Ph.D.
+ Steve Hwang, Ph.D.

Environmental Protection Agency
Office of Pesticide Programs
* John R. "Jack" Fowle III, Ph.D., DABT
+ Vicki Dellarco, Ph.D.
+ Tina Levine, Ph.D.
Deborah McCall
Christine Augustyniak, Ph.D. (*U.S. Coordinator, OECD Test Guidelines Program*)
Office of Pollution Prevention and Toxics
Jerry Smrchek, Ph.D. (*U.S. Coordinator, OECD Test Guidelines Program*, to July 2009)
Office of Research and Development
Suzanne McMaster, Ph.D. (to Dec. 2008)
Julian Preston, Ph.D. (to July 2009)
Stephanie Padilla, Ph.D. (to July 2009)
Office of Science Coordination and Policy
Karen Hamernik, Ph.D. (to July 2009)

Food and Drug Administration
Office of the Commissioner
* Suzanne Fitzpatrick, Ph.D., DABT
Center for Biologics Evaluation and Research
Richard McFarland, Ph.D., M.D.
Ying Huang, Ph.D.
Center for Devices and Radiological Health
Melvin E. Stratmeyer, Ph.D.
Vasant G. Malshet, Ph.D., DABT
Center for Drug Evaluation and Research
+ Abigail C. Jacobs, Ph.D.
Paul C. Brown, Ph.D.
Center for Food Safety and Applied Nutrition
David G. Hattan, Ph.D.
Robert L. Bronaugh, Ph.D.
Center for Veterinary Medicine
Devaraya Jagannath, Ph.D.
M. Cecilia Aguila, D.V.M.
National Center for Toxicological Research
Paul Howard, Ph.D.
Donna Mendrick, Ph.D.
William T. Allaben, Ph.D. (to Jan. 2009)
Office of Regulatory Affairs
Lawrence D'Hoostelaere, Ph.D.

National Cancer Institute
* T. Kevin Howcroft, Ph.D.
Chand Khanna, D.V.M., Ph.D.
Alan Poland, M.D. (to Oct. 2008)

National Institute of Environmental Health Sciences
* William S. Stokes, D.V.M., DACLAM
+ Raymond R. Tice, Ph.D.
Rajendra S. Chhabra, Ph.D., DABT
Jerrold J. Heindel, Ph.D.

National Institute for Occupational Safety and Health
* Paul Nicolaysen, V.M.D.
+ K. Murali Rao, M.D., Ph.D.

National Institutes of Health
* Margaret D. Snyder, Ph.D.

National Library of Medicine
* Pertti (Bert) Hakkinen, Ph.D.
+ Jeanne Goshorn, M.S.

Occupational Safety and Health Administration
* Surender Ahir, Ph.D.

* Principal agency representative
+ Alternate principal agency representative

Acknowledgements

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Ocular Toxicity Working Group (OTWG)

U.S. Consumer Product Safety Commission
Marilyn L. Wind, Ph.D.
Adrienne Layton, Ph.D.

U.S. Department of Defense
Harry Salem, Ph.D.

U.S. Department of Transportation
Steve Hwang, Ph.D.

U.S. Environmental Protection Agency
Office of Pesticide Programs
Meta Bonner, Ph.D.
Jonathan Chen, Ph.D.
John R. "Jack" Fowle III, Ph.D., DABT
Masih Hashim, D.V.M., Ph.D.
Karen Hicks
Marianne Lewis
Debbie McCall
Timothy McMahon, Ph.D.
Mark Perry
John Redden
Jenny Tao, Ph.D.
Office of Research and Development
Andrew Geller, Ph.D.
Office of Science Coordination and Policy
Karen Hamernik, Ph.D.

U.S. Food and Drug Administration
Center for Drug Evaluation and Research
Paul Brown, Ph.D.
Wiley Chambers, M.D.
Abigail (Abby) Jacobs, Ph.D.
Jill Merrill, Ph.D., DABT (OTWG Chair)
Center for Food Safety and Applied Nutrition
Robert Bronaugh, Ph.D.
Donnie Lowther
Office of the Commissioner
Suzanne Fitzpatrick, Ph.D., DABT

National Institute of Environmental Health Sciences
Warren Casey, Ph.D., DABT
Mark F. Cesta, D.V.M, DACVP
Raymond (Buck) Grissom, Ph.D.
William Stokes, D.V.M., DACLAM

Occupational Safety and Health Administration
Surender Ahir, Ph.D.

European Centre for the Validation of Alternative Methods – Liaison
João Barroso, Ph.D.
Thomas Cole, Ph.D.
Valerie Zuang, Ph.D.

Japanese Center for the Validation of Alternative Methods – Liaison
Hajime Kojima, Ph.D.

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

National Institute of Environmental Health Sciences

William Stokes, D.V.M., DACLAM
Director; Project Officer

Warren Casey, Ph.D., DABT
Deputy Director

Deborah McCarley
Special Assistant; Assistant Project Officer

NICEATM Support Contract Staff (Integrated Laboratory Systems [ILS], Inc.)

David Allen, Ph.D.
Jonathan Hamm, Ph.D.
Nelson Johnson
Brett Jones, Ph.D.
Elizabeth Lipscomb, Ph.D.
Linda Litchfield
Steven Morefield, M.D.
Catherine Sprankle
James Truax, M.A.
Linda Wilson

Statistical Consultant for ILS, Inc.

Joseph Haseman, Ph.D.

Additional Reviewers for the *In Vitro* Ocular Corrosion and Irritation Test Methods Background Review Documents

Chantra Eskes, Eng., Ph.D.
ECVAM
Ispra, Italy

Robert L Guest Bsc, CBiol, MlBiol
SafePharm Laboratories, Ltd.
Derby, United Kingdom

John Harbell, Ph.D.
Institute for In Vitro Sciences
Gaithersburg, Maryland

Penny Jones
Unilever Research
Sharnbrook, United Kingdom

Menk Prinsen
TNO Nutrition & Food Research Institute
Zest, The Netherlands

Horst Spielmann, Dr.med.
ZEBET
Berlin, Germany

Companies and Individuals that Provided *In Vitro* and/or *In Vivo* Data for the HET-CAM Test Method Background Review Document

ICCVAM and NICEATM gratefully acknowledge the following individuals and institutions that submitted data to NICEATM for the evaluation of alternative ocular safety testing methods and approaches.

Cosmetics, Toiletry, and Fragrance Association
Carol Eisenmann, Ph.D.

ECVAM
Chantra Eskes, Ph.D.

Johnson & Johnson Pharmaceutical R&D
Freddy van Goethem, Ph.D.
Philippe Vanparys, Ph.D.

National Institute of Health Sciences
Yasuo Ohno, Ph.D.

U.S. Food and Drug Administration
Donnie Lowther

ZEBET
Manfred Liebsch, Ph.D.
Horst Spielmann, Dr. med.

This page intentionally left blank

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 (CPSC), U.S. Environmental Protection Agency (EPA), U.S. Food and Drug Administration, and U.S. Occupational Safety and Health 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. 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 Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) previously evaluated the validation status of the bovine corneal opacity and permeability (BCOP), isolated chicken eye (ICE), isolated rabbit eye (IRE), and hen's egg test–chorioallantoic membrane (HET-CAM) assays for the identification of ocular corrosives or severe (irreversible) ocular irritants. ICCVAM's evaluation used the EPA (EPA 2003a), United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UN 2007), and European Union (EU 2001) regulatory hazard classification systems. In ICCVAM's assessment, the performance of the BCOP and ICE test methods substantiated their use in testing some substances for regulatory hazard classification. The IRE and HET-CAM test methods lacked sufficient performance and/or sufficient data to substantiate their use for regulatory hazard classification.

ICCVAM recommended that the BCOP and ICE should be used in a tiered-testing strategy in which positive substances can be classified as ocular corrosives or severe irritants without animal testing. In accordance with the ICCVAM Authorization Act of 2000 (Public Law 106-545), these recommendations were made available to the public and provided to U.S. Federal agencies for consideration in the ICCVAM *Test Method Evaluation Report* – In Vitro *Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives* (ICCVAM 2006b). The ICCVAM recommendations were accepted by U.S. Federal agencies, and *in vitro* test methods may now be used instead of the Draize rabbit eye test for certain regulatory testing purposes.

ICCVAM is now reviewing the validation status of these *in vitro* test methods for identification of nonsevere ocular irritants (that is, those that induce reversible ocular damage [EPA Category II, III; EU Category R36, GHS Category 2A, 2B]) and substances Not Classified as irritant (GHS NC or Not Labeled, EPA Category IV, FHSA Not Labeled, or EU Not Labeled) according to the GHS (UN 2007), EPA (EPA 2003a), FHSA (FHSA 2005), and EU (EU 2001) classification systems. The Federal Hazardous Substances Act (FHSA) classification system (FHSA 2005) as defined in the "Test for Eye Irritants" (i.e., "Irritant" or Not Labeled [as an irritant]) and published in 16 CFR 1500.42 (CPSC 2003) is also provided in the current background review documents. The FHSA classification system was not used in the previous analyses of test methods used for the identification of severe ocular irritants or corrosives because the FHSA classification is limited to irritants and is not intended to identify corrosive substances or to differentiate between severe and nonsevere irritants.

Accordingly, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the ICCVAM Ocular Toxicity Working Group (OTWG) prepared draft background review documents that summarize the current validation status of each test

method based on published studies and other data and information submitted in response to a June 7, 2007, *Federal Register* request (72 FR 31582, available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_10966.pdf). The background review documents form the basis for draft ICCVAM test method recommendations, which are provided in separate documents. Liaisons from the European Centre for the Validation of Alternative Methods and the Japanese Centre for the Validation of Alternative Methods will provide input and contribute to the OTWG throughout the evaluation process.

An international independent scientific peer review panel (Panel) met in public session on May 19-21, 2009, to develop conclusions and recommendations on the *in vitro* BCOP, ICE, IRE, and HET-CAM test methods. 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 can use the subsequent independent Panel report to deliberate and develop their own test method recommendations (ICCVAM Peer Review Panel Report [ICCVAM 2009] available to the public for comment on July 12, 2009). The Panel considered these BRDs and evaluated the extent to which the available information supports the draft ICCVAM test method recommendations.

ICCVAM provided the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) with the draft BRD and draft Test Method Evaluation Report, the Panel's report, and all public comments. SACATM discussed these at their June 25-26, 2009, meeting, where public stakeholders were given another opportunity to comment. After SACATM's meeting, ICCVAM considered the SACATM comments, the Panel report, and all public comments before finalizing the Background Review Document and test method recommendations. These recommendations 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 background review document, including the following staff from the NICEATM support contractor, Integrated Laboratory Systems, Inc.: David Allen, Jon Hamm, Nelson Johnson, Elizabeth Lipscomb, Linda Litchfield, Steven Morefield, Gregory Moyer, Catherine Sprankle, and Jim Truax. We also thank the members of the OTWG, chaired by Karen Hamernik, Ph.D. (U.S. EPA) and Jill Merrill, Ph.D. (U.S. Food and Drug Administration), and ICCVAM representatives who reviewed and commented on draft versions. We also thank Valerie Zuang, Ph.D., and Dr. Hajime Kojima, Ph.D., the liaisons to the OTWG from the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods, respectively, for their participation.

Marilyn Wind, Ph.D.
Deputy Associate Executive Director
Directorate for Health Sciences
U.S. Consumer Product Safety Commission
Chair, ICCVAM

William S. Stokes, D.V.M., DACLAM
Rear Admiral/Assistant Surgeon General, U.S. Public Health Service
Director, NICEATM
Executive Director, ICCVAM

Executive Summary

In October 2003, the U.S. Environmental Protection Agency (EPA) submitted to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) a nomination requesting the evaluation of several activities related to reducing, refining, and replacing the use of rabbits in the current *in vivo* Draize rabbit eye test (69 FR 13859 [March 24, 2004]). In response to this nomination, ICCVAM evaluated the validation status of the bovine corneal opacity and permeability (BCOP), hen's egg test–chorioallantoic membrane (HET-CAM), isolated chicken eye (ICE), and isolated rabbit eye (IRE) test methods. To evaluate how well these test methods identify ocular corrosives and severe irritants, ICCVAM used the EPA (2003a), European Union (EU 2001), and United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UN 2007) classification systems.

ICCVAM considered the performance of two of these *in vitro* test methods, the BCOP and the ICE, to be sufficient to support their use in testing certain types of substances for regulatory hazard classification. The IRE and HET-CAM test methods lacked sufficient performance and/or sufficient data to support their use for regulatory hazard classification. ICCVAM recommended that the BCOP and ICE test methods should be used in a tiered-testing strategy that would classify positive substances as ocular corrosives or severe irritants without animal testing. These recommendations were accepted by U.S. Federal agencies, and, as a result, *in vitro* test methods may now be used instead of conventional tests for certain regulatory testing purposes.

ICCVAM is now reviewing the validation status of these *in vitro* test methods to identify nonsevere ocular irritants (those that cause reversible ocular damage [EPA Category II and III; EU R36; GHS Category 2A and 2B]) and substances not labeled as irritants (EPA Category IV; EU Not Labeled; GHS Not Classified) according to the EPA (2003a), EU (2001), and GHS (UN 2007) classification systems. The FHSA classification system, which is based on the testing guidelines and associated criteria included in 16 CFR 1500.42 (CPSC 2003), is also included in these evaluations. The FHSA classification system was not used in the original analyses (ability of the test methods to identify ocular corrosives and severe irritants) because the FHSA ocular hazard category that is assigned based on results from the Draize rabbit eye test (Draize et al. 1944) does not distinguish between ocular corrosives and severe irritants and less severe irritants. For this reason, an evaluation to identify ocular corrosives and severe irritants using the FHSA classification system was not possible.

Because the FHSA classification system (FHSA 2005) is based on a sequential testing strategy that uses up to 18 animals, only a small percentage of the substances in the HET-CAM database would be classifiable if the FHSA criteria were strictly applied. To maximize the number of substances included in these analyses, "proportionality" criteria were applied for the purpose of assigning an FHSA classification to test results that would require additional testing according to the FHSA sequential testing strategy. These "proportionality" criteria (FHSA-20% and FHSA-67%) are as follows:

- FHSA-20% is based on the proportion of positive animals needed to identify a substance as an irritant using the FHSA sequential testing strategy, where 20% of the animals must demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥1 positive animal in a 3- to 5-animal test or ≥2 positive animals in a 6-animal test.
- FHSA-67% is based on the proportion of positive animals needed to identify a substance as an irritant using the "first test" of the FHSA sequential testing strategy, where 67% of the animals must demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled as an irritant if ≤1/6

animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥2/3, 3/4, 4/5, or 4/6 positive animals. If 1/3, 1/4, 2/4, 1/5, 2/5, 3/5, 2/6, or 3/6 animals were positive, further testing would be required.

Together, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the ICCVAM Ocular Toxicity Working Group prepared draft background review documents (BRDs) that summarize the available data and information regarding the validity (usefulness and limitations) of each test method. This BRD summarizes all available information for the HET-CAM test method and its current validation status, including what is known about its reliability and accuracy, and the scope of the substances tested. Original data for the HET-CAM test method will be maintained for future use so that these performance statistics may be updated as additional information becomes available.

HET-CAM Test Method Protocol

The HET-CAM test method uses the vascular fetal membrane of chicken embryos. The HET-CAM test method is proposed to provide information on the effects that may occur in the conjunctiva of the eye following test substance administration. It is assumed that acute effects induced by a test substance on the small blood vessels and proteins of this soft tissue membrane are similar to effects induced by the same test substance in the eye of a treated rabbit. The membrane is evaluated for the development of irritant endpoints (hyperemia, hemorrhage, and coagulation) and qualitative assessments of the irritation potential of test substances are made.

Validation Database

No new HET-CAM data have been obtained since ICCVAM evaluated the HET-CAM test method for identifying ocular corrosives and severe irritants (ICCVAM 2006a). Therefore, the same database was used in the current evaluation. The HET-CAM validation database contains a total of 260 substances and formulations. The most commonly tested chemical classes are alcohols, carboxylic acids, and formulations. The most commonly tested product classes are solvents, shampoos, surfactants, and cosmetics. Analyses of each of the HET-CAM protocols indicate that the Irritation Score (A), or IS(A), analysis method performed best when evaluating substances not labeled as irritants. The available IS(A) database includes 63 test substances, 58 to 60 of which had sufficient *in vivo* data to be assigned an ocular irritancy hazard classification, depending on the classification system used. These 58 to 60 substances comprise 43 cosmetic and personal care product formulations (including 25 surfactant-based formulations and 18 oil/water emulsions) and 17 individual substances (including seven alcohols; no other classes were represented by more than three substances).

In order to calculate the appropriate EPA (2003a), EU (2001), FHSA (2005), and GHS (UN 2007) ocular irritancy hazard classifications, detailed *in vivo* data consisting of cornea, iris, and conjunctiva scores for each animal at 24, 48, and 72 hours following test substance administration and/or assessment of the presence or absence of lesions at 7, 14, and 21 days are needed. Some of the test substances had only limited *in vivo* data and could not be used to evaluate test method accuracy and reliability. To maximize the number of substances included in the FHSA analyses, “proportionality” criteria (FHSA-20% and FHSA-67%), as outlined above, were applied for the purpose of assigning a FHSA classification to test results that would require additional testing according to the FHSA sequential testing strategy.

HET-CAM Test Method Accuracy

Identification of All Ocular Hazard Categories

ICCVAM evaluated how well the HET-CAM test method identified all categories of ocular irritation potential as defined by the EPA (2003a), GHS (UN 2007), and EU (2001) classification systems. For

these evaluations, the IS(A) analysis method was used. Because the FHSA classification system does not distinguish between ocular corrosives and severe irritants and less severe irritants, an evaluation for all ocular hazard categories using the FHSA classification system was not possible. Analyses were also performed excluding specific chemical classes and/or physical properties that were previously identified as discordant in the HET-CAM test method (alcohols, surfactant formulations, and oil/water emulsions) relative to the *in vivo* hazard classification (ICCVAM 2006a).

As shown in **Table 1**, overall correct classifications ranged from 38% (23/60) to 41% (24/59) when using the entire database, depending on the hazard classification system used. When discordant classes are excluded, overall correct classifications improved to a range of 62% (5/8) to 78% (7/9), depending on the classification system used. However, too few substances (0–2) are in the moderate category (EPA Category II, GHS Category 2A, EU R36) to adequately evaluate the performance of the HET-CAM test method for this irritant category. Similarly, while 18 substances are classified as mild (EPA Category III) for the EPA system, only five are classified as GHS Category 2B (the EU system does not distinguish mild irritants).

Distinguishing Substances Not Labeled as Irritants from All Other Hazard Categories

ICCVAM also evaluated how well the HET-CAM test method distinguished substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other ocular hazard categories (EPA Category I, II, III; EU R41, R36; FHSA Irritant; GHS Category 1, 2A, 2B) as defined by the EPA (2003a), GHS (UN 2007), EU (2001), and FHSA (2005) classification systems. Analyses were also performed excluding specific chemical classes and/or physical properties that were previously identified as discordant in the HET-CAM test method (alcohols, surfactant formulations, and oil/water emulsions) relative to the *in vivo* hazard classification (ICCVAM 2006a).

As shown in **Table 2**, overall accuracy ranged from 62% (36/58) to 80% (44/55), depending on the hazard classification system used. The lowest false negative rate (0% [0/31 and 0/26]) was noted for the GHS and EU classification systems, followed by 3% (1/39) for FHSA-67% criteria, and 9% (4/45 and 4/47) for the EPA and FHSA-20% classification systems. All four false negatives for the EPA classification system were oil/water emulsions that were classified as EPA Category III substances based on Draize rabbit eye test data. The false negatives identified using the FHSA-20% and FHSA-67% criteria were the same oil/water emulsions identified by the EPA classification system. The lowest false positive rate (60% [9/15]) was noted for the EPA classification system, followed by 63% (10/16) for the FHSA-20% and FHSA-67% criteria, and 64% (18/28) and 69% (22/32) for the GHS and EU classification systems, respectively.

The exclusion of discordant classes improved accuracy (ranged from 75% [6/8] to 100% [9/9 and 10/10] when discordant classes were removed versus 62% [36/58] to 80% [44/55] for overall accuracy, depending on the hazard classification system used). However, the discordant substances comprised at least 84% of the substances in each classification system, so the performance of each classification system was based on ten or fewer substances.

Table 1 Performance of the HET-CAM Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA, GHS, and EU Classification Systems[1]

Hazard Classification System	Overall Correct Classification	Severe[2]		Moderate[3]			Mild[4]			Not Labeled[5]	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Overall (EPA)	38% (23/60)	48% (12/25)	52% (13/25)	50% (1/2)	50% (1/2)	0% (0/2)	56% (10/18)	22% (4/18)	22% (4/18)	60% (9/15)	40% (6/15)
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions[6]	78% (7/9)	100% (6/6)	0% (0/6)	50% (1/2)	50% (1/2)	0% (0/2)	100% (1/1)	0% (0/1)	0% (0/1)	- (0/0)	- (0/0)
Overall (GHS)	41% (24/59)	50% (13/26)	50% (13/26)	- (0/0)	- (0/0)	- (0/0)	80% (4/5)	20% (1/5)	0% (0/5)	64% (18/28)	36% (10/28)
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions	67% (6/9)	86% (6/7)	14% (1/7)	- (0/0)	- (0/0)	- (0/0)	100% (1/1)	0% (0/1)	0% (0/1)	100% (1/1)	0% (0/1)
Overall (EU)	40% (23/58)	50% (12/24)	50% (12/24)	50% (1/2)	50% (1/2)	0% (0/2)	NA	NA	NA	69% (22/32)	31% (10/32)
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions	62% (5/8)	100% (5/5)	0% (5/5)	100% (1/1)	0% (0/1)	0% (0/1)	NA	NA	NA	100% (2/2)	0% (0/2)

Abbreviations: EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = Globally Harmonized System; HET-CAM = hen's egg test–chorioallantoic membrane; NA = not applicable.

[1] EPA classification system (EPA 2003a); GHS classification system (UN 2007); EU classification system (EU 2001). Because the FHSA classification system does not distinguish between ocular corrosives/severe irritants and less severe irritants, an evaluation for all ocular hazard categories using the FHSA classification system was not possible.

[2] Severe = EPA Category I; GHS Category 1; EU R41.

[3] Moderate = EPA Category II; GHS Category 2A; EU R36.

[4] Mild = EPA Category III; GHS Category 2B.

[5] Not Labeled = EPA Category IV; GHS Not Classified: EU Not Labeled.

[6] Alcohols, surfactant formulations, and oil/water emulsions were previously identified as discordant in the HET-CAM test method relative to the *in vivo* hazard classification (ICCVAM 2006a).

HET-CAM Test Method Reliability

Interlaboratory Reproducibility

Previous quantitative and qualitative evaluations of the reliability of the HET-CAM test method have been conducted (ICCVAM 2006a). Because the database used for the current evaluation of the HET-CAM test method has not changed, the quantitative evaluation of test method reliability remains unchanged. Additional qualitative analyses of interlaboratory reproducibility were conducted to evaluate how well the HET-CAM hazard classifications agreed among the five participating laboratories from the interlaboratory validation study (Hagino et al. 1999). These evaluations were based on the use of the HET-CAM test method (1) to identify all ocular hazard categories according to the EPA, EU, or GHS systems, and (2) to distinguish substances not labeled as irritants (EPA Category IV, GHS Not Classified, EU Not Labeled) from all other ocular hazard categories (EPA Categories I, II, III; GHS Categories 1, 2A, 2B; EU R41, R36). Because the performance of the HET-CAM test method was similar for the EPA and FHSA hazard classification systems, additional reliability analyses were not conducted for the FHSA hazard classification system.

Using the first approach (identifying all ocular hazard categories), there was 100% agreement among the five laboratories for a majority of the Draize ocular corrosives and severe irritants correctly classified by the HET-CAM test method based on all three classification systems. There was 100% agreement for 63% [5/8] of the correctly identified EPA Category I substances and 100% agreement for 71% [5/7] of the correctly identified GHS Category 1 or EU R41 substances. There was 100% agreement among the five laboratories for the one moderate irritant in the database (EPA Category II or EU R36; no GHS Category 2A substances were included), which was overpredicted by the HET-CAM test method. There was 100% agreement for the mild ocular irritants (EPA Category III, GHS Category 2B; the EU does not have a mild irritant category), which were uniformly overpredicted by the HET-CAM test method. For the Hagino et al. (1999) database, all of the substances not classified as irritants based on Draize data (EPA Category IV, EU Not Labeled, GHS Not Classified) were overpredicted by the HET-CAM test method. There was 100% agreement among the five laboratories for 86% (6/7) or 75% (3/4) of these substances for the EU and GHS classification systems, respectively. By comparison, for the two EPA Category IV substances tested, there was either 100% or 80% agreement among the five laboratories.

Using the second approach (distinguishing substances not labeled as irritants from all other ocular hazard categories), there was 100% agreement among the five laboratories for 76% (13/17) to 94% (16/17) of the substances tested by the HET-CAM test method, depending on the classification system used.

There was 100% agreement among the five laboratories for 100% (13/13) of the substances correctly identified as irritants according to the EPA classification system (Category I, II, or III). While neither of the EPA Category IV substances were correctly identified by the HET-CAM test method, there was 60% agreement among the five laboratories for 100% (2/2) of the EPA Category IV substances that were overpredicted by the HET-CAM test method.

There was 100% agreement among the five laboratories for 63% (5/8) of the substances correctly identified as an irritant according to the EU classification system (R36 or R41). There was at least 60% agreement among the five laboratories for the remaining three substances correctly classified as an irritant. While none of the EU Not Labeled substances were correctly identified by the HET-CAM test method, there was 100% agreement among the five laboratories for 86% (6/7) of these substances that were overpredicted by the HET-CAM test method.

There was 100% agreement among the five laboratories for 100% (11/11) of the substances correctly identified as irritants according to the GHS classification system (Category 1, 2A, or 2B). While none of the GHS Not Classified substances were correctly identified by the HET-CAM test method, there

was 100% agreement among the five laboratories for 75% (3/4) of these substances that were overpredicted by the HET-CAM test method.

Table 2 Accuracy of the HET-CAM IS(A) Test Method in Distinguishing Substances Not Labeled as Irritants from All Other Hazard Categories, as Defined by the EPA, GHS, EU, and FHSA Classification Systems

Hazard Classification System	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall (EPA)[1]	60	78	47/60	91	41/45	40	6/15	60	9/15	9	4/45
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions[2]	9	100	9/9	100	9/9	-	0/0	0	0/9	-	0/0
Overall (GHS)[3]	59	69	41/59	100	31/31	36	10/28	64	18/28	0	0/31
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions	9	89	8/9	100	8/8	0	0/1	100	1/1	0	0/8
Overall (EU)[4]	58	62	36/58	100	26/26	31	10/32	69	22/32	0	0/26
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions	8	75	6/8	100	6/6	0	0/2	100	2/2	0	0/6
Overall (FHSA-20%)[5]	63	78	49/63	91	43/47	38	6/16	63	10/16	9	4/47
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions	10	100	10/10	100	10/10	-[6]	-	-	-	0	0/10
Overall (FHSA-67%)[5]	55	80	44/55	97	38/39	38	6/16	63	10/16	3	1/39
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions	9	100	9/9	100	9/9	-[6]	-	-	-	0	0/9

Abbreviations: EPA = U.S. Environmental Protection Agency; EU = European Union; FHSA = U.S. Federal Hazardous Substances Act; GHS = Globally Harmonized System; HET-CAM = hen's egg test–chorioallantoic membrane; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] EPA classification system (EPA 2003a): Category IV vs. Category I/II/III.

[2] Alcohols, surfactant formulations, and oil/water emulsions were previously identified as discordant in the HET-CAM test method relative to the *in vivo* hazard classification (ICCVAM 2006a).

[3] GHS classification system (UN 2007): Not Classified vs. Category 1/2A/2B.

[4] EU classification system (EU 2001): Not Labeled vs. R41/R36.

[5] FHSA classification system (FHSA 2005): Not Labeled vs. Irritant. To maximize the number of substances included in the FHSA analyses, “proportionality” criteria (FHSA-20% and FHSA-67%) were applied for the purpose of assigning a FHSA classification to test results that would require additional testing according to the FHSA sequential testing strategy.

[6] No substances were classified as Not Labeled by FHSA or as nonirritants in HET-CAM, so specificity and the false positive rate could not be determined.

1.0 Introduction

1.1 Background

The current rabbit eye test method identifies both irreversible (e.g., corrosion) and reversible ocular effects. It also provides quantitative scoring with which to categorize the severity of reversible effects such as mild, moderate, or severe irritation. Current U.S. Environmental Protection Agency ocular testing guidelines and the United Nations (UN) Globally Harmonized System (GHS) of Classification and Labelling of Chemicals indicate that if serious ocular damage is anticipated (e.g., a lesion considered to be irreversible or persisting for 21 days), then a test on a single animal may be considered. If serious damage is observed, no further animal testing is necessary (EPA 1998; UN 2007). If no serious damage is observed, additional test animals (1 or 2 rabbits) may be evaluated sequentially until concordant irritant or nonirritant responses are observed based on the GHS (UN 2007) or until unequivocal results are obtained in a minimum of three animals according to the EPA test guideline (EPA 1998). In the FHSA classification system (FHSA 2005), which is based on the testing guidelines and associated criteria included in 16 CFR 1500.42 (CPSC 2003), corrosive substances are identified by other test methods (e.g., Draize skin test or human accidental exposure data) and excluded from further irritant testing.

In 2006, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) finished evaluating the hen's egg test–chorioallantoic membrane (HET-CAM) test method to identify ocular corrosives and severe irritants (ICCVAM 2006a). ICCVAM concluded that the HET-CAM test method was not suitable for identifying ocular corrosives and severe irritants (i.e., EPA Category I, GHS Category 1, EU R41) (ICCVAM 2006b), but this recommendation could be revised as additional data become available.

ICCVAM is now evaluating the usefulness and limitations of the HET-CAM test method for identifying nonsevere irritants (i.e., those that induce reversible ocular damage [EPA Category II and III; EU R36; GHS Category 2A and 2B]) and substances not labeled as irritants (i.e., EPA Category IV; EU Not Labeled; FHSA Not Labeled; GHS Not Classified) according to the EPA, EU, FHSA, and GHS classification systems (EPA 2003a; EU 2001; FHSA 2005; UN 2007). However because the FHSA classification system (2005) is based on a sequential testing strategy, which uses up to 18 animals, only a small percentage of the substances in the ICE database would be classifiable if the FHSA criteria were strictly applied. In order to maximize the number of substances included in these analyses, "proportionality" criteria (i.e., FHSA-20% and FHSA-67%) were applied for the purpose of assigning an FHSA classification for test results that would require additional testing according to the FHSA sequential testing strategy (see **Section 4.1**).

As part of the evaluation process, this background review document (BRD) has been prepared to describe the current validation status of the HET-CAM test method, including what is known about its reliability and accuracy, its applicability domain, the numbers and types of substances tested, and the availability of a standardized protocol. An ICCVAM expert panel used this BRD when reviewing the HET-CAM as a method to identify all categories of ocular irritants and substances not labeled as irritants.

Parallel reviews of the bovine corneal opacity and permeability (BCOP), isolated chicken eye (ICE), and isolated rabbit eye (IRE), test methods are being conducted. The expert panel report and the analyses presented in the BRDs will be used to support ICCVAM recommendations on the proposed standardized test method protocols, proposed list of recommended reference substances, and additional optimization and/or validation studies that may be necessary to further develop and characterize the usefulness and limitations of these methods.

For a more detailed discussion of the background of the HET-CAM test method, including its scientific basis and regulatory rationale and applicability, see the *ICCVAM Background Review Document—Current Status of* In Vitro *Test Methods for Identifying Ocular Corrosives and Severe Irritants: Hen's Egg Test – Chorioallantoic Membrane* (ICCVAM 2006a).

1.2 Use of the HET-CAM Test Method in Overall Strategy of Hazard or Safety Assessment

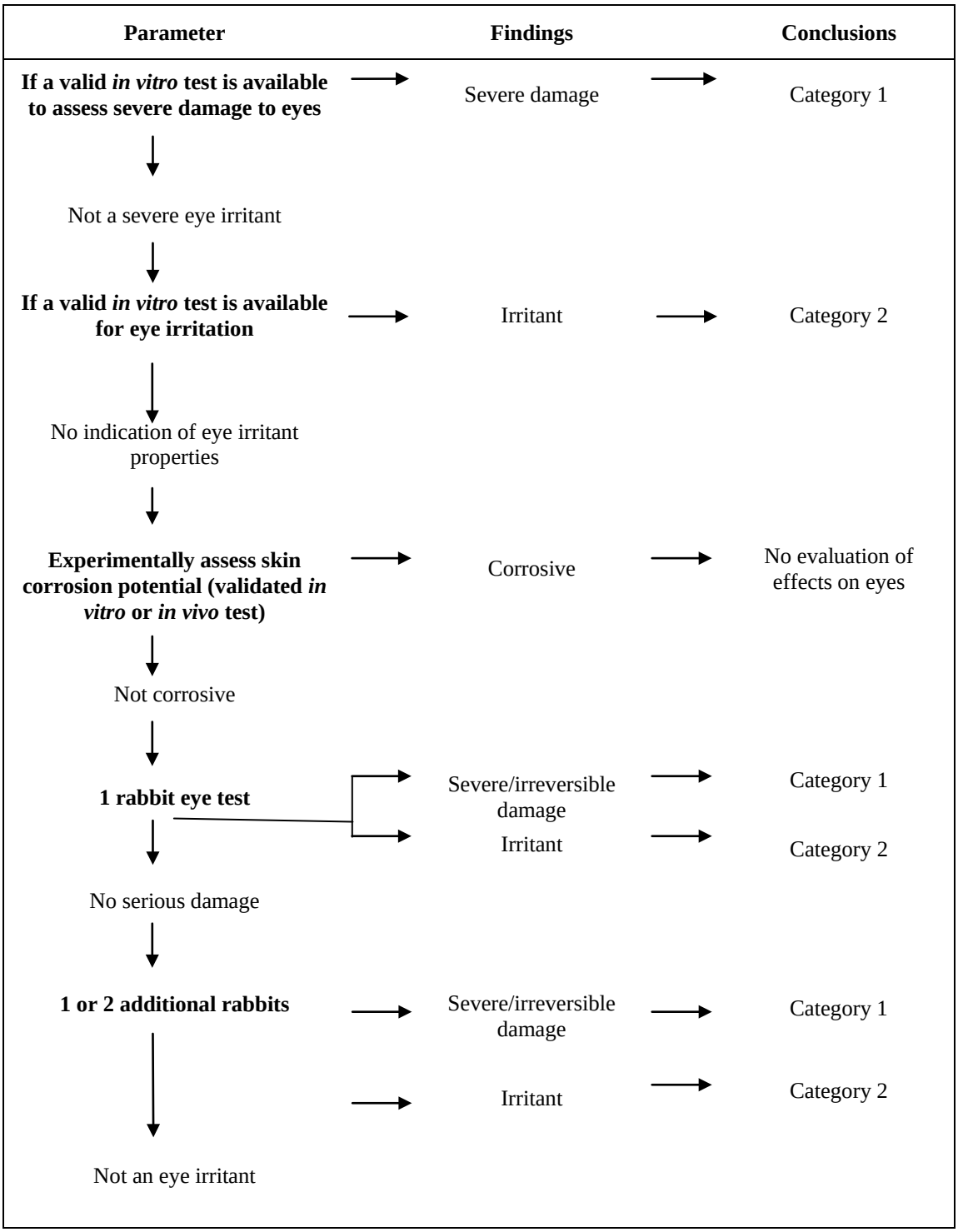
As shown in **Figure 1-1**, the GHS allows for use of validated and accepted *in vitro* methods to identify ocular corrosives/severe irritants and ocular irritants without further testing. The HET-CAM test method is currently not recommended for identification of ocular corrosives and severe irritants in a tiered-testing strategy for regulatory classification and labeling for use in the GHS testing scheme (UN 2007). ICCVAM is now further evaluating the usefulness and limitations of the HET-CAM test method for identifying nonsevere irritants and substances not labeled as irritants.

1.3 Validation of the HET-CAM Test Method

The ICCVAM Authorization Act of 2000 (Sec. 4([c]) mandates that "each Federal Agency … shall ensure that any new or revised … test method … is determined to be valid for its proposed use prior to requiring, recommending, or encouraging [its use]" [A16] (Public Law 106-545).

Validation is the process that establishes the reliability and relevance of a test method for a specific purpose (ICCVAM 2003). *Relevance* is defined as the extent to which a test method will correctly predict or measure the biological effect of interest (ICCVAM 2003). For the HET-CAM test method described in the ICCVAM 2006 BRD (ICCVAM 2006a), relevance is restricted to how well the test method identifies substances that are capable of producing corrosive or severe irritant effects to the eye. For the current BRD, relevance is based on how well the test method identifies substances that are capable of producing nonsevere ocular irritation or substances not labeled as irritants.

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



Abbreviations: GHS = Globally Harmonized System

[1] Adapted from UN (2007).

Reliability is defined as the reproducibility of a test method within and among laboratories. Reliability should be based on its performance with a diverse set of substances that (1) represent the types of

chemical and product classes likely to be tested and (2) cover the range of responses that need to be identified. The validation process will provide data and information to allow U.S. Federal agencies to develop guidance on the development and use of the HET-CAM test method as part of a tiered-testing approach to evaluating substances' eye irritation potential.

The first stage in this evaluation is the preparation of a BRD that presents and evaluates the relevant data and information about the test method, including its mechanistic basis, proposed uses, reliability, and performance characteristics (ICCVAM 2003). This BRD summarizes the available information on the HET-CAM test method. Where adequate data are available, the qualitative and quantitative performance of the test method are evaluated.

1.4 Search Strategies and Selection of Citations for the HET-CAM BRD

The HET-CAM test method data summarized in this BRD are based on information found in the peer-reviewed scientific literature as detailed in the ICCVAM *Background Review Document—Current Status of* In Vitro *Test Methods for Identifying Ocular Corrosives and Severe Irritants: Hen's Egg Test – Chorioallantoic Membrane Test Method* (ICCVAM 2006a). A literature search for HET-CAM studies published between January 2005 and January 2009 used the same terminology and information databases used in the 2006 ICCVAM BRD (ICCVAM 2006a). The research revealed four studies that included information on HET-CAM protocols or contained data on test substances. While no *in vivo* reference data were included in any of the four citations, *in vivo* data for six of nine substances included in one study were available from the National Toxicology Program Interagency Center for the Validation of Alternative Toxicological Methods (NICEATM) database of Draize eye test results. However, because these substances were included in the original analyses (and the HET-CAM results from the new study agreed with the previous results), the database used in the HET-CAM performance analysis is the same as the database used in the ICCVAM *Background Review Document—Current Status of* In Vitro *Test Methods for Identifying Ocular Corrosives and Severe Irritants: Hen's Egg Test – Chorioallantoic Membrane Test Method* (ICCVAM 2006a).

2.0 Hen's Egg Test–Chorioallantoic Membrane Test Method Protocol Components

The HET-CAM protocol first described by Luepke (1985) uses a vascular fetal membrane, the chorioallantoic membrane (CAM), which is composed of the fused chorion and allantois. The CAM has been proposed as a model for a living membrane (such as the conjunctiva) because it comprises a functional vasculature. Additionally, evaluation of coagulation (i.e., protein denaturation) may reflect corneal damage that may be produced by the test substance. The acute effects induced by a test substance on the small blood vessels and proteins of this soft tissue membrane are proposed to be similar to effects induced by the same test substance in the eye of a treated rabbit.

Since the initial description of the HET-CAM test method, several studies have been conducted to evaluate the feasibility of using HET-CAM as a complete replacement for the *in vivo* rabbit ocular test. Most of these reports describe a HET-CAM test method protocol that is similar but not identical to the original protocol. These differences include the breed of hen from which eggs are obtained, the endpoints evaluated, data collection procedures, and methods used to analyze the data.

To date, no single HET-CAM test method protocol has gained wide acceptance as a standardized protocol. However, for a general description of how the HET-CAM test method is conducted, see the ICCVAM *Background Review Document—Current Status of* In Vitro *Test Methods for Identifying Ocular Corrosives and Severe Irritants: Hen's Egg Test – Chorioallantoic Membrane Test Method* (2006a). Briefly, during a HET-CAM study, the test substance is applied to the surface of the CAM. The CAM is subsequently evaluated for development of irritant endpoints: hemorrhage (bleeding), vascular lysis (blood vessel disintegration), and coagulation (intra- and extravascular protein

denaturation). Depending on the method used to collect data on the endpoints (e.g., time to development, severity of observed effect), qualitative assessments of the irritation potential of test substances are made. As detailed in **Section 6.0**, analyses of each of the HET-CAM analysis methods indicate that the irritation score (A) (IS[A]) analysis method achieved the best performance when evaluating substances not labeled as irritants. Therefore, the IS(A) method is described here. For a description of the other HET-CAM analysis methods (i.e., Q-score, mtc10, ITS, and S-score), see the 2006 ICCVAM BRD (ICCVAM 2006a).

2.1 The Irritation Score (IS) Analysis Method

For those test method protocols that assigned a score to each of the endpoints evaluated at preset time intervals, the values assigned to each endpoint were added to give an irritation score (IS) value for the test substance (i.e., IS[A] analysis method). The possible IS values range from 0 (for test substances that do not induce development of any of the toxic endpoints of interest over the range of time intervals) to 21 (for test substances that induced development of all three toxic endpoints within 30 seconds of application of the test substance) (Luepke 1985).

For those test method protocols that noted the time that a specific endpoint was first observed, the IS value was calculated (i.e., IS[B] analysis method) using the following formula (Kalweit et al. 1987, 1990):

$$\left(\left(\frac{(301-\textit{Hemorrhage time})}{300}\right)\times 5\right)+\left(\left(\frac{(301-\textit{Lysis time})}{300}\right)\times 7\right)+\left(\left(\frac{(301-\textit{Coagulation time})}{300}\right)\times 9\right)$$

where:
Hemorrhage time = time (in seconds) of the first appearance of blood hemorrhages
Lysis time = time (in seconds) of the first appearance of vessel lysis
Coagulation time = time (in seconds) of the first appearance of protein coagulation

The IS value, when calculated using this formula, has a maximal value of 21.

When the development of hyperemia, injection, or another toxic endpoint was evaluated instead of vessel lysis, the time to first appearance for the alternative endpoint replaced the lysis time point.

2.1.1 IS Classification Scheme

For studies that used the analysis methods developed by Luepke (1985) or Kalweit et al. (1987, 1990), the accuracy analysis presented in this BRD (see **Section 6.0**) used the ocular irritancy classification scheme described in **Table 2-1**. Therefore, substances with IS(A) or IS(B) values of 9 or greater were classified as severe irritants for the purposes of this analysis. The rationale for the decision criteria used in this classification scheme were not provided, and the correlation of these categories to irritancy categories described by the EPA (2003), GHS (UN 2007), and EU (2001) classification systems is unknown.

Table 2-1 IS Classification Scheme Used to Classify Substances for Accuracy Analysis[1]

HET-CAM Score Range	Irritation Category
0 to 0.9	Not Labeled
1 to 4.9	Slight Irritation
5 to 8.9	Moderate Irritation
9 to 21	Severe Irritation

[1] According to Luepke (1985) and Kalweit et al. (1987, 1990).

3.0 Substances Used for Validation of the HET-CAM Test Method

3.1 Rationale for the Substances or Products Selected for Use

Validation studies for *in vitro* ocular test methods should ideally evaluate an adequate sample of test substances and products from chemical and product classes that would be evaluated using the *in vivo* rabbit eye test method. Test substances with a wide range of *in vivo* ocular responses (e.g., corrosive/severe irritant to not labeled) also should be assessed to determine any limit to the range of responses that can be evaluated by the *in vitro* test method.

Although new HET-CAM data were identified among four studies published since the ICCVAM evaluation of HET-CAM for identification of ocular corrosives and severe irritants (ICCVAM 2006a), the only substances for which *in vivo* reference data were available were already included in the original HET-CAM database. Therefore, the same database was used in the current evaluation (i.e., Bagley et al. 1992; Balls et al. 1995; CEC 1991; Gettings et al. 1991, 1994, 1996; Gilleron et al. 1996, 1997; Hagino et al. 1999; Kojima et al. 1995; Spielmann et al. 1996;Vinardell and Macián, 1994). As detailed in **Section 6.0**, analyses of each of the multiple HET-CAM protocols indicates that the IS(A) analysis method achieved the best performance when evaluating substances not labeled as irritants. The available database for the IS(A) includes a total of 63 test substances, of which *in vivo* reference data sufficient to assign an ocular irritancy classification are available for 58 - 60 substances depending upon the classification system.

Table 3-1 and **Table 3-2** show the chemical classes and product classes for the test substances included in the original assessment. Information, including substance name, Chemical Abstracts Service Registry Number (CASRN), chemical and/or product class, concentration(s) tested, purity, supplier or source, and literature reference for the test substance are provided in **Annex I**. If not assigned in the study report, the product class was sought from other sources, including the National Library of Medicine's ChemIDplus® database. Chemical classes were assigned to each substance using a standard classification scheme based on the National Library of Medicine Medical Subject Headings (MeSH®) classification system (available at: http://www.nlm.nih.gov/mesh), which ensures consistency in classifying substances among all *in vitro* ocular test methods under consideration. Importantly, a substance could be assigned to more than one chemical or product class.

As shown in **Table 3-1**, the chemical classes with the greatest amount of HET-CAM data are alcohols (n=75), carboxylic acids (n= 51), and formulations (n=53). Of the 504 substances included in **Annex II**, 28 substances, including formulations and mixtures of unknown composition, could not be assigned a specific chemical class.

Table 3-1 Chemical Classes Tested in the HET-CAM Test Method

Chemical Class	# of Substances
Acyl halide	2
Alcohol	75
Aldehyde	9
Alkali	4
Amide	2
Amidine	6
Amine	34
Amino acid	7
Carbohydrate	1
Carboxylic acid	51
Ester	34
Ether	38
Formulation	53
Heterocyclic compound	37
Hydrocarbon, acyclic	5
Hydrocarbon, cyclic	5
Inorganic boron compound	2

Chemical Class	# of Substances
Inorganic salt	14
Imide	4
Ketone	15
Lactone	5
Nitrile	3
Nitro compound	3
Onium compound	22
Organic salt	50
Organometallic compound	2
Organophosphorous compound	1
Organosilicon compound	6
Phenol	4
Polycyclic compound	11
Organic sulfur compound	18
Unknown	28
Urea	3

As shown in **Table 3-2**, the most common product classes tested in the HET-CAM test method are solvents (n=13), hair shampoos (n=13), surfactants (n=17), and cosmetics (n=14). Of the 504 substances included in **Annex II**, 167 were unable to be classified within a product class.

As described in **Section 6.0**, analyses of each of the multiple HET-CAM protocols indicates that the IS(A) analysis method achieved the best performance when evaluating substances not labeled as irritants. The total available database for the IS(A) analysis method includes 63 substances, for which 58–60 substances have available *in vivo* reference data sufficient to assign an ocular irritancy classification depending upon the classification system. Among these substances are 43 cosmetic and personal care product formulations (including 25 surfactant-based formulations and 18 oil/water emulsions) and 17 individual substances (including seven alcohols; no other classes represented by more than three substances).

Table 3-2 Product Classes Tested in the HET-CAM Test Method

Product Class	# of Substances
Aerosol formulation ingredient	1
Antifreezing agent	1
Anti-infective agent, Anti-bacterial agent	2
Antiperspirant	1
Bactericide, Biocide, Fungicide, Germicide	4
Beverage	1
Cationic surface active agent	1
Chemical intermediate	6
Cleaner	1
Conditioner, Hair	2
Cosmetics	14
Cream	1
Disinfectant	1
Drug vehicle	1
Emollient	2
Fertilizer	1
Flavor ingredient	5
Fragrances	4
Industrial explosive	1

Product Class	# of Substances
Laboratory reagent	7
Lotion	3
Lubricant	1
Mouthwash	1
Neurotransmitter	2
Pesticide	5
Pharmaceutical agent, Pharmaceutical intermediate, Pharmaceutical metabolite	4
Plasticizer	2
Polymer	1
Preservative	1
Raw material	1
Shampoo, Hair	13
Solvent	13
Sunscreen	3
Surfactant	17
Synthetic flavor ingredient, Flavor ingredient	4
Synthetic intermediate	1
Unknown	167

4.0 *In Vivo* Reference Data Used for an Assessment of HET-CAM Test Method Accuracy

A detailed description of the test method protocol predominantly used to generate the *in vivo* reference data (i.e., the Draize rabbit eye test) is provided in the *ICCVAM Background Review Document—Current Status of* In Vitro *Test Methods for Identifying Ocular Corrosives and Severe Irritants: Hen's Egg Test – Chorioallantoic Membrane Test Method* (ICCVAM 2006a). There also are a number of national and international test guidelines that describe this procedure (EPA 1998; OECD 2002; CPSC 2003; EU 2004). The scoring system used for assigning an ocular hazard classification is subjective and based on a discrete scale for grading the severity of ocular lesions on the cornea, iris, and conjunctiva.

Most of the HET-CAM studies evaluated in this BRD include *in vivo* reference data generated using the basic procedures for the *in vivo* rabbit eye test method described above. These data were used by NICEATM to assign an ocular hazard classification according to the EPA (2003a), EU (2001), FHSA (2005), and the GHS (UN 2007) ocular irritancy classification systems (**Annex III**). Exceptions included the *in vivo* data used by Gilleron et al. (1996), which were obtained from the studies of Gautheron et al. (1994). According to the report by Gilleron et al., the studies were performed according to the French and European directives (European Economic Council [EEC] 1984, 1991). Substances were classified by the authors according to the EU (1993) classification system and were used to assess the *in vitro* test method accuracy.

4.1 *In Vivo* Classification Criteria Used for BRD Analysis

As described in the ICCVAM 2006 BRD (2006a), the *in vivo* rabbit eye test database that was used to analyze the accuracy of the HET-CAM test method includes studies that were conducted using from one to six rabbits. However, some of the *in vivo* classification systems considered for the accuracy analyses are designed for application to studies using no more than three rabbits. Thus, to maximize the amount of data used to evaluate the HET-CAM test method, the decision criteria for each classification system were expanded to include studies that used more than three rabbits in their evaluation. The criteria used for classification according to the EPA (2003a), GHS (UN 2007), and EU (2001) classification systems were detailed in the 2006 ICCVAM BRD. Each of these classification systems requires that the Draize scoring system be used. For these classification systems, scoring continues until the effect is cleared, but usually not beyond 21 days after the substance is applied to the eye of the rabbit. In order for a substance to be included in the accuracy evaluations in the 2006 ICCVAM BRD (2006a), the following four criteria must have been met.

- At least three rabbits were tested in the study unless a severe effect (e.g., corrosion of the cornea) was noted in a single rabbit. In such cases, substance classification could proceed based on the effects observed in fewer than three rabbits.
- A volume of 0.1 mL or 0.1 g was tested in each rabbit. A study in which a lower volume was applied to the eye could be accepted for substance classification provided that a severe effect (e.g., corrosion of the cornea, lesion persistence) was observed in a rabbit.
- Observations of the eye were made at least 24, 48, and 72 hours after test substance application if no severe effect was observed.
- Observations of the eye were made until reversibility was assessed, typically meaning that all endpoint scores were cleared. Results from a study terminated early were not used unless the reason for the early termination was documented.

If any of the above criteria were not fulfilled, then the data for that substance were not used for the accuracy analyses. The rules used for classification according to the EPA, EU, or GHS classification systems are detailed in the ICCVAM 2006 BRD (2006a).

For the FHSA classification system (FHSA 2005), the testing guidelines and associated criteria are included in 16 CFR 1500.42 (CPSC 2003). The FHSA classification system is based on using up to three sequential tests for each test substance with six animals used per test (**Table 4-1**). Decisions on further sequential testing are based on the number of positive responses in each test. The severity of effects for each endpoint (i.e., corneal ulceration and opacity, conjunctival redness and/or swelling, and iritis) is measured at 24, 48, and 72 hours after test substance administration. Positive responses include corneal ulceration (other than a fine stippling), corneal opacity or iritis ≥1, and conjunctival swelling and/or redness ≥2. In the first test, six animals are tested. If ≥4 animals are positive, the test is positive. If ≤1 animal tests positive, the test is negative. If 2/6 or 3/6 animals are positive, then a second test is performed with six additional animals. A third test is needed if 1/6 or 2/6 animals are positive with the second test.

The FHSA classification system (FHSA 2005) is a binary system, which classifies substances that test positive (according to the criteria provided in **Table 4-1)** as an irritant and substances that test negative as not requiring labeling (i.e. FHSA Not Labeled). Based on the FHSA sequential testing strategy, a substance can be classified as an eye irritant hazard with a few as 22% of the animals having a positive response (i.e., 2/6 [first test] +1/6 [second test] +1/6 [third test] = 4/18 or 22%).

Because the FHSA classification system is based on a sequential testing strategy, which uses up to 18 animals, only a small percentage of the substances in HET-CAM database would be classifiable if the FHSA criteria were strictly applied. In order to maximize the number of substances include in these analyses, "proportionality" criteria were developed by NICEATM for the purpose of assigning an FHSA classification for test results that would require additional testing according to the FHSA sequential testing strategy (**Table 4-2**).

These "proportionality" criteria (i.e., FHSA-20% and FHSA-67%) are as follows:

- (FHSA-20%) – FHSA-20% is based on the proportion of positive animals needed to identify a substance as an irritant using the FHSA sequential testing strategy, where 20% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥1 positive animal in a 3- to 5-animal test or ≥2 positive animals in a 6-animal test.
- (FHSA-67%) – FHSA-67% is based on the proportion of positive animals needed to identify a substance as an irritant using the "first test" of the FHSA sequential testing strategy, where 67% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥2/3, 3/4, 4/5, or 4/6 positive animals. If 1/3, 1/4, 2/4, 1/5, 2/5, 3/5, 2/6, or 3/6 animals were positive, further testing would be required.

Table 4-1 FHSA Classification System (16 CFR 1500.42)[1,2]

Positive Response for a Single Rabbit[3] ≥1 of the following at 24, 48, and/or 72 hours	*In Vivo* Effect
• Corneal ulceration (other than a fine stippling) • Corneal opacity (CO) ≥1 • Iritis (IR) ≥1 • Conjuctival redness (CR) and/or chemosis (CC) ≥2	First Test – If ≥4/6 animals are positive, the test is positive. If ≤1 animal is positive, the test is negative. If 2/6 or 3/6 animals are positive, the test is repeated using a different group of six animals. Second Test – If ≥3/6 animals are positive, the test is positive. If 0/6 animals are positive, the test is negative. If 1/6 or 2/6 animals are positive, the test is repeated using a different group of six animals. Third Test – Should a third test be needed, the test is positive if ≥1/6 animals are positive. If 0/6 animals are positive, the test is negative.

Abbreviations: CC = conjunctival chemosis; CFR = Code of Federal Regulations; CO = corneal opacity; CR = conjunctival redness; FHSA = Federal Hazardous Substances Act; IR = iritis.

[1] For the FHSA Classification System (2005), the testing guidelines and associated criteria are included in 16 CFR 1500.42 (CPSC 2003).

[2] At least three animals per test (one animal screen for corrosive/severe irritants permitted). Maximum score in any animal used for classification.

[3] The following scores are considered positive: CO or IR ≥1 or CR or CC ≥2. Therefore, CO and IR scores of 0 or CR and CC scores ≤1 are considered negative.

Table 4-2 Proposed FHSA "Proportionality" Criteria

No. of Animals in Test	FHSA-20%[1]		FHSA-67%[1]		
	NL	Irritant	NL	Irritant	Further Testing Required[2]
3	0/3	≥1 (≥33%)	0/3	≥2 (≥67%)	1/3
4	0/4	≥1 (≥25%)	0/4	≥3 (≥75%)	1/4, 2/4
5	0/5	≥1 (≥20%)	0/5	≥4 (≥80%)	1/5, 2/5, 3/5
6	0/6, 1/6	≥2 (≥33%)	0/6, 1/6	≥4 (≥67%)	2/6, 3/6

Abbreviations: CPSC = U.S. Consumer Product Safety Commission; FHSA = Federal Hazardous Substances Act; NL = Not Labeled (as an irritant); No. = number.

[1] FHSA-20% and FHSA-67% analysis methods are based on the proportionality of positive animals needed to identify a substance as an irritant.

[2] For FHSA-67%, Further Testing Required refers to substances that do not meet adequate positive or negative criteria to be classified.

4.2 *In Vivo* Data Quality

Ideally, all data supporting the validity of a test method should be obtained and reported in accordance with Good Laboratory Practice (GLP) guidelines. GLP guidelines are nationally and internationally recognized rules designed to produce high-quality laboratory records (OECD 1998; EPA 2003b, 2003c; FDA 2003). These guidelines provide an internationally standardized approach

for the conduct of studies, reporting requirements, archival of study data and records, and information about the test protocol, thereby ensuring the integrity, reliability, and accountability of a study.

The extent to which the *in vivo* rabbit eye studies that were used to provide the comparative data in the published HET-CAM validation studies complied with GLP guidelines is based on the information provided in the published reports. Based on the available information, the reports that were identified as following GLP guidelines or used data obtained according to GLP guidelines were Gettings et al. (1991, 1994, 1996), Balls et al. (1995), Spielmann et al. (1996), and Hagino et al. (1999).

5.0 Hen's Egg Test–Chorioallantoic Membrane Test Method Data and Results

The following twelve published reports contained sufficient data for an accuracy analysis of the HET-CAM test method for the identification of all categories of ocular irritation: CEC (1991), Gettings et al. (1991, 1994, 1996), Bagley et al. (1992), Vinardell and Macián (1994), Balls et al. (1995), Kojima et al. (1995), Gilleron et al. (1996, 1997), Spielmann et al. (1996), and Hagino et al. (1999).

5.1 Availability of Copies of Original Data Used to Evaluate the Accuracy and Reliability

On March 24, 2004, NICEATM published a *Federal Register* notice requesting original HET-CAM data for substances that also had been tested *in vivo* using the standard rabbit eye test (69 FR 13589; available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_04_6487.pdf). A second request was published on February 28, 2005 (70 FR 9661; available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_05_3831.pdf). In addition, NICEATM contacted authors of selected published HET-CAM studies and requested the original HET-CAM data. In response to these efforts, the following *in vitro* data were obtained:

- Summaries of HET-CAM results (e.g., Q-scores) for the 60 substances evaluated by Balls et al. (1995) from the European Centre for the Validation of Alternative Methods (ECVAM). The summary data included the substance name and the average HET-CAM score for the substance.
- *In vitro* data for the substances evaluated in Spielmann et al. (1996) from Drs. H. Spielmann and M. Liebsch. The data included the overall HET-CAM scores obtained by each laboratory for each substance evaluated. *In vitro* data for two control substances also were provided.
- Individual endpoint scores for each egg evaluated for substances described in Gilleron et al. (1996, 1997) from Drs. Philippe Vanparys and Freddy Van Goethem. *In vitro* data for four control substances also were provided.

5.2 Description of the Statistical Approaches Used to Evaluate the Resulting Data

The approach used to analyze HET-CAM study data varied and depended on the method used to collect the data. For test method protocols that evaluated the time to development of endpoints (i.e., hemorrhage, lysis, coagulation) that are correlated with ocular corrosivity or irritation, an IS, Q-score, or mean time of coagulation (mtc) value was calculated. For test method protocols that evaluated the severity of the toxic response, an S-score was calculated. For test method protocols that evaluated the lowest test substance concentration needed to produce a minimal response on the CAM, the irritation threshold concentration was determined. The irritation threshold concentration was typically combined with the IS for the test substance to evaluate ocular irritation or corrosivity potential of a substance.

The accuracy analysis in this BRD focuses on the ability of the HET-CAM test method to identify all irritant hazard categories (i.e., moderate and mild irritants) and/or substances not labeled as irritants as defined by the EPA, GHS, and EU classification systems (EPA 2003a; UN 2007; EU 2001). However, multiple irritancy schemes have been developed for HET-CAM, and different scoring methods and decision criteria were used. No single uniform irritancy classification scheme was developed for HET-CAM. Furthermore, the *in vitro* hazard classifications were not always consistent with or applicable to those based on Draize rabbit eye test data used by the U.S. (EPA 2003a), the GHS (UN 2007), or the EU (EU 2001). However, some investigators have tried to correlate HET-CAM scores with the ocular irritation classification scheme described by the Federal Hazardous Substances Act classification system (CPSC 1988) and the EU classification system (EU 1992) (Gettings et al. 1991, 1994, 1996; Spielmann et al. 1996, respectively).

To evaluate the ability of HET-CAM to identify all ocular hazard categories or substances not labeled as irritants, NICEATM assigned HET-CAM results obtained using each of the different analysis methods an ocular irritancy classification based on the *in vitro* classification system most commonly used for that particular data analysis method. Thus, substances were classified in categories based on the *in vitro* score. Categories ranged from substances not labeled as irritants to ocular corrosives or severe irritants (see **Section 2.0**). Some investigators (e.g., Gettings et al. 1996) classified the ocular irritancy potential of test substances using two or more different analysis methods. In such cases, these data were reclassified according to the approach used most commonly for each *in vitro* classification scheme, and an accuracy assessment was conducted for each analysis method.

NICEATM's preliminary evaluation using the various analysis methods (see **Section 6.1** and **Annex III**) indicated that only the IS(A) analysis method had adequate accuracy to conduct a study of mild/moderate ocular irritation based on rabbit eye test data. Therefore, the data was limited to 63 test substances obtained from Bagley et al. (1992), Gettings et al. (1994, 1996), Kojima et al. (1995), and Hagino et al. (1999).

5.3 Summary of Results

A total of 260 test substances were evaluated in 383 HET-CAM studies for which comparative *in vivo* data were available (ICCVAM 2006a). A summary of results used to evaluate test method accuracy appears in **Annex III**. This table, sorted by reference, provides the following specifics, if provided:

- Name
- CASRN (if available)
- Chemical class
- Product class
- Concentration tested
- Form tested
- Calculated *in vitro* score
- *In vitro* irritation classification of the test substance (based on the irritation classification schemes in **Section 5.3**)
- *In vivo* reference classifications (i.e., EPA, GHS, EU)
- Literature source

Other supporting information, such as purity of the test substance, was included in the table to the extent that this information was available. If not provided, the CASRN was obtained from various sources, including the National Library of Medicine's ChemIDplus® database (available at http://chem2.sis.nlm.nih.gov/chemidplus). All substances with the same CASRN were listed under the same name, regardless of the synonym used in the original report. Chemical and product classes were assigned to each test substance based on the National Library of Medicine's Medical Subject Heading classification system (MeSH®; available at http://www.nlm.nih.gov/mesh). **Annex I**

provides information on the names, synonyms, CASRN, and chemical/product class, where available, for each substance. **Annex II** provides the *in vitro* HET-CAM test method data sorted by reference and alphabetically by substance name.

5.4 Use of Coded Chemicals and Compliance with GLP Guidelines

Ideally, all data supporting the validity of a test method should be obtained and reported in accordance with GLP guidelines and with the use of coded chemicals (OECD 1998; EPA 2003b, 2003c; FDA 2003). The data quality was evaluated by reviewing the methods section in literature references and the submitted reports. Thus, data quality presented in the reviewed literature references can be evaluated only to the extent such information was provided in the published reports. Based on the available information, the following reports were identified as following GLP guidelines or using data obtained according to GLP guidelines: Gettings et al. (1991, 1994, 1996), Balls et al. (1995), Spielmann et al. (1996), and Hagino et al. (1999).

Detailed information on coding procedures used in different studies is provided in Section 3.4 of the ICCVAM 2006 BRD (2006a).

6.0 Hen's Egg Test–Chorioallantoic Membrane Test Method Accuracy

6.1 Accuracy of the HET-CAM Test Method

A critical component of an ICCVAM evaluation of a test method's validation status is an assessment of the proposed test method's accuracy compared to that of the current reference test method (ICCVAM 2003). This aspect of assay performance is typically evaluated by calculating:

- *Accuracy* (concordance): the proportion of correct outcomes (positive and negative) of a test method
- *Sensitivity*: the proportion of all positive substances that are classified as positive
- *Specificity*: the proportion of all negative substances that are classified as negative
- *Positive predictivity*: the proportion of correct positive responses among substances testing positive
- *Negative predictivity*: the proportion of correct negative responses among substances testing negative
- *False positive rate*: the proportion of all negative substances that are falsely identified as positive
- *False negative rate*: the proportion of all positive substances that are falsely identified as negative

ICCVAM evaluated the ability of the HET-CAM test method to identify all categories of ocular irritation potential as defined by the EPA, GHS, and EU classification systems (EPA 2003a; UN 2007; EU 2001). Given that the "Test for Eye Irritants" (16 CFR 1500.42) used for FHSA classification does not discriminate severe or corrosive effects from eye irritation in the rabbit, an evaluation for all ocular hazard categories using the FHSA classification system was not performed. This same analysis was also performed with specific chemical classes and/or physical properties excluded based on their previous identification as discordant in the HET-CAM test method (ICCVAM 2006a).

These evaluations were conducted on the overall data set created by combining results from the reports discussed in **Section 5.0**, then assigning an overall ocular irritancy classification for each substance (see **Annex II** and **III**). When the same substance was evaluated in multiple laboratories, an overall HET-CAM classification was based on the majority classification among all of the studies. When there were an equal number of differing irritancy classifications for substances (e.g., two tests

classified a substance as not labeled and two tests classified the same substance as a mild irritant), the more severe irritancy classification was used for the overall classification for the substance (mild irritant, in this case).

ICCVAM analyzed HET-CAM performance compared to the Draize rabbit eye test for each classification system (i.e., EPA, GHS, EU) using each of the six HET-CAM protocols (i.e., IS[A], IS[B], Q-score, S-score, IS, and irritation threshold concentration protocols, see **Annex III**). With the exception of the IS(A) and IS(B) protocols, all analysis methods had at least one *in vivo* moderate or severe irritant substance classified *in vitro* as not labeled as an irritant (i.e., EPA Category IV, GHS Not Labeled as Irritant, EU Not Labeled). However, the IS(B) overclassified most of the Not Classified Substances (e.g., HET-CAM IS[B] overclassified 93% [39/42] of the GHS Not Labeled as Irritant substances). Therefore, more extensive analyses of the HET-CAM test method described in the following sections were restricted to the IS(A) protocol.

6.1.1 GHS Classification System: HET-CAM Test Method Accuracy

Five studies (Bagley et al. 1992; Gettings et al. 1994; Gettings et al. 1996; Hagino et al. 1999; Kojima et al. 1995) contained HET-CAM data for 63 substances, 59 of which had sufficient *in vivo* data to be assigned GHS ocular irritant classifications (UN 2007) (see **Annex III**). For three of these studies (Gettings et al.1994, 1996; Hagino et al. 1999), ICCVAM evaluated each individual study separately. Individual analyses were not conducted on the other two studies (Bagley et al. 1992; Kojima et al. 1995) because they contained data for only one and two substances, respectively. Based on *in vivo* rabbit eye test data, 44% (26/59) of substances were classified as Category 1; none was classified as Category 2A; 8% (5/59) were classified as Category 2B, and 47% (28/59) were not classified as irritants. Four substances could not be classified due to lack of adequate animal data and are so noted in **Annex III**.

Identification of Category 1 Substances (Ocular Corrosives/Severe Irritants)

The HET-CAM test method correctly identified 50% (13/26) of the Category 1 substances (**Table 6-1**). Among the remaining 50% (13/26) of Category 1 substances underpredicted by HET-CAM, 42% (11/26) were classified as Category 2A and 8% (2/26) were classified as Category 2B.

Identification of Category 2A Substances (Moderate Ocular Irritants)

No substances were identified as GHS Category 2A irritants *in vivo,* and the HET-CAM test method did not mislabel any other substances as moderate ocular irritants (**Table 6-1**).

Identification of Category 2B Substances (Mild Ocular Irritants)

For the five substances that could be evaluated, the HET-CAM test method correctly identified 20% (1/5) as Category 2B, while 80% (4/5) were overpredicted and 0% (0/5) were underpredicted (**Table 6-1**).

Identification of Not Classified Substances

For the 28 substances that could be evaluated, the HET-CAM test method correctly identified 36% (10/28) as substances not classified as irritants, while 64% (18/28) were overpredicted (**Table 6-1**).

Ability to Distinguish Substances Not Classified as Irritants from All Other Classes

In addition to evaluating the ability of the HET-CAM test method to identify each individual ocular hazard category according to the GHS classification system, ICCVAM also evaluated the ability of the HET-CAM test method to distinguish ocular substances not classified as irritants from all irritant

classes.[1] For the 59 substances considered, the HET-CAM test method had an overall accuracy of 69% (41/59), a sensitivity of 100% (31/31), a specificity of 36% (10/28), a false positive rate of 64% (18/28), and a false negative rate of 0% (0/31) (**Table 6-2**).

As detailed below, the results from each individual study were also evaluated separately.

Gettings et al. (1994): Based upon the *in vivo* rabbit data, 18 substances were assigned a GHS classification. The HET-CAM test method, by comparison, has an accuracy of 50% (9/18), sensitivity of 100% (1/1), specificity of 47% (8/17), false positive rate of 53% (9/17), and a false negative rate of 0% (0/1) (**Table 6-2**).

Gettings et al. (1996): Based on the *in vivo* rabbit data, 24 substances could be assigned a GHS classification. Among these 24 substances, the HET-CAM test method has an accuracy of 83% (20/24), sensitivity of 100% (18/18), specificity of 33% (2/6), false positive rate of 67% (4/6), and a false negative rate of 0% (0/18) (**Table 6-2**).

[1] The ICCVAM 2006 BRD provides an evaluation of the HET-CAM test method for distinguishing ocular corrosives and severe irritants from all other classes (ICCVAM 2006a). Because the database of HET-CAM test method results has not changed, this analysis is not repeated here.

Table 6-1 Performance of the HET-CAM Test Method (IS[A]) in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the GHS Classification System,[1] by Study and Overall

Data Source	Overall Correct Classification	Severe (Category 1)		Moderate (Category 2A)			Mild (Category 2B)			Not Classified as Irritant	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Gettings et al. (1994)	50% (9/18)	100% (1/1)	0% (0/1)	0% (0/0)	0% (0/0)	0% (0/0)	0% (0/0)	0% (0/0)	0% (0/0)	53% (9/17)	47% (8/17)
Gettings et al. (1996)	29% (7/24)	25% (4/16)	75% (12/16)	0% (0/0)	0% (0/0)	0% (0/0)	50% (1/2)	50% (1/2)	0% (0/2)	67% (4/6)	33% (2/6)
Hagino et al. (1999)	53% (8/15)	100% (8/8)	0% (0/8)	0% (0/0)	0% (0/0)	0% (0/0)	100% (3/3)	0% (0/3)	0% (0/3)	100% (4/4)	0% (0/4)
Overall[2]	41% (24/59)	50% (13/26)	50% (13/26)	0% (0/0)	0% (0/0))	0% (0/0)	80% (4/5)	20% (1/5)	0% (0/5)	64% (18/28)	36% (10/28)

Abbreviations: GHS = Globally Harmonized System; HET-CAM = hen's egg test–chorioallantoic membrane.

[1] GHS classification system (UN 2007).

[2] Overall data set contains 59 test substances that were assigned a GHS classification and includes one additional test substance from Bagley et al. (1992) and one from Kojima et al. (1995) that were not included as individual data sources. One additional substance from Kojima et al. (1995) was not included because it was classified *in vitro* as Category1/Category 2A in the rabbit eye test.

Table 6-2 Accuracy of the HET-CAM Test Method (IS[A]) in Distinguishing Substances Not Classified as Irritants from All Other Irritant Classes, as Defined by the GHS Classification System,[1] by Study and Overall

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Gettings et al. (1994)	18	50	9/18	100	1/1	47	8/17	53	9/17	0	0/1
Gettings et al. (1996)	24	83	20/24	100	18/18	33	2/6	67	4/6	0	0/18
Hagino et al. (1999)	15	73	11/15	100	11/11	0	0/4	100	4/4	0	0/11
Overall[2]	59	69	41/59	100	31/31	36	10/28	64	18/28	0	0/31

Abbreviations: GHS = Globally Harmonized System; HET–CAM = hen's egg test–chorioallantoic membrane; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] GHS classification system (UN 2007): Not Classified as Irritant vs Category 1/2A/2B.

[2] Overall data set contains 59 test substances that were assigned a GHS hazard classification. Data from one additional test substance from Bagley et al. (1992) and one from Kojima et al. (1995) were not included as individual data sources. One additional substance from Kojima et al. (1995) was not included because it was classified *in vitro* as Category1/Category 2A in the rabbit eye test.

Hagino et al. (1999): Based upon the *in vivo* rabbit data, 15 substances could be assigned a GHS classification. Among these 15 substances, the HET-CAM test method has an accuracy of 73% (11/15), sensitivity of 100% (11/11), specificity of 0% (0/4), false positive rate of 100% (4/4), and a false negative rate of 0% (0/11) (**Table 6-2**).

Performance of the HET-CAM Test Method with Discordant Classes Excluded

Because the IS(A) analysis method is the focus of the evaluation of HET-CAM for identifying all hazard categories, separate analyses were also conducted for all chemical classes and specific physical properties of interest represented in this database of 59 substances by at least five substances (i.e., surfactant-based formulations, oil/water emulsions, and alcohols). The results indicate that alcohols tend to be overpredicted by HET-CAM: 75% (4/6) of alcohols classified as Category 2B or Not Classified as Irritant based on Draize test results, and depending on the classification system used, were overpredicted by HET-CAM by at least one hazard category. Similarly, the HET-CAM test method overpredicted 53% (9/17) of the oil/water emulsions identified as Not Classified as Irritant by at least one hazard category. By comparison, surfactant formulations classified as Category 1 based on Draize results tended to be underpredicted by HET-CAM: 75% (12/16) were underpredicted by HET-CAM as Category 2A or 2B. However, none of these substances was underpredicted as Not Classified as Irritant.

Given the proportion of substances in the HET-CAM IS(A) database represented by these chemical and product classes (i.e., 85% [50/59] of the substances are included in one of these three categories), separate analyses without these discordant substances are not particularly informative. However, because of the associated discordance with each type, overall performance, particularly for Category 1 substances, can be improved by excluding surfactant-based formulations (see **Table 6-3**).

When the ability of the HET-CAM test method to distinguish Not Classified as Irritant substances from all other irritant classes was evaluated with the specific chemical and product classes removed, the greatest improvement in false positive rate occurred when alcohols and surfactant formulations were excluded. The false positive rate decreased from 64% (18/28) to 56% (10/18). However, because the false negative rate for the overall database is 0% (0/31), this rate remained constant regardless of which chemical or product class(es) were excluded (**Table 6-4**).

Further analysis of substances for which hazard classification was underpredicted by HET-CAM according to chemical class indicated that carboxylic acids had the highest proportion of underpredicted substances (25% [1/4]). Because 98% of the entire HET-CAM IS(A) database is made up of liquid substances, the physical form of underpredicted substances was liquids. Among the 16 Category 1 surfactants, HET-CAM underpredicted 75% (12/16) (**Table 6-5**).

According to the GHS classification system, the most overpredicted substances (false positives) were alcohols, of which HET-CAM overpredicted 75% (6/8). Because 98% of the entire HET-CAM IS(A) database is made up of liquid substances, the physical form of underpredicted substances was liquids. Only one of the surfactants tested in HET-CAM was overpredicted (**Table 6-5**).

Table 6-3 Performance of the HET-CAM Test Method (IS[A]) in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the GHS Classification System,[1] with Discordant Chemical and Physical Classes Excluded

HET-CAM Database	Overall Correct Classification	Severe (Category 1)		Moderate (Category 2A)			Mild (Category 2B)			Not Classified as Irritant	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Overall	41% (24/59)	50% (13/26)	50% (13/26)	- (0/0)	- (0/0)	- (0/0)	80% (4/5)	20% (1/5)	0% (0/5)	64% (18/28)	36% (10/28)
Without Alcohols	43% (22/51)	46% (11/24)	54% (13/24)	- (0/0)	- (0/0)	- (0/0)	67% (2/3)	33% (1/3)	0% (0/3)	58% (14/24)	42% (10/24)
Without Surfactant Formulations	49% (17/35)	90% (9/10)	10% (1/10)	- (0/0)	- (0/0)	- (0/0)	100% (3/3)	0% (0/3)	0% (0/3)	64% (14/22)	36% (8/22)
Without Oil/Water Emulsions	41% (15/41)	48% (12/25)	52% (13/25)	- (0/0)	- (0/0)	- (0/0)	80% (4/5)	20% (1/5)	0% (0/5)	82% (9/11)	18% (2/11)
Without Alcohols and Surfactant Formulations	56% (15/27)	87% (7/8)	12% (1/8)	- (0/0)	- (0/0)	- (0/0)	100% (1/1)	0% (0/1)	0% (0/1)	56% (10/18)	44% (8/18)
Without Alcohols and Oil/Water Emulsions	39% (13/33)	44% (10/23)	56% (13/23)	- (0/0)	- (0/0)	- (0/0)	67% (2/3)	33% (1/3)	0% (0/3)	71% (5/7)	29% (2/7)
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions	67% (6/9)	86% (6/7)	14% (1/7)	- (0/0)	- (0/0)	- (0/0)	100% (1/1)	0% (0/1)	0% (0/1)	100% (1/1)	0% (0/1)

Abbreviations: GHS = Globally Harmonized System; HET-CAM = hen's egg test–chorioallantoic membrane.

[1] GHS classification system (UN 2007).

Table 6-4 Accuracy of the HET-CAM Test Method (IS[A]) in Distinguishing Substances Not Classified as Irritants from All Other Irritant Classes, as Defined by the GHS Classification System,[1] with Discordant Chemical and Physical Classes Excluded

HET-CAM Database	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall	59	69	41/59	100	31/31	36	10/28	64	18/28	0	0/31
Without Alcohols	51	73	37/51	100	27/27	42	10/24	58	14/24	0	0/27
Without Surfactant Formulations	35	60	21/35	100	13/13	36	8/22	64	14/22	0	0/13
Without Oil/Water Emulsions	41	78	32/41	100	30/30	18	2/11	82	9/11	0	0/30
Without Alcohols and Surfactant Formulations	27	63	17/27	100	9/9	44	8/18/	56	10/18	0	0/9
Without Alcohols and Oil/Water Emulsions	33	85	28/33	100	26/26	29	2/7	71	5/7	0	0/26
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions	9	89	8/9	100	8/8	0	0/1	100	1/1	0	0/8

Abbreviations: GHS = Globally Harmonized System; HET-CAM = hen's egg test–chorioallantoic membrane; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] GHS classification system (UN 2007).

Table 6-5 Performance of the HET-CAM Test Method (IS[A]) Using the GHS Classification System[1] in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property

Category	N	Underprediction (*In Vivo/In Vitro)*						Overprediction (*In Vivo/In Vitro)*					
		Severe (Category 1)			Moderate (Category 2A)		Mild (Category 2B)	Moderate (Category 2A)	Mild (Category 2B)		NC (Not Classified)		
		NC	2B	2A	NC	2B	NC	1	2A	1	2B	2A	1
Overall	59	0% (0/26)	8% (2/26)	42% (11/26)	-	-	0% (0/5)	-	20% (1/5)	60% (3/5)	32% (9/28)	14% (4/28)	18% (5/28)
Chemical Class[2]													
Alcohol	8	0% (0/2)	0% (0/2)	0% (0/2)	-	-	0% (0/2)	-	0% (0/2)	100% (2/2)	0% (0/4)	50% (2/4)	50% (2/4)
Carboxylic acid	5	0% (0/4)	0% (0/4)	25% (1/4)	-	-	0% (0/1)	-	0% (0/1)	100% (1/1)	-	-	-
Organic salt	6	0% (0/6)	0% (0/6)	17% (1/6)	-	-	-	-	-	-	-	-	-
Properties of Interest													
Liquids	58	0% (0/25)	8% (2/25)	40% (10/25)	0% (0/5)	-	0% (0/2)	-	20% (1/5)	60% (3/5)	32% (9/28)	14% (4/28)	18% (5/28)
Solids	0	-	-	-	-	-	-	-	-	-	-	-	-
Pesticide	0	-	-	-	-	-	-	-	-	-	-	-	-
Surfactant—Total	24	0% (0/16)	12% (2/16)	62% (10/16)	-	-	0% (0/2)	-	50% (1/2)	0% (0/2)	0% (0/6)	0% (0/6)	0% (0/6)
-nonionic	-	-	-	-	-	-	-	-	-	-	-	-	-
-anionic	-	-	-	-	-	-	-	-	-	-	-	-	-
-cationic	-	-	-	-	-	-	-	-	-	-	-	-	-

Category	N	Underprediction (*In Vivo/In Vitro*)						Overprediction (*In Vivo/In Vitro*)					
		Severe (Category 1)			Moderate (Category 2A)		Mild (Category 2B)	Moderate (Category 2A)	Mild (Category 2B)		NC (Not Classified)		
		NC	2B	2A	NC	2B	NC	1	2A	1	2B	2A	1
Overall	59	0% (0/26)	8% (2/26)	42% (11/26)	-	-	0% (0/5)	-	20% (1/5)	60% (3/5)	32% (9/28)	14% (4/28)	18% (5/28)
Oil/Water Emulsion	18	0% (0/1)	0% (0/1)	0% (0/1)	-	-	-	-	-	-	24% (4/17)	12% (2/17)	18% (3/17)
pH—Total	0	-	-	-	-	-	-	-	-	-	-	-	-
-acidic (pH <7.0)	-	-	-	-	-	-	-	-	-	-	-	-	-
-basic (pH >7.0)	-	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: GHS = Globally Harmonized System; HET-CAM = hen's egg test – chorioallantoic membrane; NC = Not Classified as Irritant.

[1] GHS classification system (UN 2007).

[2] Chemical classes included in this table are represented by at least five substances tested in the HET-CAM test method, and assignments are based upon National Library of Medicine medical subject heading (MeSH) categories (www.nlm.nih.gov/mesh) as defined in **Annex I**.

Table 6-6 Performance of the HET-CAM Test Method (IS[A]) in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA Classification System[1], by Study and Overall

Data Source	Overall Correct Classification	Severe (Category I)		Moderate (Category II)			Mild (Category III)			Not Labeled (Category IV)	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Gettings et al. (1994)	33% (6/18)	100% (1/1)	0% (0/1)	0% (0/0)	0% (0/0)	0% (0/0)	38% (3/8)	12% (1/8)	50% (4/8)	56% (5/9)	44% (4/9)
Gettings et al. (1996)	36% (9/25)	24% (4/17)	76% (13/17)	0% (0/0)	0% (0/0)	0% (0/0)	25% (1/4)	75% (3/4)	0% (0/4)	50% (2/4)`	50% (2/4)
Hagino et al. (1999)	47% (7/15)	100% (7/7)	0% (0/7)	100% (1/1)	0% (0/1)	0% (0/1)	100% (5/5)	0% (0/5)	0% (0/5)	100% (2/2)	0% (0/2)
Overall[2]	38% (23/60)	48% (12/25)	52% (13/25)	50% (1/2)	50% (1/2)	0% (0/2)	56% (10/18)	22% (4/18)	22% (4/18)	60% (9/15)	40% (6/15)

Abbreviations: EPA = U.S. Environmental Protection Agency; HET-CAM = hen's egg test–chorioallantoic membrane.

[1] EPA classification system (EPA 2003a).

[2] Overall data set includes 60 test substances that were assigned an EPA hazard classification based on rabbit eye test data. Data from one test substance from Bagley et al. (1992) and one from Kojima et al. (1995) were not included as individual data sources. One substance from Kojima et al. (1995) was classified as a GHS Category 1/2A and could not be used in the analysis.

6.1.2 EPA Classification System: HET-CAM Test Method Accuracy

Five studies (Bagley et al. 1992; Gettings et al. 1994; Kojima et al. 1995; Gettings et al. 1996; Hagino et al. 1999) contained HET-CAM test method data on 63 substances, 60 of which had sufficient *in vivo* data to be assigned an ocular irritancy classification according to the EPA classification system (EPA 2003a) (see **Annex III**). Based on results from *in vivo* rabbit eye experiments, 42% (25/60) were classified as severe irritants (i.e., Category I), 3% (2/60) were classified as moderate irritants (Category II), 30% (18/60) were classified as mild irritants (Category III), and 25% (15/60) were classified as not labeled as irritant (Category IV). Three substances could not be classified according to the EPA classification system due to the lack of adequate animal data and are so noted in **Annex III**.

Identification of Category I Substances (Ocular Corrosives/Severe Irritants)

The HET-CAM test method correctly identified 48% (12/25) of the Category I substances (**Table 6-6**). Among the remaining 52% (13/25) Category I substances that were underpredicted by HET-CAM, 40% (10/25) were classified as Category II, and 12% (3/25) were classified as Category III.

Identification of Category II Substances (Moderate Ocular Irritants)

For the two substances that could be evaluated, the HET-CAM test method correctly identified 50% (1/2) as Category II while 50% (1/2) were overpredicted and 0% (0/2) were underpredicted (**Table 6-6**).

Identification of Category III (Mild Ocular Irritants)

For the 18 substances that could be evaluated, the HET-CAM test method correctly identified 22% (4/18) as Category III while 56% (10/18) were overpredicted and 22% (4/18) were underpredicted (**Table 6-6**).

Identification of Category IV Substances

For the 15 substances that could be evaluated, the HET-CAM test method correctly identified 40% (6/15) as substances not labeled as irritants while 60% (9/15) were overpredicted (**Table 6-6**).

Ability to Distinguish Category IV Substances from All Other Classes

In addition to evaluating the ability of the HET-CAM test method to identify each individual ocular hazard category according to the EPA classification system, ICCVAM also evaluated the ability of the HET-CAM test method to distinguish ocular substances not labeled as irritants from all irritant classes.[2] Among the 60 substances considered, the HET-CAM test method had an overall accuracy of 78% (47/60), a sensitivity of 91% (41/45), a specificity of 40% (6/15), a false positive rate of 60% (9/15), and a false negative rate of 9% (4/45) (**Table 6-7**).

As detailed below, the results from each individual study were also evaluated separately.

Gettings et al. (1994): Based upon the *in vivo* rabbit data, 18 substances were assigned an EPA classification. The HET-CAM test method, by comparison, has an accuracy of 50% (9/18), sensitivity of 56% (5/9), specificity of 44% (4/9), false positive rate of 56% (5/9), and a false negative rate of 44% (4/9) (**Table 6-7**).

[2] The ICCVAM 2006 BRD (2006a) provides an evaluation of the HET-CAM test method for distinguishing ocular corrosives and severe irritants from all other classes (ICCVAM 2006a). Because the database of HET-CAM test method results has not changed, this analysis is not repeated here.

Table 6-7 Accuracy of the HET-CAM Test Method (IS[A]) in Distinguishing Category IV Substances from All Other Irritant Classes as Defined by the EPA Classification System,[1] by Study and Overall

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Gettings et al. (1994)	18	50	9/18	56	5/9	44	4/9	56	5/9	44	4/9
Gettings et al. (1996)	25	92	23/25	100	21/21	50	2/4	50	2/4	0	0/21
Hagino et al. (1999)	15	87	13/15	100	13/13	0	0/2	100	2/2	0	0/13
Overall[2]	60	78	47/60	91	41/45	40	6/15	60	9/15	9	4/45

Abbreviations: EPA = U.S. Environmental Protection Agency; HET-CAM = hen's egg test–chorioallantoic membrane; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] EPA classification system (EPA 2003a): Category IV vs. Categories I/II/III.

[2] Overall database includes 60 test substances that were assigned an EPA hazard classification based on rabbit eye test data. Data on one test substance from Bagley et al. (1992) and another substance from Kojima et al. (1995) were not included as individual data sources. One substance from Kojima et al. (1995) was classified as a GHS Category 1/2A and, therefore, was not used in the analysis either.

Gettings et al. (1996): Based upon the *in vivo* rabbit data, 25 substances were assigned an EPA classification. The HET-CAM test method, by comparison, has an accuracy of 92% (23/25), sensitivity of 100% (21/21), specificity of 50% (2/4), false positive rate of 50% (2/4), and a false negative rate of 0% (0/21).

Hagino et al. (1999): Based upon the *in vivo* rabbit data, 15 substances were assigned an EPA classification. The HET-CAM test method, by comparison, has an accuracy of 87% (13/15), sensitivity of 100% (13/13), specificity of 0% (0/2), false positive rate of 100% (2/2), and a false negative rate of 0% (0/13).

Performance of the HET-CAM Test Method with Discordant Classes Excluded

Because the IS(A) analysis method is the focus of the evaluation of HET-CAM for identifying all hazard categories, separate analyses were also conducted for all chemical classes and specific physical properties of interest represented in this database of 60 substances by at least five substances (i.e., surfactant-based formulations, oil/water emulsions, and alcohols).

Given the proportion of substances in the HET-CAM IS(A) database represented by these chemical and product classes (i.e., 85% [51/60] of the substances are included in one of these three categories), separate analyses without these discordant substances are not particularly informative. However, because of the associated discordance with each type, overall performance, particularly for the ocular corrosive and severe irritant category, can be improved by excluding certain product types (see **Table 6-8**). The results indicate that HET-CAM tends to overpredict alcohols. All seven alcohols (100%) classified as Category III or IV based on Draize test results were overpredicted by HET-CAM by at least one hazard category. Similarly, 47% (8/17) of the oil/water emulsions classified as Category III or IV based on Draize test results were overpredicted by HET-CAM by at least one hazard category. By comparison, surfactant formulations classified as Category I based on Draize results tended to be underpredicted by HET-CAM (73% [13/17] were underpredicted by HET-CAM as Category II or III). However, none of these substances was underpredicted as Category IV.

When the ability of the HET-CAM test method to distinguish Category IV substances from all other irritant classes was evaluated with the specific chemical and product classes removed, the greatest improvement in false positive rate occurred when alcohols and surfactant-based formulations were excluded. The false positive rate decreased from 60% (9/15) to 56% (5/9). The false negative rate for the overall database, 9% (4/45), could be reduced to 0% (0/30) by excluding oil/water emulsions from the database (**Table 6-9**).

Among the four false negatives for the EPA system, 100% (4/4) were EPA Category III substances based on Draize data. For 100% (4/4) of these substances, the categorization was based on conjunctival redness (**Table 6-10**). All of the false negative substances were oil/water emulsions.

Table 6-8 Performance of the HET-CAM Test Method (IS[A]) in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA Classification System,[1] with Discordant Chemical and Physical Classes Excluded

HET-CAM Database	Overall Correct Classification	Severe (Category I)		Moderate (Category II)			Mild (Category III)			Not Labeled (Category IV)	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Overall	41% (24/59)	50% (13/26)	50% (13/26)	0% (0/0)	0% (0/0)	0% (0/0)	80% (4/5)	20% (1/5)	0% (0/5)	64% (18/28)	36% (10/28)
Without Alcohols	42% (22/52)	46% (11/24)	54% (13/24)	50% (1/2)	50% (1/2)	0% (0/2)	38% (5/13)	31% (4/13)	31% (4/13)	54% (7/13)	46% (6/13)
Without Surfactant Formulations	40% (14/35)	100% (8/8)	0% (0/8)	50% (1/2)	50% (1/2)	0% (0/2)	64% (9/14)	7% (1/14)	29% (4/14)	64% (7/11)	36% (4/11)
Without Oil/Water Emulsions	37% (15/41)	48% (12/25)	52% (13/25)	0% (0/0)	0% (0/0)	0% (0/0)	80% (4/5)	10% (1/5)	0% (0/5)	82% (9/11)	18% (2/11)
Without Alcohols and Surfactant Formulations	48% (13/27)	100% (7/7)	0% (0/7)	50% (1/2)	50% (1/2)	0% (0/2)	44% (4/9)	11% (1/9)	44% (4/9)	56% (5/9)	44% (4/9)
Without Alcohols and Oil/Water Emulsions	47% (16/34)	43% (10/23)	57% (13/23)	50% (1/2)	50% (1/2)	0% (0/2)	40% (2/5)	60% (3/5)	0% (0/5)	50% (2/4)	50% (2/4)
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions	78% (7/9)	100% (6/6)	0% (0/6)	50% (1/2)	50% (1/2)	0% (0/2)	100% (1/1)	0% (0/1)	0% (0/1)	-	-

Abbreviations: EPA = U.S. Environmental Protection Agency; HET-CAM = hen's egg test – chorioallantoic membrane

[1] EPA classification system (EPA 2003a).

Table 6-9 Accuracy of the HET-CAM Test Method (IS[A]) in Distinguishing EPA Category IV from All Other Irritant Classes as Defined by the EPA Classification System,[1] with Discordant Chemical and Physical Classes Excluded

HET-CAM Database	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.[3]	%	No.	%	No.	%	No.	%	No.
Overall	60	78	47/60	91	41/45	40	6/15	60	9/15	9	4/45
Without Alcohols	52	87	45/52	100	39/39	46	6/13	54	7/13	10	4/39
Without Surfactant Formulations	35	80	28/35	100	24/24	29	4/14	82	9/11	17	4/24
Without Oil/Water Emulsions	41	78	32/41	100	30/30	18	2/11	82	9/11	0	0/30
Without Alcohols and Surfactant Formulations	27	81	22/27	100	18/18	44	4/9	56	5/9	44	4/18
Without Alcohols and Oil/Water Emulsions	34	94	32/34	100	30/30	50	2/4	50	2/4	0	0/30
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions	9	100	9/9	100	9/9	-	0/0	0	0/9	-	0/0

Abbreviations: EPA = U.S. Environmental Protection Agency; HET-CAM = hen's egg test – chorioallantoic membrane; N = number of substances included in this analysis/the total number of substances in the study; No. = data used to calculate the percentage.

[1] EPA classification system (EPA 2003a): Category IV vs. Categories I/II/III.

Table 6-10 HET-CAM False Negative Substances[1] Using the EPA Classification System[2]

Substance	*In Vivo* Scores		
	N	Corneal Opacity: Score (Day Cleared)[3]	Conjunctival Redness: Score (Day Cleared)[3]
HZA	6	-	N=1 2(2) N=1 2(3)
HZC	6	-	N=1 2(2)
HZV	6	-	N=2 2(2)
HZW	6	-	N=4 2(2) N=1 2(3)

Abbreviations: EPA = U.S. Environmental Protection Agency; HET-CAM = hen's egg test–chorioallantoic membrane; N = number of animals

[1] False negative compounds are those that test as nonirritants *in vitro* but are mild, moderate, or severe ocular irritants/corrosive *in vivo*, i.e., EPA Category I, II, or III.

[2] EPA classification system (EPA 2003a).

[3] For the purposes of this evaluation, *clearing* is defined in the EPA hazard classification system as opacity or iritis scores = 0 or redness or chemosis scores = 1.

Further analysis of substances for which hazard classification was underpredicted by HET-CAM according to chemical class indicated that carboxylic acids had the highest proportion of underpredicted substances (25% [1/4]). Because the entire HET-CAM IS(A) database is made up of liquid substances, the physical form of underpredicted substances was liquids. Among the 17 Category I surfactants, 73% (13/17) were underpredicted (**Table 6-11**).

According to the EPA classification system, the most overpredicted substances (false positives) were alcohols, of which 100% (7/7) were overpredicted. Because 98% (59/60) of the entire HET-CAM IS(A) database is made up of liquid substances, the physical form of overpredicted substances was liquids. Three of the surfactants tested in HET-CAM were overpredicted (**Table 6-11**).

6.1.3 EU Classification System: HET-CAM Test Method Accuracy

Five studies (Bagley et al. 1992; Gettings et al. 1994; Kojima et al. 1995; Gettings et al. 1996; Hagino et al. 1999) contained HET-CAM test method data on 63 substances, 58 of which had sufficient *in vivo* data to be assigned an ocular irritancy classification according to the EU classification system (EU 2001) (see **Annex III**). Based on results from *in vivo* rabbit eye tests, 41% (24/58) were classified as R41 (severe irritants), 3% (2/58) were classified as R36 (moderate irritants), and 55% (32/58) were classified as Not Labeled. Five substances could not be classified according to the EU classification system due to the lack of adequate animal data and are so noted in **Annex III**.

Identification of Category R41 Substances (Ocular Corrosives/Severe Irritants)

The HET-CAM test method correctly identified 50% (12/24) of the R41 substances (**Table 6-12**). Among the remaining 50% (12/24) of R41 substances that were underpredicted by HET-CAM, 42% (10/24) were classified as R36, and 8% (2/24) were classified as Not Labeled.

Identification of Category R36 Substances (Moderate Ocular Irritants)

For the two substances that could be evaluated, the HET-CAM test method correctly identified 50% (1/2) as R36, while 50% (1/2) were underpredicted and 0% (0/2) were overpredicted (**Table 6-12**).

Table 6-11 Under- and Overprediction of the HET-CAM Test Method Using the EPA Classification System[1] in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property

Category	N	Underprediction (*In Vivo/In Vitro*)						Overprediction (*In Vivo/In Vitro*)					
		Severe (Category I)			Moderate (Category II)		Mild (Category III)	Moderate (Category II)	Mild (Category III)		Not Labeled (Category IV)		
		IV	III	II	IV	III	IV	I	II	I	III	II	I
Overall	60	0% (0/25)	12% (3/25)	40% (10/25)	0% (0/2)	0% (0/2)	40% (4/10)	50% (1/2)	50% (5/10)	50% (5/10)	40% (6/15)	0% (0/15)	20% (3/15)
Chemical Class[2]													
Alcohol	8	0% (0/1)	0% (0/1)	0% (1/1)	-	-	0% (0/5)	-	40% (2/5)	60% (3/5)	50% (1/2)	0% (0/2)	50% (1/2)
Carboxylic acid	6	0% (0/4)	0% (0/4)	25% (1/4)	-	-	0% (0/2)	-	0% (0/2)	100% (2/2)	-	-	-
Organic salt	6	0% (0/6)	0% (0/6)	17% (1/6)	-	-	-	-	-	-	-	-	-
Properties of Interest													
Liquids	59	0% (0/25)	12% (3/25)	40% (10/25)	-	-	22% (4/18)	-	28% (5/18)	28% (5/18)	40% (6/15)	0% (0/15)	20% (3/15)
Solids	0	-	-	-	-	-	-	-	-	-	-	-	-
Pesticide	0	-	-	-	-	-	-	-	-	-	-	-	-
Surfactant—Total	25	0% (0/17)	18% (3/17)	59% (10/17)	-	-	0% (0/4)	-	25% (1/4)	0% (0/4)	50% (2/4)	0% (0/4)	0% (0/4)
-nonionic	-	-	-	-	-	-	-	-	-	-	-	-	-
-anionic	-	-	-	-	-	-	-	-	-	-	-	-	-
-cationic	-	-	-	-	-	-	-	-	-	-	-	-	-

Continued

Table 6-11 Under- and Overprediction of the HET-CAM Test Method Using the EPA Classification System[1] in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property (continued)

Category	N	Underprediction (*In Vivo/In Vitro)*						Overprediction (*In Vivo/In Vitro)*					
		Severe (Category I)			Moderate (Category II)		Mild (Category III)	Moderate (Category II)	Mild (Category III)		Not Labeled (Category IV)		
		IV	III	II	IV	III	IV	I	II	I	III	II	I
Overall	60	0% (0/25)	12% (3/25)	40% (10/25)	0% (0/2)	0% (0/2)	40% (4/10)	50% (1/2)	50% (5/10)	50% (5/10)	40% (6/15)	0% (0/15)	20% (3/15)
Properties of Interest (continued)													
Oil/Water Emulsion	18	0% (0/1)	0% (0/1)	0% (0/1)	-	-	50% (4/8)	-	25% (2/8)	13% (1/8)	33% (3/9)	0% (0/9)	22% (2/9)
pH—Total	0	-	-	-	-	-	-	-	-	-	-	-	-
-acidic (pH <7.0)	-	-	-	-	-	-	-	-	-	-	-	-	-
-basic (pH >7.0)	-	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: EPA = Environmental Protection Agency; HET-CAM = hen's egg test – chorioallantoic membrane; N = number of animals.

[1] EPA classification system (EPA 2003a).

[2] Chemical classes included in this table are represented by at least five substances tested in the HET-CAM test method, and assignments are based on the National Library of Medicine's medical substance headings (MeSH) classifications (www.nlm.nih.gov/mesh) as defined in **Annex I**.

Table 6-12 Performance of the HET-CAM Test Method (IS[A]) in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EU Classification System,[1] by Study and Overall

Data Source	Overall Correct Classification	Severe (R41)		Moderate (R36)			Mild			Not Labeled	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Gettings et al. (1994)	50% (9/18)	100% (1/1)	0% (0/1)	0% (0/0)	0% (0/0)	0% (0/0)	NA	NA	NA	53% (9/17)	47% (8/17)
Gettings et al. (1996)	29% (7/24)	25% (4/16)	75% (10/16)	0% (0/1)	100% (1/1)	0% (0/1)	NA	NA	NA	71% (5/7)	29% (2/7)
Hagino et al. (1999)	47% (7/15)	100% (7/7)	0% (0/7)	100% (1/1)	0% (0/1)	0% (0/1)	NA	NA	NA	100% (7/7)	0% (0/7)
Overall[2]	40% (23/58)	50% (12/24)	50% (12/24)	50% (1/2)	50% (1/2)	0% (0/2)	NA	NA	NA	69% (22/32)	31% (10/32)

Abbreviations: EU = European Union; HET-CAM = hen's egg test–chorioallantoic membrane; NA = not applicable.

[1] EU classification system (EU 2001).

[2] Overall data set includes one additional test substance from Bagley et al. (1992).

Identification of Not Labeled Substances

For the 32 substances that could be evaluated, the HET-CAM test method correctly identified 31% (10/32) as substances not labeled as irritants, while 69% (22/32) were overpredicted (**Table 6-12**).

Ability to Distinguish Not Labeled Substances from All Other Classes

In addition to evaluating the ability of the HET-CAM test method to identify each individual ocular hazard category according to the EU classification system, ICCVAM also evaluated the ability of the HET-CAM test method to distinguish ocular substances not labeled as irritants from all other irritant classes.[3] Among the 58 substances considered, the HET-CAM test method has an overall accuracy of 62% (36/58), a sensitivity of 100% (26/26), a specificity of 31% (10/32), a false positive rate of 69% (22/32), and a false negative rate of 0% (0/26) (**Table 6-13**).

As detailed below, the results from each individual study were also evaluated separately.

Gettings et al. (1994): Based upon the *in vivo* rabbit data, 18 substances were assigned an EU classification. The HET-CAM test method, by comparison, has an accuracy of 50% (9/18), sensitivity of 100% (1/1), specificity of 47% (8/17), false positive rate of 53% (9/17), and a false negative rate of 0% (0/1) (**Table 6-13**).

Gettings et al. (1996): Based upon the *in vivo* rabbit data, 24 substances were assigned a EU classification. The HET-CAM test method, by comparison, has an accuracy of 79% (19/24), sensitivity of 100% (17/17), specificity of 29% (2/7), false positive rate of 61% (5/7), and a false negative rate of 0% (0/17) (**Table 6-13**).

Hagino et al. (1999): Based upon the *in vivo* rabbit data, 15 substances were assigned a EU classification. The HET-CAM test method, by comparison, has an accuracy of 53% (8/15), sensitivity of 100% (8/8), specificity of 0% (0/7), false positive rate of 100% (7/7), and a false negative rate of 0% (0/26) (**Table 6-13**).

Performance of the HET-CAM Test Method with Discordant Classes Excluded

Because the IS(A) analysis method is the focus of the evaluation of HET-CAM for identifying all hazard categories, separate analyses were also conducted for all chemical classes and specific physical properties of interest represented in this database of 58 substances by at least five substances (i.e., surfactant-based formulations, oil/water emulsions, and alcohols).

Given the proportion of substances in the HET-CAM IS(A) database represented by these chemical and product classes (i.e., 88% [51/58] of the substances are included in one of these three categories), separate analyses without these discordant substances are not particularly informative. However, because of the associated discordance with each type, overall performance, particularly for the ocular corrosive and severe irritant category, can be improved by excluded certain product types (see **Table 6-14**). The results indicate that HET-CAM tends to overpredict alcohols (i.e., 83% [5/6] of alcohols classified as Not Labeled based on Draize test results were overpredicted by HET-CAM by at least one hazard category). Similarly, 53% (9/17) of the oil/water emulsions were overpredicted by HET-CAM by at least one hazard category. By comparison, surfactant formulations classified as R41 based on Draize results tended to be underpredicted by HET-CAM (75% [12/16] were underpredicted by HET-CAM as R36). However, none of these substances was underpredicted as Not Labeled.

When the ability of the HET-CAM test method to distinguish Not Labeled substances from all other irritant classes was evaluated with the specific chemical and product classes removed, the greatest

[3] The ICCVAM 2006 BRD provides an evaluation of the HET-CAM test method for distinguishing ocular corrosives and severe irritants from all other classes (ICCVAM 2006a). Because the database of HET-CAM test method results has not changed, this analysis is not repeated here.

improvement in false positive rate occurred when alcohols and surfactant formulations were excluded. The false positive rate decreased from 69% (22/32) to 58% (11/19). However, because the false negative rate for the overall database is 0% (0/31), this rate remained constant regardless of which chemical or product class(es) were excluded (**Table 6-15**).

Further analysis of substances for which hazard classification was underpredicted by HET-CAM according to chemical class indicated that carboxylic acids had the highest proportion of underpredicted substances (25% [1/4]). Because the entire HET-CAM IS(A) database is made up of liquid substances, the physical form of underpredicted substances was liquids. Among the 16 R41 surfactant formulations, 75% (12/16) were underpredicted (**Table 6-16**).

According to the EU classification system, the most overpredicted substances (false positives) were alcohols, of which 83% (5/6) were overpredicted. Because the entire HET-CAM IS(A) database is made up of liquid substances, the physical form of underpredicted substances was liquids. One of the Not Labeled surfactant formulations tested in HET-CAM was overpredicted (**Table 6-16**).

6.1.4 FHSA Classification System: HET-CAM Test Method Accuracy

The three studies (Gettings et al. 1994; Gettings et al. 1996; Hagino et al. 1999) contained HET-CAM test method data on 64 substances, 63 and 55 of which had sufficient *in vivo* data to be assigned an ocular irritancy classification according to the FHSA-20% and FHSA-67% criteria, respectively. Based on results from *in vivo* rabbit eye experiments using the FHSA-20% criteria, 68% (43/63) were classified as Irritants and 10% (6/63) were classified as Not Labeled. The remaining 24% (15/64) could not be classified using the FHSA-20% criteria and are so noted in **Annex III**. Using the FHSA-67% criteria, 69% (38/55) were classified as Irritants and 11% (6/55) were classified as Not Labeled. The remaining 17% (11/64) could not be classified using the FHSA-20% criteria and are so noted in **Annex III**.

Ability to Distinguish Not Labeled Substances From Irritants

ICCVAM evaluated the ability of the HET-CAM test method to distinguish substances not labeled as irritants from irritants. Using this approach for the 63 substances classified according to the FHSA-20% criteria, the HET-CAM test method has an overall accuracy of 78% (49/63), a sensitivity of 91% (43/47), a specificity of 38% (6/16), a false positive rate of 63% (10/16), and a false negative rate of 9% (4/47) (**Table 6-17**).

Using this approach for the 55 substances classified according to the FHSA-67% criteria, the HET-CAM test method has an overall accuracy of 80% (44/55), a sensitivity of 97% (38/39), a specificity of 38% (6/16), a false positive rate of 63% (10/16), and a false negative rate of 3% (1/39) (**Table 6-18**).

As detailed below, the results from each individual study were evaluated separately.

Gettings et al. (1994): Based upon *in vivo* rabbit data using the FHSA-20% criteria (**Table 6-17**), 18 substances could be assigned a classification. Among these 18 substances, the HET-CAM test method has an accuracy of 44% (8/18), sensitivity of 50% (4/8), specificity of 40% (4/10), a false positive rate of 60% (6/10), and a false negative rate of 50% (4/8).

Based upon *in vivo* rabbit data using the FHSA-67% analysis method (**Table 6-18**), 15 substances could be assigned a classification. Among these 15 substances, the HET-CAM test method has an accuracy of 53% (8/15), sensitivity of 80% (4/5), specificity of 40% (4/10), a false positive rate of 60% (6/10), and a false negative rate of 20% (1/5).

Gettings et al. (1996): Based upon *in vivo* rabbit data using the FHSA-20% criteria (**Table 6-17**), 25 substances could be assigned a classification. Among these 25 substances, the HET-CAM test

method has an accuracy of 92% (23/25), sensitivity of 100% (21/21), specificity of 50% (2/4), a false positive rate of 50% (2/4), and a false negative rate of 0% (0/21).

Based upon *in vivo* rabbit data using the FHSA-67% criteria (**Table 6-18**), 23 substances could be assigned a classification. Among these 23 substances, the HET-CAM test method has an accuracy of 91% (21/23), sensitivity of 100% (19/19), specificity of 50% (2/4), a false positive rate of 50% (2/4), and a false negative rate of 0% (0/19).

Hagino et al. (1999): Based upon *in vivo* rabbit data using the FHSA-20% criteria (**Table 6-17**), 17 substances could be assigned a classification. Among these 17 substances, the HET-CAM test method has an accuracy of 88% (15/17), sensitivity of 100% (15/15), specificity of 0% (0/2), a false positive rate of 100% (2/2), and a false negative rate of 0% (0/15).

Based upon *in vivo* rabbit data using the FHSA-67% criteria (**Table 6-18**), 15 substances could be assigned a classification. Among these 15 substances, the HET-CAM test method has an accuracy of 87% (13/15), sensitivity of 100% (13/13), specificity of 0% (0/2), a false positive rate of 100% (2/2), and a false negative rate of 0% (0/13).

Performance of the HET-CAM Test Method with Discordant Classes Excluded

The previous ICCVAM BRD identified limitations of the HET-CAM test method based upon the false positive rate for alcohols and the false negative rates for surfactant-based formulations, many of which were oil/water emulsions when the HET-CAM is used to identify ocular corrosives and severe irritants (ICCVAM 2006a). For this reason, the performance of the HET-CAM test method in identifying FHSA irritants using the FHSA-20% criteria was evaluated with these substances excluded from the database. The overall accuracy and sensitivity improve with exclusion of all substances belonging to these discordant classes (**Table 6-19**). However, the number of available substances was reduced to ten with none classified as Not Labeled that precluded determination of specificity and the false positive rate when all of the discordant substances were removed.

Exclusion of oil/water emulsions improved performance with an increase in accuracy from 78% (49/63) to 91% (41/45) and decreased the false negative rate from 9% (4/47) to 0% (0/39) with only a 4% increase in the false positive rate (**Table 6-19**). Removal of alcohols did not affect performance significantly, but the false positive rate was reduced 21% when alcohols and oil/water emulsions were excluded while the false negative rate remained the same and accuracy increased 17%. Removal of surfactant formulations reduced accuracy to 68% (26/38) and marginally decreased sensitivity and specificity at the expense of an increase in the false negative rate from 9% (4/47) to 15% (4/26). The false negative rate increased further to 22% (4/18) if alcohols and surfactant formulations were excluded.

The four false negative substances identified using the FHSA-20% criteria overall (i.e., HZA, HZC, HZV, and HZW) are the same four substances identified as false negative substances using the EPA classification system (EPA 2003a) shown in **Table 6-10**.

The performance of the HET-CAM test method in identifying FHSA irritants using the FHSA-67% criteria also was evaluated with these substances excluded from the database. The overall accuracy and sensitivity improve with exclusion of all substances belonging to these discordant classes (**Table 6-20**). However, the number of available substances was reduced to nine with none classified as Not Labeled that precluded determination of specificity and the false positive rate when all of the discordant substances were removed.

Using the FHSA-67% criteria, the exclusion of oil/water emulsions improved performance with an increase in accuracy from 80% (44/55) to 90% (36/40) and decreased the false negative rate from 3% (1/39) to 0% (0/34) with only a 4% increase in the false positive rate (**Table 6-20**). Removal of alcohols did not affect performance significantly, but the false positive rate was reduced 21% when

alcohols and oil/water emulsions were excluded while the false negative rate remained the same and accuracy increased 15%. Removal of surfactant formulations reduced accuracy to 72% (23/32) and marginally decreased sensitivity and increased the false negative rate. The false negative rate increased further to 7% (1/14) if alcohols and surfactant formulations were excluded.

The false negative substance using the FHSA-67% criteria overall was HZW, one of the four false negative substances identified using the EPA classification system shown in **Table 6-10**.

Table 6-13 Accuracy of the HET-CAM Test Method (IS[A]) in Distinguishing Not Labeled Substances from All Other Irritant Classes, as Defined by the EU Classification System,[1] by Study and Overall

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Gettings et al. (1994)	18	50	9/18	100	1/1	47	8/17	53	9/17	0	0/1
Gettings et al. (1996)	24	79	19/24	100	17/17	29	2/7	61	5/7	0	0/17
Hagino et al. (1999)	15	53	8/15	100	8/8	0	0/7	100	7/7	0	0/8
Overall[2]	58	62	36/58	100	26/26	31	10/32	69	22/32	0	0/26

Abbreviations: EU = European Union; HET-CAM = hen's egg test – chorioallantoic membrane; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] EU classification system (EU 2001): Not Labeled vs. R41/R36.

[2] Overall data set includes one additional test substance from Bagley et al. (1992).

Table 6-14 Performance of the HET-CAM Test Method (IS[A]) in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EU Classification System,[1] with Discordant Chemical and Physical Classes Excluded

HET-CAM Database	Overall Correct Classification	Severe (R41)		Moderate (R36)			Mild			Not Labeled	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Overall	40% (23/58)	50% (12/24)	50% (12/24)	50% (1/2)	50% (1/2)	0% (0/2)	NA	NA	NA	69% (22/32)	31% (10/32)
Without Alcohols	42% (21/50)	45% (10/22)	55% (12/22)	50% (1/2)	50% (1/2)	0% (0/2)	NA	NA	NA	62% (16/26)	38% (10/26)
Without Surfactant Formulations	47% (16/34)	100% (8/8)	0% (0/8)	100% (1/1)	0% (0/1)	0% (0/1)	NA	NA	NA	68% (17/25)	32% (8/25)
Without Oil/Water Emulsions	35% (14/40)	48% (11/23)	52 (12/23)	50% (0/2)	50% (1/2)	0% (0/2)	NA	NA	NA	87% (13/15)	13% (2/15)
Without Alcohols and Surfactant Formulations	54% (14/26)	100% (6/6)	0% (0/6)	100% (0/1)	0% (0/1)	0% (0/1)	NA	NA	NA	58% (11/19)	42% (8/19)
Without Alcohols and Oil/Water Emulsions	37% (12/32)	43% (9/21)	57% (12/21)	50% (1/2)	50% (1/2)	0% (0/2)	NA	NA	NA	78% (7/9)	22% (2/9)
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions	62% (5/8)	100% (5/5)	0% (0/5)	100% (1/1)	0% (0/1)	0% (0/1)	NA	NA	NA	100% (2/2)	0% (0/2)

Abbreviations: EU = European Union; HET-CAM = hen's egg test–chorioallanotic membrane; NA = not applicable.

[1] EU classification system (EU 2001).

Table 6-15 Accuracy of the HET-CAM Test Method (IS[A]) in Distinguishing Not Labeled Substances from All Other Irritant Classes, as Defined by the EU Classification System,[1] with Discordant Chemical and Physical Classes Excluded

HET-CAM Database	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall	58	62	36/58	100	26/26	31	10/32	69	22/32	0	0/26
Without Alcohols	50	42	21/50	100	24/24	38	10/26	62	16/26	0	0/24
Without Surfactant Formulations	34	50	17/34	100	9/9	32	8/25	68	17/25	0	0/9
Without Oil/Water Emulsions	40	67	26/39	100	25/25	13	2/15	87	13/15	0	0/25
Without Alcohols and Surfactant Formulations	26	58	15/26	100	7/7	42	8/19	58	11/19	0	0/7
Without Alcohols and Oil/Water Emulsions	32	78	25/32	100	23/23	22	2/9	78	7/9	0	0/23
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions	8	75	6/8	100	6/6	0	0/2	100	2/2	0	0/6

Abbreviations: EU = European Union; HET-CAM = hen's egg test – chorioallantoic membrane; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] EU classification system (EU 2001): Not Labeled vs. R41/R36.

Table 6-16 Performance of the HET-CAM Test Method Using the EU Classification System[1] in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property

Category	N	Underprediction (*In Vivo/In Vitro*)			Overprediction (*In Vivo/In Vitro*)		
		Severe (R41)		Moderate (R36)	Moderate (R36)	Not Labeled (NL)	
		NL	R36	NL	R41	R36	R41
Overall	58	8% (2/24)	42% (10/24)	50% (1/2)	0% (0/2)	68% (15/22)	32% (7/22)
Chemical Class[2]							
Alcohol	8	0% (0/2)	0% (0/2)	50% (1/2)	0% (0/2)	33% (2/6)	50% (3/6)
Carboxylic Acid	5	0% (0/4)	25% (1/4)	-	-	0% (0/1)	100% (1/1)
Organic salt	2	0% (0/5)	20% (1/5)	100% (1/1)	0% (0/1)	-	-
Properties of Interest							
Liquids	58	8% (2/24)	42% (10/24)	50% (1/2)	50% (1/2)	16% (5/32)	25% (8/32)
Solids	0	-	-	-	-	-	-
Pesticide	0	-	-	-	-	-	-
Surfactant-Total	24	0% (0/16)	62% (12/16)	100% (1/1)	0% (0/1)	14% (1/7)	0% (0/7)
-nonionic	-	-	-	-	-	-	-
anionic	-	-	-	-	-	-	-
cationic	-	-	-	-	-	-	-

continued

Table 6-16 Performance of the HET-CAM Test Method Using the EU Classification System[1] in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property (continued)

Category	N	Underprediction (*In Vivo/In Vitro)*			Overprediction (*In Vivo/In Vitro)*		
		Severe (R41)		Moderate (R36)	Moderate (R36)	Not Labeled (NL)	
		NL	R36	NL	R41	R36	R41
Overall	58	8% (2/24)	42% (10/24)	50% (1/2)	0% (0/2)	68% (15/22)	32% (7/22)
Properties of Interest (continued)							
Oil/Water Emulsion	18	0% (0/1)	0% (0/1)	-	-	35% (6/17)	18% (3/17)
pH-Total	0	-	-	-	-	-	-
-acidic (pH <7.0)	-	-	-	-	-	-	-
-basic (pH >7.0)	-	-	-	-	-	-	-

Abbreviations: EU = European Union; HET-CAM = hen's egg test–chorioallantoic membrane; N = number of animals; NL = Not Labeled (as irritant).

[1] EU classification system (EU 2001).

[2] Chemical classes included in this table are represented by at least five substances tested in the HET-CAM test method, and assignments are based upon National Library of Medicine medical subject heading (MeSH) categories (www.nlm.nih.gov/mesh) as defined in **Annex I**.

Table 6-17 Accuracy of the HET-CAM Test Method (IS[A]) in Distinguishing Substances Not Labeled as Irritants from Irritants, as Defined by the FHSA-20% Classification System,[1] by Study and Overall

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Gettings et al. (1994)	18	44	8/18	50	4/8	40	4/10	60	6/10	50	4/8
Gettings et al. (1996)	25	92	23/25	100	21/21	50	2/4	50	2/4	0	0/21
Hagino et al. (1999)	17	88	15/17	100	15/15	0	0/2	100	2/2	0	0/15
Overall[2]	63	78	49/63	91	43/47	38	6/16	63	10/16	9	4/47

Abbreviations: FHSA = U.S. Federal Hazardous Substances Act; HET-CAM = hen's egg test–chorioallantoic membrane; N = number of classifiable substances; No. = number on which the percentage is calculated.

[1] FHSA classification system (16 CFR 1500.42): Irritant or Not Labeled as an Irritant. FHSA-20% analysis method is based on use of proportionality in which an irritant is identified by a positive response (i.e., CO or IR >1 and/or CR or CC≥2) in ≥1/3, 1/4, 1/5 or ≥2/6 animals (20 to 33% positive). Substances that do not produce a positive response in 3, 4, 5, or 6 animals or that produce a positive response in 1/6 animals are not classified as irritants, and therefore do not require labeling.

[2] Because Bagley et al. (1992) and Kojima et al. (1995) contain only one and two classifiable substances, respectively, data from these studies were included only in the overall analysis and were not evaluated separately.

Table 6-18 Accuracy of the HET-CAM Test Method in Distinguishing Substances Not Labeled as Irritants from Irritants, as Defined by the FHSA-67% Classification System,[1] by Study and Overall

Data Source	N[2]	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.[3]	%	No.	%	No.	%	No.	%	No.
Gettings et al. (1994)	15	53	8/15	80	4/5	40	4/10	60	6/10	20	1/5
Gettings et al. (1996)	23	91	21/23	100	19/19	50	2/4	50	2/4	0	0/19
Hagino et al. (1999)	15	87	13/15	100	13/13	0	0/2	100	2/2	0	0/13
Overall[2]	55	80	44/55	97	38/39	38	6/16	63	10/16	3	1/39

Abbreviations: FHSA = Federal Hazardous Substances Act; HET-CAM = hen's egg test–chorioallantoic membrane; N = number of substances included in this analysis; No. = number on which the percentage is calculated.

[1] FHSA classification system (16 CFR 1500.42): Irritant or not labeled. FHSA-67% analysis method is based on use of proportionality in which an irritant is identified by a positive response (i.e., CO or IR >1 and/or CR or CC≥2) in ≥2/3, 3/4, 4/5 or 4/6 animals (67% to 80% positive). Substances that do not produce a positive response in 3, 4, 5, or 6 animals or that produce a positive response in 1/6 animals are not classified as irritants, and therefore do not require labeling.

[2] Because Bagley et al. (1992) and Kojima et al. (1995) contain only one and two classifiable substances, respectively, data from these studies were included only in the overall analysis and were not evaluated separately. The FHSA-67% Inconclusive substances were not included in the calculations. One of these was from the Bagley et al. (1992) study; therefore, the overall correct classification values increase by two rather than by three substances.

Table 6-19 Accuracy of the HET-CAM Test Method (IS[A]) in Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes, as Defined by the FHSA-20% Classification System,[1] with Discordant Chemical and Physical Classes Excluded

HET-CAM Database	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall	63	78	49/63	91	43/47	38	6/16	63	10/16	9	4/47
Without Alcohols	53	77	41/53	90	35/39	43	6/14	57	8/14	10	4/39
Without Surfactant Formulations	38	68	26/38	85	22/26	33	4/12	67	8/12	15	4/26
Without Oil/Water Emulsions	45	91	41/45	100	39/39	33	2/6	67	4/6	0	0/39
Without Alcohols and Surfactant Formulations	28	64	18/28	78	14/18	40	4/10	60	6/10	22	4/18
Without Alcohols and Oil/Water Emulsions	35	94	33/35	100	31/31	50	2/4	50	2/4	0	0/31
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions	10	100	10/10	100	10/10	-[2]	-	-	-	0	0/10

Abbreviations: FHSA = U.S. Federal Hazardous Substances Act; HET-CAM = hen's egg test–chorioallantoic membrane; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] FHSA classification system (16 CFR 1500.42): Irritant or Not Labeled as an Irritant. FHSA-20% analysis method is based on use of proportionality in which an irritant is identified by a positive response (i.e., CO or IR >1 and/or CR or CC≥2) in ≥1/3, 1/4, 1/5 or ≥2/6 animals (20% to 33% positive). Substances that do not produce a positive response in 3, 4, 5, or 6 animals or that produce a positive response in 1/6 animals are not classified as irritants, and are therefore do not require labeling.

[2] No substances were classified as Not Labeled by FHSA or as nonirritants in HET-CAM, therefore specificity and the false positive rate could not be determined.

Table 6-20 Accuracy of the HET-CAM Test Method (IS[A]) in Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes, as Defined by the FHSA-67% Classification System,[1] with Discordant Chemical and Physical Classes Excluded

HET-CAM Database	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall	55	80	44/55	97	38/39	38	6/16	63	10/16	3	1/39
Without Alcohols	47	81	38/47	97	32/33	43	6/14	57	8/14	3	1/33
Without Surfactant Formulations	32	72	23/32	95	19/20	33	4/12	67	8/12	5	1/20
Without Oil/Water Emulsions	40	90	36/40	100	34/34	33	2/6	67	4/6	0	0/34
Without Alcohols and Surfactant Formulations	24	71	17/24	93	13/14	40	4/10	60	6/10	7	1/14
Without Alcohols and Oil/Water Emulsions	32	94	30/32	100	28/28	50	2/4	50	2/4	0	0/28
Without Alcohols, Surfactant Formulations, and Oil/Water Emulsions	9	100	9/9	100	9/9	-[2]	-	-	-	0	0/9

Abbreviations: FHSA = U.S. Federal Hazardous Substances Act; HET-CAM = hen's egg test–chorioallantoic membrane; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] FHSA classification system (16 CFR 1500.42): Irritant or not labeled. FHSA-67% analysis method is based on use of proportionality in which an irritant is identified by a positive response (i.e., CO or IR >1 and/or CR or CC≥2) in ≥2/3, 3/4, 4/5 or 4/6 animals (67% to 80% positive). Substances that do not produce a positive response in 3, 4, 5, or 6 animals or that produce a positive response in 1/6 animals are not classified as irritants, and are therefore do not require labeling.

[2] No substances were classified as Not Labeled by FHSA or as Nonirritants in HET-CAM; therefore, specificity and the false positive rate could not be determined.

7.0 HET-CAM Test Method Reliability

An assessment of test method reliability (intralaboratory repeatability and intra- and interlaboratory reproducibility) is essential to any evaluation of the performance of an alternative test method (ICCVAM 2003). Quantitative and qualitative evaluations of HET-CAM test method reliability have been conducted previously (ICCVAM 2006a). Because the database used for the current evaluation of the HET-CAM test method has not changed, the quantitative evaluation of test method reliability remains unchanged. However, additional qualitative analyses of test method reproducibility were conducted to evaluate the extent of agreement in HET-CAM hazard classifications among the laboratories. Given that the performance of the BCOP test method was similar for the EPA and FHSA hazard classification systems, additional reliability analyses were not conducted for the FHSA hazard classification system.

7.1 Interlaboratory Reproducibility of Hazard Classification Category Using the GHS Classification System

Fifteen of 17 substances tested had sufficient data to be classified using the GHS system (UN 2007). Of four Not Classified and three Category 2B substances, none was correctly identified by HET-CAM. None of the 15 GHS-classified substances tested was classified Category 2A by HET-CAM. However, eight substances classified as GHS Category 1 were correctly identified by the HET-CAM test method.

To evaluate the extent of agreement in irritant classifications among laboratories (i.e., Category 1, 2A, and 2B = + and Not Classified = -), regardless of the individual hazard classification, NICEATM compared *in vivo* and *in vitro* data (**Table 7-1**).

For 11 substances, there was 100% agreement between the *in vivo* and *in vitro* classifications (i.e., +/+). For four substances that were overpredicted *in vitro* (i.e., -/+), there was 100% agreement for 75% (3/4) of the substances and 80% agreement for 25% (1/4) of the substances. For two substances that could not be assigned GHS classifications, there was 100% agreement on the *in vitro* classifications (i.e., ?/+).

NICEATM could not assess the agreement between laboratories for substances not labeled as irritants compared to all other classes, because the HET-CAM test method did not produce any Not Classified classifications. Overall, however, there was 100% agreement for 94% (16/17) of the substances and 80% agreement for 6% (1/17) of the substances.[4]

The extent of agreement for a test substance was also evaluated among the five laboratories based on prediction of the individual GHS hazard category (**Table 7-2**). Of four Not Classified substances, all were overpredicted with 100% agreement by 75% (3/4) of the laboratories and 80% agreement by 25% (1/4) of the laboratories. All three Category 2B substances were overpredicted with 100% (3/3) agreement among the five laboratories. No Category 2A substances were identified.

All eight substances were correctly predicted as Category 1 with 100% agreement for 63% (5/8) of the substances, 80% agreement for 13% (1/8) of the substances, and 60% agreement for 25% (2/8) of the substances.

[4] Because the database of HET-CAM test method results has not changed since the 2006 ICCVAM BRD, the qualitative evaluation of reproducibility is not repeated here.

Table 7-1 Interlaboratory Variability of Hagino et al. (1999) Compared to the *In Vivo* Rabbit Eye Test in Predicting Ocular Hazard Categories for Severe Irritants or Corrosives (1) from Nonsevere Irritants (2A, 2B) and Substances Not Classified, as Defined by the GHS Classification System[1]

Report	Analysis Method[2]	Classification (*In Vivo*/*In Vitro*)[3]	# of Labs	N	Substances with 100% Agreement among Labs[4]	Substances with 80% Agreement among Labs[4]
Hagino et al. (1999)	IS(A)	+/+	5	11	11 (100%)	0
		+/-	5	0	0	0
		-/+	5	4	3 (75%)	1 (25%)
		-/-	5	0	0	0
		?/-	5	0	0	0
		?/+	5	2	2 (100%)	0
		Total	5	17	16 (94%)	1 (6%)

Abbreviations: GHS = Globally Harmonized System; N = number of substances.

[1] GHS classification system (UN 2007).

[2] Analysis method used to transform the sample data into HET-CAM scores. IS(A) = method described in Luepke (1985); IS(B)-10 and IS(B)-100 = method described in Kalweit et al. (1987).

[3] A "+" indicates that the substance was assigned an overall classification of corrosive or a severe irritant (Category 1). A "-" indicates that the substance was assigned an overall classification of nonsevere irritant (Category 2A or 2B) or Not Classified. A "?" indicates that, due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects; insufficient dose volume), a GHS classification could not be made. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro.*

[4] Number in parentheses indicates percentage of tested chemicals.

None of the eight Category 1 substances was incorrectly identified. However, all four Not Classified substances and the three Category 2B substances, 4/4 (100%) and 3/3 (100%), respectively, were incorrectly identified (**Table 7-2**).

There was no agreement among the five participating laboratories in incorrect classification of 0/8 (0%) of the GHS Category 1 substances. All were correctly classified. There was 100% agreement in overclassifying 100% (3/3) of the GHS Category 2B substances, 100% agreement in overclassifying 75% (3/4) of the substances, and 80% agreement in overclassifying 25% (1/4) of the Not Classified substances (**Table 7-2**).

7.2 Interlaboratory Reproducibility of Hazard Classification Category Using the EPA Classification System

Fifteen of 17 substances tested had sufficient data to be classified using the EPA system (EPA 2003a). Of two Category IV, five Category III, and one Category II substances, none (0% [0/2], 0% [0/5], and 0% [0/1], respectively) was correctly identified by the HET-CAM test method. However, seven substances classified as EPA Category I were correctly identified by HET-CAM (100% [7/7]).

To evaluate the extent of agreement in irritant classifications among laboratories (i.e., Category 1, 2A, and 2B = + and Not Labeled = -), regardless of the individual hazard classification, NICEATM compared *in vivo* and *in vitro* data (**Table 7-3**).

For 13 substances, there was 100% agreement among the *in vivo* and *in vitro* classifications (i.e., +/+). There was 60% agreement for both (100% [2/2]) of the substances that were overpredicted *in vitro* (i.e., -/+). For two substances that could not be assigned an EPA classification, there was 100% agreement on the *in vitro* classifications (i.e., ?/+) for 50% (1/2) of the substances and 60% agreement for 50% (1/2) of the substances.

NICEATM could not assess the agreement between laboratories for substances not labeled as irritants compared to all other classes, because the HET-CAM test method did not produce any Not Labeled classifications. Overall, however, there was 100% agreement for 82% (14/17) of the substances and 60% agreement for 18% (3/17) of the substances.[5]

The extent of agreement for a test substance was also evaluated among the five laboratories based on prediction of the individual EPA hazard category (**Table 7-4**). Both Category IV substances were overpredicted with 100% agreement by 50% (1/2) of the laboratories and with 80% agreement by 50% (1/2) of the laboratories. All five Category III substances were overpredicted with 100% agreement among the five laboratories. One Category II substance was overpredicted with 100% agreement among the five laboratories. All seven substances were correctly predicted as Category I substances with 100% agreement for 71% (5/7) of the substances and 80% agreement for 29% (2/7) of the substances.

None of the seven Category 1 substances was incorrectly identified. However, both Category IV, all five Category III, and the one Category II substance (i.e., 100% [2/2], 100% [5/5], and 100%, respectively) were incorrectly identified by the HET-CAM test method (**Table 7-4**).

There was no agreement among the five participating laboratories in incorrectly classifying any (0% [0/7]) of the EPA Category I substances. All were correctly classified. There was 100% agreement in overclassifying 50% (1/2) and 80% agreement in overclassifying 50% (1/2) of the EPA Category IV substances. For Category III substances, there was 100% agreement in overclassifying 5/5 substances. There was 100% agreement in overclassifying the Category II substance.

---

[5] Because the database of HET-CAM test method results has not changed since the 2006 ICCVAM BRD (2006a), the qualitative evaluation of reproducibility is not repeated here.

Table 7-2 Interlaboratory Variability of Hagino et al. (1999) Compared to the *In Vivo* Rabbit Eye Test in Predicting Each Ocular Hazard Category (1, 2A, 2B) and Substances Not Classified, as Defined by the GHS Classification System[1]

***In Vivo* Classification (No.)[2]**	***In Vitro* Classification**	**N**	**# of Labs**	**Substances with 100% Agreement Among Labs**	**Substances with 80% Agreement Among Labs**	**Substances with 60% Agreement Among Labs**
Not Classified (4)	Actual	0	5	0	0	0
	Over	4	5	3 (75%)	1 (25%)	0
Category 2B (3)	Under	0	5	0	0	0
	Actual	0	5	0	0	0
	Over	3	5	3 (100%)	0	0
Category 2A (0)	Under	0	5	0	0	0
	Actual	0	5	0	0	0
	Over	0	5	0	0	0
Category 1 (8)	Under	0	5	0	0	0
	Actual	8	5	5 (63%)	1 (13%)	2 (25%)

Abbreviations: GHS = Globally Harmonized System; N = number of substances; No. = number of substances classified.

[1] GHS classification system (UN 2007).

[2] Due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects), a GHS classification could not be made for two substances. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro.*

Table 7-3 Interlaboratory Variability of Hagino et al. (1999) Compared to the *In Vivo* Rabbit Eye Test in Predicting Ocular Hazard Category I (Severe Irritants or Corrosives) from Nonsevere Irritants (Category II, III) and Substances Not Labeled (Category IV), as Defined by the EPA Classification System[1]

Report	Analysis Method[2]	Classification (*In Vivo*/*In Vitro*)[3]	# of Labs	N	Substances with 100% Agreement Among Labs[4]	Substances with 60% Agreement Among Labs[4]
Hagino et al. (1999)	IS(A)	+/+	5	13	13 (100%)	0
		+/-	5	0	0	0
		-/+	5	2	0	2 (100%)
		-/-	5	0	0	0
		?/-	5	0	0	0
		?/+	5	2	1 (50%)	1 (50%)
		Total	5	17	14 (82%)	3 (18%)

Abbreviations: EPA = U.S. Environmental Protection Agency; N = number of substances.

[1] EPA classification system (EPA 2003a).

[2] Analysis method used to transform the sample data into HET-CAM scores. IS(A) = method described in Luepke (1985); IS(B)-10 and IS(B)-100 = method described in Kalweit et al. (1987).

[3] A "+" indicates that the substance was assigned an overall classification of corrosive or a severe irritant (Category 1). A "-" indicates that the substance was assigned an overall classification of nonsevere irritant (Category 2A or 2B) or Not Labeled. A "?" indicates that, due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects; insufficient dose volume), a GHS classification could not be made. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro.*

[4] Number in parentheses indicates percentage of tested chemicals.

Table 7-4 Interlaboratory Variability of Hagino et al. (1999) Compared to the *In Vivo* Rabbit Eye Test in Predicting Each Ocular Hazard Category for Severe Irritants or Corrosives (I), Irritants (II, III), and Substances Not Labeled (Category IV), as Defined by the EPA Classification System[1]

In Vivo Classification (No.)[2]	*In Vitro* Classification	# of Labs	N	Substances with 100% Agreement Among Labs	Substances with 80% Agreement Among Labs
Category IV (2)	Actual	5	0	0	0
	Over	5	2	1 (50%)	1 (50%)
Category III (5)	Under	5	0	0	0
	Actual	5	0	0	0
	Over	5	5	5 (100%)	0
Category II (1)	Under	5	0	0	0
	Actual	5	0	0	0
	Over	5	1	1 (100%)	0
Category 1 (7)	Under	5	0	0	0
	Actual	5	7	5 (71%)	2 (29%)

Abbreviations: EPA = U.S. Environmental Protection Agency; N = number of substances; No. = number of substances classified.

[1] EPA classification system (EPA 2003a).

[2] Due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects), an EPA classification could not be made for two substances. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro.*

7.3 Interlaboratory Reproducibility of Hazard Classification Category Using the EU Classification System

Fifteen of 17 substances tested had sufficient data to be classified using the EU system (EU 2001). Of seven Not Labeled and one R36 substances, none (0% [0/7] and 0% [0/1], respectively) were correctly identified by HET-CAM. However, all seven substances classified as EU R41 were correctly identified by the HET-CAM test method (100%).

To evaluate the extent of agreement in irritant classifications among laboratories (i.e., Category 1, 2A, and 2B = + and Not Labeled = -), regardless of the individual hazard classification, NICEATM compared *in vivo* and *in vitro* data (**Table 7-5**).

For eight substances, there was 100% agreement among the *in vivo* and *in vitro* classifications for 63% (5/8), 80% agreement for 25% (2/8), and 60% agreement for 13% (1/8). For seven substances that were overpredicted *in vitro* (i.e., -/+), there was 100% agreement for 86% (6/7) and 80% agreement for 14% (1/7) of the substances. There was 100% agreement on the *in vitro* classification (i.e.,?/+) of both substances that could not be assigned an EU classification.

NICEATM could not assess the agreement between laboratories for substances not labeled as irritants compared to all other classes, because the HET-CAM test method did not produce any Not Labeled classifications.

The extent of agreement for a test substance was also evaluated among the five laboratories based on prediction of the individual EU hazard category (**Table 7-6**).

All seven Not Labeled substances were overpredicted with 100% agreement by 86% (6/7) of the laboratories and with 80% agreement by 14% (1/7) of the laboratories.

The one R36 substance was overpredicted with 100% agreement among the five laboratories.

Seven R41 substances were overpredicted with 100% agreement among the five laboratories for 71% (5/7), 80% agreement for 14% (1/7), and 60% agreement for 14% (1/7) of the substances.

None of the seven R41 substances was incorrectly identified. However, all seven Not Labeled, one Category R36, and seven R41 substances (i.e., 100% [7/7], 100% [1/1], and 100% [7/7], respectively) were incorrectly identified by HET-CAM (**Table 7-6**).

There was no agreement among the five participating laboratories in incorrectly classifying any (0/7) of the EU R41 substances; all were correctly classified. There was 100% agreement in overclassifying 86% (6/7) and 80% agreement in overclassifying 14% (1/7) of the EPA substances not labeled as irritants. For R36 substances, there was 100% agreement in overclassifying 1/1 substance.

7.4 Common Chemical or Product Classes Among Test Substances with Discordant Interlaboratory Results Using the GHS Classification System

There were insufficient data with which to determine the effect of discordant chemicals on the interlaboratory analyses.

Table 7-5 Interlaboratory Variability of Hagino et al. (1999) Compared to the *In Vivo* Rabbit Eye Test in Predicting Ocular Hazard Categories for Severe Irritants or Corrosives (R41) from Irritants (R36) and Substances Not Labeled, as Defined by the EU Classification System[1]

Report	Analysis Method[2]	Classification (*In Vivo*/*In Vitro*)[3]	# of Labs	N	Substances with 100% Agreement Among Labs	Substances with 80% Agreement Among Labs	Substances with 60% Agreement Among Labs
Hagino et al. (1999)	IS(A)	+/+	5	8	5 (63%)	2 (25%)	1 (13%)
		+/-	5	0	0	0	0
		-/+	5	7	6 (86%)	1 (14%)	0
		-/-	5	0	0	0	0
		?/-	5	0	0	0	0
		?/+	5	2	2 (100%)	0	0
		Total	5	17	13 (76%)	3 (18%)	1 (6%)

Abbreviations: EU = European Union; N = number of substances.

[1] EU classification system (2001).

[2] Analysis method used to transform the sample data into HET-CAM scores. IS(A) = method described in Luepke (1985); IS(B)-10 and IS(B)-100 = method described in Kalweit et al. (1987).

[3] A "+" indicates that the substance was assigned an overall classification of severe irritant or corrosive (R41). A "-" indicates that the substance was assigned an overall classification of nonsevere irritant (R36) or Not Labeled. A "?" indicates that, due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects; insufficient dose volume), an EU classification could not be made. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro.*

Table 7-6 Interlaboratory Variability of Hagino et al. (1999) Compared to the *In Vivo* Rabbit Eye Test in Predicting Each Ocular Hazard Category for Severe Irritants or Corrosives (R41), Irritants (R36), and Substances Not Labeled, as Defined by the EU Classification System[1]

In Vivo Classification (No.)[2]	Classification *(In Vitro)*	# of Labs	N	Substances with 100% Agreement Among Labs	Substances with 80% Agreement Among Labs	Substances with 60% Agreement Among Labs
NL (7)	Actual	5	0	0	0	0
	Over	5	7	6 (86%)	1 (14%)	0
R36 (1)	Under	5	0	0	0	0
	Actual	5	0	0	0	0
	Over	5	1	1 (100%)	0	0
R41 (7)	Under	5	0	0	0	0
	Actual	5[2]	7	5 (71%)	1 (14%)	1 (14%)

Abbreviations: EU = European Union; N = number of substances; NL = Not Labeled (as irritant); No. = number of substances classified.

[1] EU classification system (2001).

[2] Due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects), an EU classification could not be made for two substances. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro.*

8.0 Test Method Data Quality

The same database was used in this assessment and the 2006 ICCVAM *Background Review Document: Current Status of* In Vitro *Test Methods for Identifying Ocular Corrosives and Severe Irritants: Hen's Egg Test – Chorioallantoic Membrane*, in which test method data quality is evaluated (ICCVAM 2006a).

9.0 Other Scientific Reports and Reviews

NICEATM obtained two studies that were not discussed in the 2006 BRD (ICCVAM 2006a) but that contain HET-CAM data: de Silva et al. (1992) and Boue-Grabot et al. (1995).

De Silva et al. (1992) presented the results of a HET-CAM study of 60 chemicals and 41 cosmetic formulations. The chemicals were tested at 10% of their *in vivo* test concentration, whereas the cosmetic formulations were tested neat. The researchers used the test method of Luepke (1985) with a fixed time point IS(A) analysis method (i.e., 0.5, 2, and 5 minutes). Intralaboratory reproducibility was evaluated using a double-blind study of 20 surfactants tested at concentrations of 1% and 10%. Spearman's coefficient rho was greater than 0.9 ($p < 10^{-8}$) for the two concentrations. For the 60 chemicals, HET-CAM scores (i.e., maximum score of 21) were correlated with three EEC ocular irritation classes (i.e., Class I = Not Labeled, Class II = R36, and Class III = R41). Class I substances were clearly distinguished from Class II substances. Sensitivity, specificity, and concordance were 91%, 88%, and 90%, respectively, when an IS(A) score of 9 was used to differentiate Class I from Class II substances.

In de Silva et al., the false positive rate was 5% (3/60), and the false negative rate was 5% (3/60). The false negative substances were one Class II or severe irritant (acetaldehyde) and two nonsevere irritants (n-butanol and a nonionic surfactant). The HET-CAM scores for 21 formulations (i.e., make-up removers, shower gels, and shampoos) studied without rinsing, and 20 formulations (i.e., creams and body milks) washed off after a 20-second contact were compared to Draize MAS values, resulting in Spearman rank correlation coefficients of rho = 0.77 ($p < 10^{-2}$) and rho = 0.76 ($p < 10^{-2}$), respectively. The authors suggest that the HET-CAM test method, with optimization, is potentially useful in a battery of *in vitro* test methods for the screening of new ingredients and formulations. These data were not used in the HET-CAM performance analyses in this BRD because original Draize data were not available to derive regulatory classifications based on the current EPA, GHS, and EU classification systems (EPA 2003a; UN 2007; EU 2001).

In Boue-Grabot (1995), 103 cosmetics and toiletries were tested in the HET-CAM test method using the fixed time point method (i.e., 0, 0.5, 2 and 5 minutes) of Luepke (1985). In this method, the CAM is observed for the appearance of vasodilation, hemorrhage, or coagulation at each time point, and numerical scores are assigned. The IS was converted to a mean chorioallantoic irritation index (MCA), and the HET-CAM results (i.e., nonirritant, slightly irritant, moderately irritant, or very irritant) were compared to the Draize test using the maximal ocular irritation index (IOMA) with an identical irritation classification scheme. Results were expressed in terms of correlation ($r = 0.657$, $p < 0.001$) between the MCA and IOMA values. Accuracy was 92%, sensitivity was 80%, specificity was 94%, the false negative rate was 2%, and the false positive rate was 6%. A cytotoxicity test was used to further reduce the false positive and false negative rates. No individual HET-CAM or Draize data were provided in this study, so the data could not be used in the performance analysis.

NICEATM found five additional studies containing HET-CAM data in the peer-reviewed literature from 2005 to 2009 (Dahl 2007; Debbasch et al. 2005; Mancebo et al. 2008; Mehling et al. 2007; Vinardell and Mitjans 2006). From these studies, seven test substances were identified with *in vitro* scores and *in vivo* data using the Draize rabbit eye test. However, the Draize rabbit eye test data and

HET-CAM results for all seven test substances were included in the accuracy analyses reported in the ICCVAM BRD (2006a). Consequently, they have already been considered in the current evaluation.

Getttings et al. (1996b) used the original Draize data and new low volume eye test (LVET) data to evaluate new *in vitro* test method data, including HET-CAM using the IS(A) and IS(B) analysis methods, on 10 hydroalcoholic formulations that were originally published in Gettings et al. (1991). The authors suggest that the performance of the *in vitro* test methods, including HET-CAM, conformed no better (or worse) with the LVET than with the Draize test method. No individual animal data were provided to enable regulatory classification. Therefore, these data were not used in the current HET-CAM performance analyses.

In Debbasch et al. (2005), 12 coded make-up removers were applied to the external eyelid and tested in the HET-CAM, BCOP, and the corneal epithelial cell line (CEPI) test methods, as well as a clinical in-use test under ophthalmological control. Three hundred microliters of undiluted test product was applied to the CAM of 9-day-old fertilized eggs (White Leghorn chicken, four per product). Corneal opacity was determined using an adapted spectrophotometer and barrier disruption by fluorescein uptake using OD_{490} nm. *In vitro* scores were classified according to Gautheron et al. (1994) and Harbell and Curren (1998). However, no *in vivo* rabbit eye data were reported, and these data have not been obtained. For this reason, the results from this study were not included in the HET-CAM performance analyses detailed in this BRD.

In Vinardell and Mitjans (2006), several industrial and laboratory solvents were tested for potential eye irritation using the HET-CAM test method. The test substances were applied on the membrane of fertile eggs (Leghorn SA31, six per solvent) in a constant volume of 0.3 mL at 37ºC. The membrane, blood vessels, and albumen were examined for 5 minutes. The time of appearance, in seconds, of each irritant effect was recorded. No *in vivo* rabbit reference data were reported, but the Draize rabbit eye test data and HET-CAM results for 7/9 of these substances were included in the accuracy analyses reported in the ICCVAM BRD (2006a). Consequently, they have in turn already been considered in the current evaluation.

In Dahl (2007), 27 dental adhesive products in a total of 36 solutions based on four adhesive concepts (i.e., self-etch 1 step, self-etch 2 step, etch and rinse 2 steps, or etch and rinse 3 steps) were evaluated in the HET-CAM test method. The potential of dental adhesives to evoke irritation relevant to the biocompatibility of dental adhesives with regard to pulpal and mucous membrane exposure was assessed. An IS was obtained over a 5-minute observation period based on the time of first appearance of hemorrhage, vascular lysis, or coagulation in the chorioallantoic membrane. Substances were applied in a volume of 0.3 mL (n=3 eggs in two experiments). Products were classified based on conversion of the HET-CAM IS to a mean irritation score (i.e., nonirritant, slight irritant, moderate irritant, or strong irritant). Sixteen solutions were identified as strong irritants and found among all adhesive concept groups except the newest, self-etch 1 step. However, all substances in the self-etch 1 step group were classified as moderate irritants with IS scores close to those of a strong irritant. The results suggested that dental adhesives have the potential to cause an irritant reaction if exposed to oral mucosa. This HET-CAM data could not be used in the BRD performance analysis because no corresponding Draize data were provided.

Mehling et al. (2007) tested 18 proprietary surfactants using the red blood cell test, HET-CAM, and the SkinEthic™ ocular tissue model. Following the standard operating procedure of the Colipa project (INVITTOX Protocol No. 96), 300 microliters of test solution diluted in water were applied to the exposed CAM. The intensity of the subsequent reactions (i.e., hemorrhage, lysis, and coagulation) was semiquantitatively assessed on a scale of 0 to 3. No *in vivo* rabbit reference data were reported in this study; therefore, it was not included in the HET-CAM performance analysis detailed in this BRD.

In Mancebo et al. (2008), 14 proprietary formulations generally used in agriculture were tested in acute dermal toxicity and in eye irritation/corrosion tests. Three substances were tested using the

HET-CAM method and the acute eye irritation/corrosion test. Three hundred microliters of each test substance was applied to the CAM of fertile eggs (Lohman, six per substance) and observed for 5 minutes. The three endpoints for this study were hemorrhage, vessel lyses, and coagulation. Although mean *in vivo* rabbit eye data and corresponding irritation levels and HET-CAM IS values were reported in the study, the original animal data were not provided. Thus the study was not included in the HET-CAM performance analyses detailed in this BRD.

Several other studies on HET-CAM were reported. For example, Budai et al. (2004) tested three pesticide formulations in the HET-CAM test method using the IS(B) analysis method, but only qualitative results and no corresponding Draize data were provided. Tavaszi and Budai (2006) provided IS(B) scores for HET-CAM data but no corresponding Draize data on six agrochemical pesticides. Tavaszi and Budai (2007) reported HET-CAM data on six additional agrochemical formulations using the IS(B) analysis method and converted the scores to qualitative irritation indices that were compared to qualitative Draize results based on the maximum mean total score (MMTS). This data could not be used for regulatory classification and was not included in the performance analyses. Tavaszi et al. (2008) performed similar analyses on six additional agrochemical formulations.

10.0 How the HET-CAM Test Method Will Refine, Reduce, or Replace Animal Use

ICCVAM promotes the scientific validation and regulatory acceptance of new methods that refine, reduce, or replace animal use where scientifically feasible. Refinement, reduction, and replacement are known as the "three Rs" of animal protection. These principles of humane treatment of laboratory animals are described as:

- Refining experimental procedures such that animal suffering is minimized
- Reducing animal use through improved science and experimental design
- Replacing animal models with non-animal procedures (e.g., *in vitro* technologies), where possible (Russell and Burch 1992)

The HET-CAM test method has the potential to refine and reduce animal use in eye irritation testing. The HET-CAM test method would refine animal use by the *in vitro* identification of ocular corrosives and severe irritants, nonsevere irritants, or substances not labeled as irritants when used in a tiered-testing scheme. Substances identified as corrosives or severe irritants would be excluded from *in vivo* testing. Furthermore, the ability to identify mild and moderate ocular irritants would eliminate the need for *in vivo* testing, thus sparing rabbits from the pain associated with these types of substances. The HET-CAM test method can also reduce animal use because the test method does not use live animals. Use of the HET-CAM test method in lieu of one that uses live animals or animals used as a food source (e.g., BCOP, ICE, IRE) would further reduce the number of animals in a tiered-testing strategy.

10.1 Requirement for the Use of Animals

The HET-CAM test method has been designed so as not to require the use of animals. International regulations provide for the protection of animals used for experimental or other scientific purposes. For test methods using an animal embryo or fetus, some provisions indicate when an animal embryo or fetus is considered an animal and is therefore protected by the regulations. According to some of these regulations, a bird is considered a protected animal (thus the test is considered an *in vivo* and not *in vitro* test) when more than half of the gestation or incubation period has elapsed (Day 10.5 of the 21-day incubation period for a chicken embryo) (Animals [Scientific Procedures] Act 1986; EU 1986). The Public Health Service Policy, with which all National Institutes of Health (NIH)-funded research projects must comply, applies to all live vertebrate species. The NIH Office of Laboratory

Animal Welfare has provided written guidance in this area, interpreting “live vertebrate animal” to apply to avians (e.g., chick embryos) only after hatching (Kulpa-Eddy J, personal communication; NIH 2000).

It has been proposed that at incubation Day 9, the embryonic differentiation of the chicken central nervous system is sufficiently incomplete that suffering from pain perception is unlikely to occur (MSPCA 2005; Liebsch M, personal communication). Evaluations suggest that there are few sensory fibers present at Day 9 in the avian embryo and that significant development of the sensory nerve ending occurs between incubation Days 11 and 14 (Romanoff 1960). Studies also have suggested that the extraembryonal vascular systems (e.g., yolk sac, CAM) are not sensitive to pain (Rosenbruch 1997; Spielmann H, personal communication). Combined, these studies suggest that at incubation Day 9 the developing embryo perceives little or no pain during the conduct of the HET-CAM test method.

11.0 Practical Considerations

Practical considerations for the HET-CAM test method are detailed in the *Background Review Document: Current Status of* In Vitro *Test Methods for Identifying Ocular Corrosives and Severe Irritants: Hen’s Egg Test – Chorioallantoic Membrane Test Method* (ICCVAM 2006a).

12.0 References

Animals (Scientific Procedures) Act 1986. 1986. Chapter 14.

Bagley D, Bruner L, DeSilva O, Cottin M, O'Brien K, Uttley M, et al. 1992. An evaluation of five potential alternatives *in vitro* to the rabbit eye irritation test *in vivo*. Toxic In Vitro 6:275-284.

Balls M, Botham P, Bruner L, Spielmann H. 1995. The EC/HO international validation study on alternatives to the Draize eye irritation test. Toxic In Vitro 9:871-929.

Boue-Grabot M, Bernardin G, Chaumond S, Pinon JF. 1995. Alternative methods: hen's egg chorioallantoic membrane and in vitro cytotoxicity - a complementary approach. Intl J Cosmetic Science 17:207-215.

Budai P, Varnagy L, Fejes S, Somlyay IM, Linczmayer K, Pongracz A. 2004. Irritative effects of some pesticides and a technical component on tissue structure of the chorioallantoic membrane. Comm App Biol Sci 69:807-809.

CEC. 1991. Collaborative Study on the Evaluation of Alternative Methods to the Eye Irritation Test. Doc. XI/632/91/V/E/1/131/91 Parts I and II.

CPSC. 2003. Commercial practices. Hazardous Substances and Articles; Administration and Enforcement Regulations. 16 CFR 1500.42.

Dahl JE. 2007. Potential of dental adhesives to induce mucosal irritation evaluated by the HET-CAM method. Acta Odontologica Scandinavica 65:275-283.

De Silva O, Rougier A, Dossou KG. 1992. The HET-CAM test: a study of the irritation potential of chemicals and formulations. Altern Lab Anim 20:432-437.

Debbasch C, Ebenhahn C, Dami N, Pericoi M, Van den Berghe C, Cottin M, et al. 2005. Eye irritation of low-irritant cosmetic formulations: correlation of *in vitro* results with clinical data and product comparison. Food Chem Toxicol 43:155-165.

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.

EEC. 1984. Commission Directive 84/449/EEC of 25 April 1984 adapting to technical progress for the sixth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (Acute Toxicity–Eye Irritation. Annex V, Part B.5). Official Journal of the European Communities L251:109.

EEC. 1991. Commission Directive 91/325/EEC of 1 March 1991 adapting to technical progress for the twelfth 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 L180:52–56.

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

EPA. 2003b. Good Laboratory Practice Standards. Toxic Substances Control Act. 40 CFR 792.

EPA. 2003c. Good Laboratory Practice Standards. Federal Insecticide, Fungicide, and Rodenticide Act. 40 CFR 160.

EU. 1986. Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Official Journal of the European Communities L358:1-28.

EU. 1992. Commission Directive 92/69/EEC of 31 July 1992 adapting to technical progress for the seventeenth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L383:113-115.

EU. 1993. Commission Directive 93/21/EEC of 27 April 1993 adapting to technical progress for the 18th time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L110A:20–21.

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 L225:1-333.

EU. 2004. Manual of Decisions for Implementation of the Sixth and Seventh Amendments to Directive 67/548/EEC on Dangerous Substances (Directives 79/831/EEC and 92/32/EEC). European Commission, Joint Research Centre. Report n. EUR 20519 EN, updated version of July 2004. Available: http://ecb.jrc.it/new-chemicals [accessed 14 October 2004].

FDA. 2003. Good Laboratory Practice for Nonclinical Laboratory Studies. 21 CFR 58.

Federal Hazardous Substances Act (FHSA). 2005. Public Law 86-613.

Gautheron P, Giroux J, Cottin M, Audegond L, Morilla A, Mayordomo-Blanco L, et al. 1994. Interlaboratory assessment of the bovine corneal opacity and permeability (BCOP) assay. Toxicol In Vitro 8:381-392.

Gettings S, Dipasquale L, Bagley D, Casterton P, Chudkowski M, Curren R, et al. 1994. The CTFA evaluation of alternatives program: an evaluation of *in vitro* alternatives to the Draize primary eye irritation test. (Phase II) oil/water emulsions. Food Chem Toxicol 32:943-976.

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 Toxicol 34:79-117.

Gettings S, Teal J, Bagley D, Demetrulias J, DiPasquale L, Hintze K, et al. 1991. The CTFA evaluation of alternatives program: an evaluation of *in vitro* alternatives to the Draize primary eye irritation test. (Phase 1) hydro-alcoholic formulations; (Part 2) data analysis and biological significance. In Vitro Toxicol 4:247-288.

Gilleron L, Coecke S, Sysmans M, Hansen E, van Oproy S, Marzin D, et al. 1996. Evaluation of a modified HET-CAM assay as a screening test for eye irritancy. Toxicol In Vitro 10:431-446.

Gilleron L, Coecke S, Sysmans M, Hansen E, van Oproy S, Marzin D, et al. 1997. Evaluation of the HET-CAM-TSA method as an alternative to the Draize eye irritation test. Toxicol In Vitro 11:641-644.

Hagino S, Kinoshita S, Tani N, Nakamura T, Ono N, Konishi K, et al. 1999. Interlaboratory validation of *in vitro* eye irritation tests for cosmetic ingredients. (2) chorioallantoic membrane (CAM) test. Toxicol In Vitro 13:99-113.

Harbell J, Curren RD. 1998. The bovine corneal opacity and permeability assay: observations on assay performance. In Vitro Mol Toxicol 11:337–341.

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

ICCVAM. 2006a. Background Review Document: Current Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants: Hen's Egg Test – Chorioallantoic Membrane Test Method. NIH Publication No. 06-4515. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

ICCVAM. 2006b. ICCVAM Test Method Evaluation Report: *In Vitro* Ocular Toxicity Methods for Identifying Severe Irritants and Corrosives. NIH Publication No. 07-4517. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

ICCVAM Authorization Act of 2000. 2000. Public Law 106-545. Available: http://iccvam.niehs.nih.gov/about/overview.htm. [accessed 21 October 2004].

Kalweit S, Besoke R, Gerner I, Spielmann H. 1990. A national validation project of alternative methods to the Draize rabbit eye test. Toxicol In Vitro 4:702-706.

Kalweit S, Gerner I, Spielmann H. 1987. Validation project of alternatives for the Draize eye test. Mol Toxicol 1:597-603.

Kojima H, Hanamura A, Miyamoto S, Sato A, Konishi H, Yoshimura I. 1995. Evaluation of seven alternative assays on the main ingredients in cosmetics as predictors of Draize eye irritation scores. Toxicol In Vitro 9:333-340.

Luepke N. 1985. Hen's egg chorioallantoic membrane test for irritation potential. Food Chem Toxicol 23:287-291.

Mancebo A, Hernandez O, Gonzalez Y, Aldana L, Carballo O. 2008. Assessment of skin and eye irritation of 14 products under the stepwise approach of the OECD. Cutan Ocul Toxicol 27:173-185.

Mehling A, Kleber M, Hensen H. 2007. Comparative studies on the ocular and dermal irritation potential of surfactants. Food Chem Toxicol 45:747-758.

Massachusetts Society for the Prevention of Cruelty to Animals (MSPCA). 2005. Animals in Education: Laws and Regulations. Available: http//www.mspca.org.

NIH. 2000. The Public Health Service Responds to Commonly Asked Questions. Available: http://grants.nih.gov.

OECD. 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring Number 1: OECD Principles on Good Laboratory Practice (as revised in 1997). ENV/MC/CHEM(98)17. Paris:Organisation for Economic Co-operation and Development.

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

Romanoff AL. 1960. The Avian Embryo: Structural and Functional Development. New York:The Macmillan Company.

Rosenbruch M. 1997. The sensitivity of chicken embryos in incubated eggs. ALTEX 14:111-113.

Russell WMS, Burch RL. 1992. The Principles of Humane Experimental Technique. 14th ed. South Mimms, Potters Bar, Herts, England:Universities Federation for Animal Welfare.

Spielmann H, Liebsch M, Kalweit S, Moldenhauer F, Wirnsberger T, Holzhütter H, et al. 1996. Results of a validation study in Germany on two *in vitro* alternatives to the Draize eye irritation test, HET-CAM test and the 3T3 NRU cytotoxicity test. Altern Lab Anim 24:741-858.

Tavaszi J, Budai P. 2006. Toxicity study of agrochemicals on chorioallantoic membrane of the egg. Comm App Biol Sci 71:101-105.

Tavaszi J, Budai P. 2007. The use of HET-CAM test in detecting the ocular irritation. Comm App Biol Sci 72:137-141.

Tavaszi J, Budai P, Palovics A, Kismanyoki A. 2008. An alternative test battery in detecting ocular irritancy of agrochemicals. Comm App Biol Sci 73:891-895.

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

Vinardell M, Macián M. 1994. Comparative study of the HET-CAM test and the Draize eye test for assessment of irritancy potential. Toxicol In Vitro 8:467-470.

Vinardell MP, Mitjans M. 2006. The chorioallantoic membrane test as a model to predict the potential human eye irritation induced by commonly used laboratory solvents. Toxicol In Vitro 20:1066-1070.

13.0 Glossary[6]

Accuracy:[*] (a) The closeness of agreement between a test method result and an accepted reference value. (b) The proportion of correct outcomes of a test method. It is a measure of test method performance and one aspect of "relevance." The term is often used interchangeably with *concordance* (see also *two-by-two* table). Accuracy is highly dependent on the prevalence of positives in the population being examined.

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

---

[6] The definitions in this Glossary are restricted to their uses with respect to the Draize rabbit eye test method and the HET-CAM test method.

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

Benchmark control: A sample containing all components of a test system and treated with a known substance (i.e., the benchmark substance) to induce a known response. The sample is processed with test substance-treated and other control samples to compare the response produced by the test substance to the benchmark substance to allow for an assessment of the sensitivity of the test method to assess a specific chemical class or product class.

Benchmark substance: A substance used as a standard for comparison to a test substance. A benchmark substance should have the following properties:

- a consistent and reliable source(s)
- structural and functional similarity to the class of substances being tested
- known physical/chemical characteristics
- supporting data on known effects
- known potency in the range of the desired response

Blepharitis: Inflammation of the eyelids.

Bulbar conjunctiva: The portion of the conjunctiva that covers the outer surface of the eye.

Chorioallantoic membrane (CAM): A vascularized respiratory fetal membrane that is composed of the chorion and allantois.

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

Coagulation: The process of a liquid becoming viscous, jellylike, or solid by chemical reaction.

Coded substances: Substances labeled by code rather than name so that they can be tested and evaluated without knowledge of their identity or anticipation of test results. Coded substances are used to avoid intentional or unintentional bias when evaluating laboratory or test method performance.

Coefficient of variation: A statistical representation of the precision of a test. It is expressed as a percentage and is calculated as follows:

$$\left(\frac{standard\ deviation}{mean}\right) \times 100\%$$

Concordance:[*] The proportion of all substances tested that are correctly classified as positive or negative. It is a measure of test method performance and one aspect of "relevance." The term is often used interchangeably with *accuracy* (see also *two-by-two table*). Concordance is highly dependent on the prevalence of positives in the population being examined.

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: 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. Opacity can be evaluated subjectively, as done in the Draize rabbit eye test, or objectively with an instrument such as an opacitometer.

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.

Endpoint:* The biological process, response, or effect assessed by a test method.

False negative:* A substance incorrectly identified as negative by a test method.

False negative rate:* The proportion of all positive substances falsely identified by a test method as negative (see *two-by-two table*). It is one indicator of test method accuracy.

False positive:* A substance incorrectly identified as positive by a test method.

False positive rate:* The proportion of all negative substances that are falsely identified by a test method as positive (see *two-by-two table*). It is one indicator of test method accuracy.

Fibrous tunic: The outer of the three membranes of the eye, comprising the cornea and the sclera; called also *tunica fibrosa oculi*.

Globally Harmonised System (GHS): A classification system presented by the United Nations that provides (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.

Good Laboratory Practices (GLP):* Regulations promulgated by the U.S. Food and Drug Administration and the U.S. Environmental Protection Agency, and principles and procedures adopted by the Organization for Economic Cooperation and Development and Japanese authorities that describe record-keeping and quality assurance procedures for laboratory records that will be the basis for data submissions to national regulatory agencies.

Hazard:* 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.

Hemorrhage: Discharge of blood from a vessel.

Hyperemia: Excess of blood in a body part.

Interlaboratory reproducibility:* A measure of whether different qualified laboratories using the same protocol and test substances can produce qualitatively and quantitatively similar results. Interlaboratory reproducibility is determined during the prevalidation and validation processes and indicates the extent to which a test method can be transferred successfully among laboratories.

Intralaboratory repeatability:* The closeness of agreement between test results obtained within a single laboratory when the procedure is performed on the same substance under identical conditions within a given time period.

Intralaboratory reproducibility:* The first stage of validation; a determination of whether qualified people within the same laboratory can successfully replicate results using a specific test protocol at different times.

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.

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.

---

* Definition used by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM 2003).

Irritation score: Value calculated by different analysis methods, which is used to classify the irritancy potential of a test substance. Also referred to as *IS*.

Irritation Threshold Concentration: The lowest concentration of a test substance required to produce a weak or slight irritant response on the CAM. Also referred to as *ITC*.

IS(A) analysis method: HET-CAM analysis method where endpoints are observed at specified time points after application of the test substance (typically 0.5, 2, and 5 minutes post exposure). At the time points, presence of an endpoint is determined and a score assigned, if it is present. The scores are totaled to yield an overall irritation score.

IS(B) analysis method: HET-CAM analysis method where endpoints are observed over the entire observation period after application of the test substance (typically 5 minutes). The time (in seconds) when an endpoint develops is noted, and the times are used to yield an overall irritation score using a mathematical formula.

Lysis: The disintegration of blood vessels.

Mean Time to Coagulation (mtc): Mean detection time for appearance of coagulation endpoint.

Negative control: An untreated sample containing all components of a test system, except the test substance solvent, which is replaced with a known nonreactive material, such as water. This sample is processed with test substance-treated samples and other control samples to determine whether the solvent interacts with the test system.

Negative predictivity:[*] The proportion of correct negative responses among substances testing negative by a test method (see *two-by-two table*). It is one indicator of test method accuracy. Negative predictivity is a function of the sensitivity of the test method and the prevalence of negatives among the substances tested.

Neuroectodermal tunic: The innermost of three membranes of the eye, comprising the retina.

Nictating membrane: The membrane that moves horizontally across the eye in some animal species (e.g., rabbit, cat) to provide additional protection in particular circumstances. It may be referred to as the *third eyelid*.

Not Labeled: (a) A substance that 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.

Nonsevere irritant: (a) A substance that causes tissue damage in the eye following application to the anterior surface of the eye; the tissue damage is reversible within 21 days of application and the observed adverse effects in the eye are less severe than observed for a severe irritant. (b) Substances that are classified as GHS Category 2A or 2B; EPA Category II, III, or IV; or EU 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.

Palpebral conjunctiva: The part of the conjunctiva that covers the inner surface of the eyelids.

---

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

Pannus: A specific type of corneal inflammation that begins within the conjunctiva, and with time spreads to the cornea. Also referred to as *chronic superficial keratitis*.

Performance:* The accuracy and reliability characteristics of a test method (see *accuracy*, *reliability*).

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

Positive control: A sample containing all components of a test system and treated with a substance known to induce a positive response, which is processed with the test substance-treated and other control samples to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay over time.

Positive predictivity:* The proportion of correct positive responses among substances testing positive by a test method (see *two-by-two table*). It is one indicator of test method accuracy. Positive predictivity is a function of the sensitivity of the test method and the prevalence of positives among the substances tested.

Prevalence:* The proportion of positives in the population of substances tested (see *two-by-two table*).

Protocol:* 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.

Q-score: HET-CAM analysis method that calculates the ratio from the irritation score of a test substance compared to the irritation score of a reference substance. This HET-CAM analysis method is typically used with transparent test substances.

Quality assurance:* A management process by which adherence to laboratory testing standards, requirements, and record keeping procedures is assessed independently by individuals other than those performing the testing.

Reduction alternative:* A new or modified test method that reduces the number of animals required.

Reference test method:* The accepted *in vivo* test method used for regulatory purposes to evaluate the potential of a test substance to be hazardous to the species of interest.

Refinement alternative:* A new or modified test method that refines procedures to lessen or eliminate pain or distress in animals or enhances animal well-being.

Relevance:* The extent to which a test method correctly predicts or measures the biological effect of interest in humans or another species of interest. Relevance incorporates consideration of the accuracy or concordance of a test method.

Reliability:* A measure of the degree to which a test method can be performed reproducibly within and among laboratories over time. It is assessed by calculating intra- and interlaboratory reproducibility and intralaboratory repeatability.

Replacement alternative:* A new or modified test method that replaces animals with nonanimal systems or one animal species with a phylogenetically lower one (e.g., a mammal with an invertebrate).

Reproducibility:* The consistency of individual test results obtained in a single laboratory (intralaboratory reproducibility) or in different laboratories (interlaboratory reproducibility) using the same protocol and test substances (see intra- and interlaboratory reproducibility).

---

* Definition used by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM 2003).

Sclera: The tough, fibrous tissue that extends from the cornea to the optic nerve at the back of the eye.

Sensitivity:* The proportion of all positive substances that are classified correctly as positive in a test method. It is a measure of test method accuracy (see *two-by-two table*).

Secondary bacterial keratitis: Inflammation of the cornea that occurs secondary to another insult that compromised the integrity of the eye.

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.

Solvent control: An untreated sample containing all components of a test system, including the solvent that is processed with the test substance-treated and other control samples to establish the baseline response for the samples treated with the test substance dissolved in the same solvent. When tested with a concurrent negative control, this sample also demonstrates whether the solvent interacts with the test system.

Specificity:* The proportion of all negative substances that are classified correctly as negative in a test method. It is a measure of test method accuracy (see *two-by-two table*).

S-score: HET-CAM analysis method that totals the severity scores for each endpoint evaluated. The highest total score is used as the S-score. This HET-CAM analysis method is typically used with nontransparent test substances.

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

Test method:* 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*.

Test method components: Structural, functional, and procedural elements of a test method that are used to develop the test method protocol. These components include unique characteristics of the test method, critical procedural details, and quality control measures.

Tiered testing: A testing strategy where all existing information on a test substance is reviewed, in a specified order, prior to *in vivo* testing. If the irritancy potential of a test substance can be assigned, based on the existing information, no additional testing is required. If the irritancy potential of a test substance cannot be assigned, based on the existing information, a step-wise animal testing procedure is performed until an unequivocal classification can be made.

Toxic keratoconjunctivitis: Inflammation of the cornea and conjunctiva due to contact with an exogenous agent. Used interchangeably with *contact keratoconjunctivitis*, *irritative keratoconjunctivitis*, and *chemical keratoconjunctivitis*.

Transferability:* The ability of a test method or procedure to be accurately and reliably performed in different, competent laboratories.

Two-by-two table:* The two-by-two table can be used for calculating accuracy (concordance) ([a+d]/[a+b+c+d]), negative predictivity (d/[c+d]), positive predictivity (a/[a+b]), prevalence

---

* Definition used by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM 2003).

([a+c]/[a+b+c+d]), sensitivity (a/[a+c]), specificity (d/[b+d]), false positive rate (b/[b+d]), and false negative rate (c/[a+c]).

		New Test Outcome		
		Positive	Negative	Total
Reference Test Outcome	Positive	a	c	a + c
	Negative	b	d	b + d
	Total	a + b	c + d	a + b + c + d

Uvea tract: The middle of three membranes of the eye, comprising the iris, ciliary body, and choroid. Also referred to as the *vascular tunic*.

Validated test method:[*] 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:[*] The process by which the reliability and relevance of a procedure are established for a specific purpose.

Vascular tunic: The middle of three membranes of the eye, comprising the iris, ciliary body, and choroid. Also referred to as the *uvea*.

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.

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

Annex I

Chemical and Product Class Information for the Substances Tested in the HET-CAM Test Method

Originally published as Appendix B of:
Background Review Document - Current Status of In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants: Hen's Egg Test - Chrorioallantoic Membrane (Het-CAM) Test Method (NIH Publication No. 06-4515)

Document is available on request from NICEATM.

This page intentionally left blank

Annex II

In Vitro Data for the IS(A) Analysis Method

This page intentionally left blank

Annex II-1

In Vitro Data for the IS(A) Analysis Method: by Reference

This page intentionally left blank

In Vitro Data for the IS(A) Analysis Method: by Reference

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Triethanolamine	102-71-6		10%	Solution		10.75		Severe			Slight	NI	IV	NI	Bagley et al. (1992)
Triton X-100	9002-93-1		1%	Solution		9		Severe			Severe	NI	IV	NI	Bagley et al. (1992)
Oil/Water Emulsion-HZA			100%	Solution		0		Nonirritant			Nonirritant	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZC			100%	Solution		0.283		Nonirritant			Nonirritant	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZE			100%	Solution		0.533		Nonirritant			Nonirritant	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZF			100%	Solution		7.33		Moderate			Moderate	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZH			100%	Solution		17.8		Severe			Severe	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZI			100%	Solution		1.97		Slight			Slight	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZJ			100%	Solution		0.917		Nonirritant			Nonirritant	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZL			100%	Solution		4.83		Slight			Slight	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZM			100%	Solution		8.33		Moderate			Moderate	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZN			100%	Solution		3.33		Slight			Slight	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZO			100%	Solution		0.5		Nonirritant			Nonirritant	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZR			100%	Solution		10.6		Severe			Severe	Category 1	Category I	R41	Gettings et al. (1994)
Oil/Water Emulsion-HZS			100%	Solution		11.6		Severe			Severe	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZT			100%	Solution		4.1		Slight			Slight	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZU			100%	Solution		0		Nonirritant			Nonirritant	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZV			100%	Solution		0.6		Nonirritant			Nonirritant	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZW			100%	Solution		0.167		Nonirritant			Nonirritant	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZY			100%	Solution		17		Severe			Severe	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)

In Vitro Data for the IS(A) Analysis Method: by Reference

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Surfactant Based Formulation 10-HZJ			10%	Solution		2.2		Slight			Slight	Nonirritant	Category IV	Nonirritant	Gettings et al. (1996)
Surfactant Based Formulation 11-HZK			10%	Solution		8.8		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 12-HZL			10%	Solution		9.6		Severe			Severe	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 13-HZM			10%	Solution		4.1		Slight			Slight	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 14-HZN			10%	Solution		6.1		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 15-HZP			10%	Solution		4.7		Slight			Slight	Nonirritant	Category III	Nonirritant	Gettings et al. (1996)
Surfactant Based Formulation 16-HZQ			10%	Solution		4.9		Slight			Slight	Nonirritant	Category III	Nonirritant	Gettings et al. (1996)
Surfactant Based Formulation 17-HZR			10%	Solution		7.7		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 18-HZS			10%	Solution		8.8		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 19-HZT			10%	Solution		0.2		Nonirritant			Nonirritant	Nonirritant	Category IV	Nonirritant	Gettings et al. (1996)
Surfactant Based Formulation 1-HZA			10%	Solution		7.8		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 20-HZU			10%	Solution		3.7		Slight			Slight	Category 2B	Category III	R36	Gettings et al. (1996)
Surfactant Based Formulation 21-HZV			10%	Solution		7.7		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 22-HZW			10%	Solution		7.8		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 23-HZX			10%	Solution		9		Severe			Severe	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 24-HZY			10%	Solution		8.7		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 25-HZZ			10%	Solution		0.7		Nonirritant			Nonirritant	Nonirritant	Category IV	Nonirritant	Gettings et al. (1996)
Surfactant Based Formulation 2-HZB			10%	Solution		4.8		Slight			Slight	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 3-HZC			10%	Solution		9.5		Severe			Severe	Category 1	Category I	R41	Gettings et al. (1996)

In Vitro Data for the IS(A) Analysis Method: by Reference

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Surfactant Based Formulation 4-HZD			10%	Solution		5.2		Moderate			Moderate	Category 2B	Category III	Nonirritant	Gettings et al. (1996)
Surfactant Based Formulation 5-HZE			10%	Solution		3.8		Slight			Slight	SCNM	Category I	SCNM	Gettings et al. (1996)
Surfactant Based Formulation 6-HZF			10%	Solution		8.3		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 7-HZG			10%	Solution		6.3		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 8-HZH			10%	Solution		1.3		Slight			Slight	Nonirritant	Category IV	Nonirritant	Gettings et al. (1996)
Surfactant Based Formulation 9-HZI			10%	Solution		9.3		Severe			Severe	Category 1	Category I	R41	Gettings et al. (1996)
Acetic acid	64-19-7	1	10%	Solution	2.4	16.5	2.89	Severe	5.00	100.00		1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Acetic acid	64-19-7	2	10%	Solution	2.4	16	3.92	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Acetic acid	64-19-7	3	10%	Solution	2.4	17.25	6.24	Severe			Severe	1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Acetic acid	64-19-7	4	10%	Solution	2.4	19.5	1.91	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Acetic acid	64-19-7	5	10%	Solution	2.4	17.5	4.04	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Butanol	71-36-3	1	10%	Suspension	7.31	8.25	2.87	Moderate	5.00	60.00		1.00	SCNM	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Butanol	71-36-3	2	10%	Suspension	7.31	10.5	1.91	Severe				1.00	SCNM	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Butanol	71-36-3	3	10%	Suspension	7.31	12	0.00	Severe			Severe	1.00	SCNM	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Butanol	71-36-3	4	10%	Suspension	7.31	12	0.00	Severe				1.00	SCNM	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Butanol	71-36-3	5	10%	Suspension	7.31	5.75	1.50	Moderate				1.00	SCNM	R41	Hagino et al. (1999)/Submitted Y. Ohno Data

In Vitro Data for the IS(A) Analysis Method: by Reference

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Cetyltrimethylammonium bromide	57-09-0	1	10%	Solution	5.89	19	1.63	Severe	5.00	100.00	Severe	1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Cetyltrimethylammonium bromide	57-09-0	2	10%	Solution	5.89	13.25	2.22	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Cetyltrimethylammonium bromide	57-09-0	3	10%	Solution	5.89	18.5	1.91	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Cetyltrimethylammonium bromide	57-09-0	4	10%	Solution	5.89	11	2.45	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Cetyltrimethylammonium bromide	57-09-0	5	10%	Solution	5.89	9	0.00	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Chlorhexidine gluconate solution (20% solution)	18472-51-0	1	10%	Solution	6.56	19	1.63	Severe	5.00	100.00	Severe	2B	II	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Chlorhexidine gluconate solution (20% solution)	18472-51-0	2	10%	Solution	6.56	13.5	1.00	Severe				2B	II	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Chlorhexidine gluconate solution (20% solution)	18472-51-0	3	10%	Solution	6.56	16	2.45	Severe				2B	II	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Chlorhexidine gluconate solution (20% solution)	18472-51-0	4	10%	Solution	6.56	11.75	4.86	Severe				2B	II	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Chlorhexidine gluconate solution (20% solution)	18472-51-0	5	10%	Solution	6.56	9	0.00	Severe				2B	II	NI	Hagino et al. (1999)/Submitted Y. Ohno Data

In Vitro Data for the IS(A) Analysis Method: by Reference

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Di(2-ethylhexyl) sodium sulfosuccinate	577-11-7	1	10%	Suspension	6.54	10	0.00	Severe	5.00	80.00	Severe	1.00	I	R36	Hagino et al. (1999)/Submitted Y. Ohno Data
Di(2-ethylhexyl) sodium sulfosuccinate	577-11-7	2	10%	Suspension	6.54	10	1.63	Severe				1.00	I	R36	Hagino et al. (1999)/Submitted Y. Ohno Data
Di(2-ethylhexyl) sodium sulfosuccinate	577-11-7	3	10%	Suspension	6.54	10.25	3.50	Severe				1.00	I	R36	Hagino et al. (1999)/Submitted Y. Ohno Data
Di(2-ethylhexyl) sodium sulfosuccinate	577-11-7	4	10%	Suspension	6.54	12	0.00	Severe				1.00	I	R36	Hagino et al. (1999)/Submitted Y. Ohno Data
Di(2-ethylhexyl) sodium sulfosuccinate	577-11-7	5	10%	Suspension	6.54	5	0.00	Moderate				1.00	I	R36	Hagino et al. (1999)/Submitted Y. Ohno Data
Diisopropanolamine	110-97-4	1	10%	Solution	11.89	8.25	2.36	Moderate	5.00	60.00	Moderate	NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Diisopropanolamine	110-97-4	2	10%	Solution	11.89	8.5	4.04	Moderate				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Diisopropanolamine	110-97-4	3	10%	Solution	11.89	9	1.15	Severe				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Diisopropanolamine	110-97-4	4	10%	Solution	11.89	12	0.00	Severe				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Diisopropanolamine	110-97-4	5	10%	Solution	11.89	5	0.00	Moderate				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data

In Vitro Data for the IS(A) Analysis Method: by Reference

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Domiphen bromide	538-71-6	1	10%	Solution	6.22	19	0.00	Severe	5.00	100.00	Severe	1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Domiphen bromide	538-71-6	2	10%	Solution	6.22	15.25	2.36	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Domiphen bromide	538-71-6	3	10%	Solution	6.22	14.75	1.50	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Domiphen bromide	538-71-6	4	10%	Solution	6.22	12.25	2.36	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Domiphen bromide	538-71-6	5	10%	Solution	6.22	9	1.22	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	1	10%	Solution	5.9	0	0.00	Nonirritant	5.00	100.00	Slight	NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	2	10%	Solution	5.9	1.25	2.50	Slight	6.00	100.00		NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	3	10%	Solution	5.9	10.5	1.91	Severe				NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	4	10%	Solution	5.9	1.5	1.73	Slight				NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	5	10%	Solution	5.9	1.25	2.50	Slight				NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data

In Vitro Data for the IS(A) Analysis Method: by Reference

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Ethanol	64-17-5	1	100%	Liquid		18.75	3.30	Severe	5.00	100.00	Severe	SCNM	SCNM	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	2	100%	Liquid		16	2.45	Severe				SCNM	SCNM	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	3	100%	Liquid		11.5	1.00	Severe				SCNM	SCNM	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	4	100%	Liquid		17	0.00	Severe				SCNM	SCNM	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	5	100%	Liquid		10.5	1.73	Severe				SCNM	SCNM	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Glycolic acid	79-14-1	1	10%	Solution	1.76	19.5	1.00	Severe	5.00	100.00	Severe	2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Glycolic acid	79-14-1	2	10%	Solution	1.76	20	1.15	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Glycolic acid	79-14-1	3	10%	Solution	1.76	17.75	3.95	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Glycolic acid	79-14-1	4	10%	Solution	1.76	12.25	2.36	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Glycolic acid	79-14-1	5	10%	Solution	1.76	12.75	2.50	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data

In Vitro Data for the IS(A) Analysis Method: by Reference

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Lactic acid	50-21-5	1	10%	Solution	1.94	20.5	1.00	Severe	5.00	100.00	Severe	SCNM	III	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	2	10%	Solution	1.94	14.25	2.06	Severe				SCNM	III	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	3	10%	Solution	1.94	19.5	1.91	Severe				SCNM	III	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	4	10%	Solution	1.94	18.5	1.91	Severe				SCNM	III	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	5	10%	Solution	1.94	10.25	2.50	Severe				SCNM	III	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	1	100%	Liquid		21	0.00	Severe	5.00	80.00	Severe	1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	2	100%	Liquid		6.25	2.50	Moderate				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	3	100%	Liquid		16	1.15	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	4	100%	Liquid		15.25	2.50	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	5	100%	Liquid		11.5	2.89	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data

In Vitro Data for the IS(A) Analysis Method: by Reference

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Monoethanolamine	141-43-5	1	10%	Solution	12.58	12	0.00	Severe	5.00	100.00	Severe	2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Monoethanolamine	141-43-5	2	10%	Solution	12.58	12	0.00	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Monoethanolamine	141-43-5	3	10%	Solution	12.58	12	0.00	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Monoethanolamine	141-43-5	4	10%	Solution	12.58	12	0.00	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Monoethanolamine	141-43-5	5	10%	Solution	12.58	12	0.00	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Potassium laurate	10124-65-9	1	10%	Solution	10.49	20	1.15	Severe	5.00	100.00	Severe	1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Potassium laurate	10124-65-9	2	10%	Solution	10.49	13.25	2.50	Severe	6.00	100.00		1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Potassium laurate	10124-65-9	3	10%	Solution	10.49	12	0.00	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Potassium laurate	10124-65-9	4	10%	Solution	10.49	12	0.00	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Potassium laurate	10124-65-9	5	10%	Solution	10.49	19.33	0.82	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data

In Vitro Data for the IS(A) Analysis Method: by Reference

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Stearyltrimethylammonium chloride	15461-40-2	1	10%	Solution	4.24	16.75	2.06	Severe	5.00	100.00	Severe	1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Stearyltrimethylammonium chloride	15461-40-2	2	10%	Solution	4.24	13	2.00	Severe	6.00	100.00		1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Stearyltrimethylammonium chloride	15461-40-2	3	10%	Solution	4.24	15.75	3.50	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Stearyltrimethylammonium chloride	15461-40-2	4	10%	Solution	4.24	13.5	1.00	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Stearyltrimethylammonium chloride	15461-40-2	5	10%	Solution	4.24	9	0.00	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data

In Vitro Data for the IS(A) Analysis Method: by Reference

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Triethanolamine	102-71-6	1	10%	Solution	11.26	1.5	2.38	Slight	5.00	80.00	Moderate	NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	2	10%	Solution	11.26	3.75	4.79	Slight	6.00	66.00		NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	3	10%	Solution	11.26	11	1.15	Severe				NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	4	10%	Solution	11.26	6	1.63	Moderate				NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	5	10%	Solution	11.26	2.5	2.89	Slight				NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	1	100%	Liquid		5	0.00	Moderate	5.00	60.00		NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	2	100%	Liquid		6.75	3.50	Moderate				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	3	100%	Liquid		11.5	1.00	Severe				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	4	100%	Liquid		12	0.00	Severe				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	5	100%	Liquid		6.75	3.50	Moderate				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5		10%	Solution	7.2	4		Slight				NI	IV	NI	Kojima et al. (1995)
Potassium laurate	10124-65-9		10%	Solution	9.4	14.3		Severe				1.00	I	R41	Kojima et al. (1995)
Sodium lauryl sulfate	151-21-3		10%	Solution	5.4	7.5		Moderate			Severe	1.00	I	R41	Kojima et al. (1995)
Stearyltrimethylammonium chloride	15461-40-2		10%	Solution	5.5	19.3		Severe				1.00	I	R41	Kojima et al. (1995)
Triton X-100	9002-93-1		10%	Solution	5.75	5		Moderate			Moderate	1.00	II	SCNM	Kojima et al. (1995)

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; Conc. = Concentration; EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = Globally Harmonized System; SCNM - Study Criteria Not Met.

[1] IS(A) represents irritation scores that were calculated using the method described in Leupke (1985); classification scheme used as described in Leupke (1985).

[2] GHS=Globally Harmonized System (UN [2003])

[3] Eye Irritant Category 1 = irreversible effects on the eye/serious damage to the eye; Category 2A = reversible effects on the eye/irritating to the eyes; Category 2B = reversible effects on the eye/mildly irritating to the eyes; Nonirritant = not an eye

[4] EPA=U.S. Environmental Protection Agency (EPA [1996]).

[5] Toxicity Category I for the Primary Eye Irritation Study = Corrosive, or corneal involvement or irritation not reversible within 21 days; Category II = Corneal involvement or irritation clearing in 8-21 days; Category III = Corneal involvement or irritation clearing in 1-7 daysl; Category IV: minimal effects clearing in less than 24 hr

[6] EU=European Union (EU [2001]).

[7] Risk phrase R41 = risk of serious damage to the eyes; R36 = irritating to the eyes; nonirritant = not an eye irritant.

This page intentionally left blank

Annex II-2

In Vitro Data for the IS(A) Analysis Method: by Substance

This page intentionally left blank

In Vitro Data for the IS(A) Analysis Method: by Substance

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Acetic acid	64-19-7	1	10%	Solution	2.4	16.5	2.89	Severe	5.00	100.00	Severe	1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Acetic acid	64-19-7	2	10%	Solution	2.4	16	3.92	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Acetic acid	64-19-7	3	10%	Solution	2.4	17.25	6.24	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Acetic acid	64-19-7	4	10%	Solution	2.4	19.5	1.91	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Acetic acid	64-19-7	5	10%	Solution	2.4	17.5	4.04	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Butanol	71-36-3	1	10%	Suspension	7.31	8.25	2.87	Moderate	5.00	60.00	Severe	1.00	SCNM	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Butanol	71-36-3	2	10%	Suspension	7.31	10.5	1.91	Severe				1.00	SCNM	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Butanol	71-36-3	3	10%	Suspension	7.31	12	0.00	Severe				1.00	SCNM	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Butanol	71-36-3	4	10%	Suspension	7.31	12	0.00	Severe				1.00	SCNM	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Butanol	71-36-3	5	10%	Suspension	7.31	5.75	1.50	Moderate				1.00	SCNM	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Cetyltrimethylammonium bromide	57-09-0	1	10%	Solution	5.89	19	1.63	Severe	5.00	100.00	Severe	1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Cetyltrimethylammonium bromide	57-09-0	2	10%	Solution	5.89	13.25	2.22	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Cetyltrimethylammonium bromide	57-09-0	3	10%	Solution	5.89	18.5	1.91	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Cetyltrimethylammonium bromide	57-09-0	4	10%	Solution	5.89	11	2.45	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Cetyltrimethylammonium bromide	57-09-0	5	10%	Solution	5.89	9	0.00	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data

In Vitro Data for the IS(A) Analysis Method: by Substance

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Chlorhexidine gluconate solution (20% solution)	18472-51-0	1	10%	Solution	6.56	19	1.63	Severe	5.00	100.00	Severe	2B	II	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Chlorhexidine gluconate solution (20% solution)	18472-51-0	2	10%	Solution	6.56	13.5	1.00	Severe				2B	II	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Chlorhexidine gluconate solution (20% solution)	18472-51-0	3	10%	Solution	6.56	16	2.45	Severe				2B	II	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Chlorhexidine gluconate solution (20% solution)	18472-51-0	4	10%	Solution	6.56	11.75	4.86	Severe				2B	II	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Chlorhexidine gluconate solution (20% solution)	18472-51-0	5	10%	Solution	6.56	9	0.00	Severe				2B	II	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Di(2-ethylhexyl) sodium sulfosuccinate	577-11-7	1	10%	Suspension	6.54	10	0.00	Severe	5.00	80.00	Severe	1.00	I	R36	Hagino et al. (1999)/Submitted Y. Ohno Data
Di(2-ethylhexyl) sodium sulfosuccinate	577-11-7	2	10%	Suspension	6.54	10	1.63	Severe				1.00	I	R36	Hagino et al. (1999)/Submitted Y. Ohno Data
Di(2-ethylhexyl) sodium sulfosuccinate	577-11-7	3	10%	Suspension	6.54	10.25	3.50	Severe				1.00	I	R36	Hagino et al. (1999)/Submitted Y. Ohno Data
Di(2-ethylhexyl) sodium sulfosuccinate	577-11-7	4	10%	Suspension	6.54	12	0.00	Severe				1.00	I	R36	Hagino et al. (1999)/Submitted Y. Ohno Data
Di(2-ethylhexyl) sodium sulfosuccinate	577-11-7	5	10%	Suspension	6.54	5	0.00	Moderate				1.00	I	R36	Hagino et al. (1999)/Submitted Y. Ohno Data
Diisopropanolamine	110-97-4	1	10%	Solution	11.89	8.25	2.36	Moderate	5.00	60.00	Moderate	NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Diisopropanolamine	110-97-4	2	10%	Solution	11.89	8.5	4.04	Moderate				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Diisopropanolamine	110-97-4	3	10%	Solution	11.89	9	1.15	Severe				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Diisopropanolamine	110-97-4	4	10%	Solution	11.89	12	0.00	Severe				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Diisopropanolamine	110-97-4	5	10%	Solution	11.89	5	0.00	Moderate				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data

In Vitro Data for the IS(A) Analysis Method: by Substance

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Domiphen bromide	538-71-6	1	10%	Solution	6.22	19	0.00	Severe	5.00	100.00	Severe	1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Domiphen bromide	538-71-6	2	10%	Solution	6.22	15.25	2.36	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Domiphen bromide	538-71-6	3	10%	Solution	6.22	14.75	1.50	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Domiphen bromide	538-71-6	4	10%	Solution	6.22	12.25	2.36	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Domiphen bromide	538-71-6	5	10%	Solution	6.22	9	1.22	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	1	10%	Solution	5.9	0	0.00	Nonirritant	5.00	100.00	Slight	NI	IV	NI	Hagino et al. (1999)/Submitted Y.
Ethanol	64-17-5	2	10%	Solution	5.9	1.25	2.50	Slight	6.00	100.00		NI	IV	NI	Hagino et al. (1999)/Submitted Y.
Ethanol	64-17-5	3	10%	Solution	5.9	10.5	1.91	Severe				NI	IV	NI	Hagino et al. (1999)/Submitted Y.
Ethanol	64-17-5	4	10%	Solution	5.9	1.5	1.73	Slight				NI	IV	NI	Hagino et al. (1999)/Submitted Y.
Ethanol	64-17-5	5	10%	Solution	5.9	1.25	2.50	Slight				NI	IV	NI	Hagino et al. (1999)/Submitted Y.
Ethanol	64-17-5		10%	Solution	7.2	4		Slight				NI	IV	NI	Kojima et al. (1995)
Ethanol	64-17-5	1	100%	Liquid		18.75	3.30	Severe	5.00	100.00	Severe	SCNM	SCNM	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	2	100%	Liquid		16	2.45	Severe				SCNM	SCNM	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	3	100%	Liquid		11.5	1.00	Severe				SCNM	SCNM	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	4	100%	Liquid		17	0.00	Severe				SCNM	SCNM	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	5	100%	Liquid		10.5	1.73	Severe				SCNM	SCNM	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data

In Vitro Data for the IS(A) Analysis Method: by Substance

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Glycolic acid	79-14-1	1	10%	Solution	1.76	19.5	1.00	Severe	5.00	100.00	Severe	2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Glycolic acid	79-14-1	2	10%	Solution	1.76	20	1.15	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Glycolic acid	79-14-1	3	10%	Solution	1.76	17.75	3.95	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Glycolic acid	79-14-1	4	10%	Solution	1.76	12.25	2.36	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Glycolic acid	79-14-1	5	10%	Solution	1.76	12.75	2.50	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	1	10%	Solution	1.94	20.5	1.00	Severe	5.00	100.00	Severe	SCNM	III	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	2	10%	Solution	1.94	14.25	2.06	Severe				SCNM	III	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	3	10%	Solution	1.94	19.5	1.91	Severe				SCNM	III	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	4	10%	Solution	1.94	18.5	1.91	Severe				SCNM	III	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	5	10%	Solution	1.94	10.25	2.50	Severe				SCNM	III	SCNM	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	1	100%	Liquid		21	0.00	Severe	5.00	80.00	Severe	1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	2	100%	Liquid		6.25	2.50	Moderate				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	3	100%	Liquid		16	1.15	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	4	100%	Liquid		15.25	2.50	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	5	100%	Liquid		11.5	2.89	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data

In Vitro Data for the IS(A) Analysis Method: by Substance

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Monoethanolamine	141-43-5	1	10%	Solution	12.58	12	0.00	Severe	5.00	100.00	Severe	2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Monoethanolamine	141-43-5	2	10%	Solution	12.58	12	0.00	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Monoethanolamine	141-43-5	3	10%	Solution	12.58	12	0.00	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Monoethanolamine	141-43-5	4	10%	Solution	12.58	12	0.00	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Monoethanolamine	141-43-5	5	10%	Solution	12.58	12	0.00	Severe				2B	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Oil/Water Emulsion-HZA			100%	Solution		0		Nonirritant			Nonirritant	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZC			100%	Solution		0.283		Nonirritant			Nonirritant	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZE			100%	Solution		0.533		Nonirritant			Nonirritant	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZF			100%	Solution		7.33		Moderate			Moderate	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZH			100%	Solution		17.8		Severe			Severe	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZI			100%	Solution		1.97		Slight			Slight	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZJ			100%	Solution		0.917		Nonirritant			Nonirritant	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZL			100%	Solution		4.83		Slight			Slight	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZM			100%	Solution		8.33		Moderate			Moderate	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZN			100%	Solution		3.33		Slight			Slight	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZO			100%	Solution		0.5		Nonirritant			Nonirritant	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZR			100%	Solution		10.6		Severe			Severe	Category 1	Category I	R41	Gettings et al. (1994)
Oil/Water Emulsion-HZS			100%	Solution		11.6		Severe			Severe	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZT			100%	Solution		4.1		Slight			Slight	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZU			100%	Solution		0		Nonirritant			Nonirritant	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)

In Vitro Data for the IS(A) Analysis Method: by Substance

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Oil/Water Emulsion-HZV			100%	Solution		0.6		Nonirritant			Nonirritant	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZW			100%	Solution		0.167		Nonirritant			Nonirritant	Nonirritant	Category III	Nonirritant	Gettings et al. (1994)
Oil/Water Emulsion-HZY			100%	Solution		17		Severe			Severe	Nonirritant	Category IV	Nonirritant	Gettings et al. (1994)
Potassium laurate	10124-65-9	1	10%	Solution	10.49	20	1.15	Severe	5.00	100.00	Severe	1.00	I	R41	Hagino et al. (1999)/Submitted Y.
Potassium laurate	10124-65-9	2	10%	Solution	10.49	13.25	2.50	Severe	6.00	100.00		1.00	I	R41	Hagino et al. (1999)/Submitted Y.
Potassium laurate	10124-65-9	3	10%	Solution	10.49	12	0.00	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y.
Potassium laurate	10124-65-9	4	10%	Solution	10.49	12	0.00	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y.
Potassium laurate	10124-65-9	5	10%	Solution	10.49	19.33	0.82	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y.
Potassium laurate	10124-65-9		10%	Solution	9.4	14.3		Severe				1.00	I	R41	Kojima et al. (1995)
Sodium lauryl sulfate	151-21-3		10%	Solution	5.4	7.5		Moderate			Severe	1.00	I	R41	Kojima et al. (1995)
Stearyltrimethylammonium chloride	15461-40-2	1	10%	Solution	4.24	16.75	2.06	Severe	5.00	100.00	Severe	1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Stearyltrimethylammonium chloride	15461-40-2	2	10%	Solution	4.24	13	2.00	Severe	6.00	100.00		1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Stearyltrimethylammonium chloride	15461-40-2	3	10%	Solution	4.24	15.75	3.50	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Stearyltrimethylammonium chloride	15461-40-2	4	10%	Solution	4.24	13.5	1.00	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Stearyltrimethylammonium chloride	15461-40-2	5	10%	Solution	4.24	9	0.00	Severe				1.00	I	R41	Hagino et al. (1999)/Submitted Y. Ohno Data
Stearyltrimethylammonium chloride	15461-40-2		10%	Solution	5.5	19.3		Severe				1.00	I	R41	Kojima et al. (1995)
Surfactant Based Formulation 1-HZA			10%	Solution		7.8		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 2-HZB			10%	Solution		4.8		Slight			Slight	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 3-HZC			10%	Solution		9.5		Severe			Severe	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 4-HZD			10%	Solution		5.2		Moderate			Moderate	Category 2B	Category III	Nonirritant	Gettings et al. (1996)

In Vitro Data for the IS(A) Analysis Method: by Substance

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Surfactant Based Formulation 5-HZE			10%	Solution		3.8		Slight			Slight	SCNM	Category I	SCNM	Gettings et al. (1996)
Surfactant Based Formulation 6-HZF			10%	Solution		8.3		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 7-HZG			10%	Solution		6.3		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 8-HZH			10%	Solution		1.3		Slight			Slight	Nonirritant	Category IV	Nonirritant	Gettings et al. (1996)
Surfactant Based Formulation 9-HZI			10%	Solution		9.3		Severe			Severe	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 10-HZJ			10%	Solution		2.2		Slight			Slight	Nonirritant	Category IV	Nonirritant	Gettings et al. (1996)
Surfactant Based Formulation 11-HZK			10%	Solution		8.8		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 12-HZL			10%	Solution		9.6		Severe			Severe	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 13-HZM			10%	Solution		4.1		Slight			Slight	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 14-HZN			10%	Solution		6.1		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 15-HZP			10%	Solution		4.7		Slight			Slight	Nonirritant	Category III	Nonirritant	Gettings et al. (1996)
Surfactant Based Formulation 16-HZQ			10%	Solution		4.9		Slight			Slight	Nonirritant	Category III	Nonirritant	Gettings et al. (1996)
Surfactant Based Formulation 17-HZR			10%	Solution		7.7		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 18-HZS			10%	Solution		8.8		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 19-HZT			10%	Solution		0.2		Nonirritant			Nonirritant	Nonirritant	Category IV	Nonirritant	Gettings et al. (1996)
Surfactant Based Formulation 20-HZU			10%	Solution		3.7		Slight			Slight	Category 2B	Category III	R36	Gettings et al. (1996)
Surfactant Based Formulation 21-HZV			10%	Solution		7.7		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 22-HZW			10%	Solution		7.8		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 23-HZX			10%	Solution		9		Severe			Severe	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 24-HZY			10%	Solution		8.7		Moderate			Moderate	Category 1	Category I	R41	Gettings et al. (1996)
Surfactant Based Formulation 25-HZZ			10%	Solution		0.7		Nonirritant			Nonirritant	Nonirritant	Category IV	Nonirritant	Gettings et al. (1996)

In Vitro Data for the IS(A) Analysis Method: by Substance

Substance Name	CASRN	Test Lab	*In Vitro* Concentration Tested	*In Vitro* Physical Form Tested	pH	IS(A)[1]	IS(A) SD	IS(A) Classification	# of Testing Labs	% Concoradance	Overall IS(A) Classification	GHS Classification[2,3]	EPA Classification[4,5]	EU Classification[6,7]	Reference
Triethanolamine	102-71-6		10%	Solution		10.75		Severe			Slight	NI	IV	NI	Bagley et al. (1992)
Triethanolamine	102-71-6	1	10%	Solution	11.26	1.5	2.38	Slight	5.00	80.00		NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	2	10%	Solution	11.26	3.75	4.79	Slight	6.00	66.00		NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	3	10%	Solution	11.26	11	1.15	Severe				NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	4	10%	Solution	11.26	6	1.63	Moderate				NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	5	10%	Solution	11.26	2.5	2.89	Slight				NI	IV	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	1	100%	Liquid		5	0.00	Moderate	5.00	60.00	Moderate	NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	2	100%	Liquid		6.75	3.50	Moderate				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	3	100%	Liquid		11.5	1.00	Severe				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	4	100%	Liquid		12	0.00	Severe				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	5	100%	Liquid		6.75	3.50	Moderate				NI	III	NI	Hagino et al. (1999)/Submitted Y. Ohno Data
Triton X-100	9002-93-1		1%	Solution		9		Severe			Severe	NI	IV	NI	Bagley et al. (1992)
Triton X-100	9002-93-1		10%	Solution	5.75	5		Moderate			Moderate	1.00	II	SCNM	Kojima et al. (1995)

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; Conc. = Concentration; EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = Globally Harmonized System; SCNM - Study Criteria Not Met.

[1] IS(A) represents irritation scores that were calculated using the method described in Leupke (1985); classification scheme used as described in Leupke (1985)

[2] GHS=Globally Harmonized System (UN [2003])

[3] Eye Irritant Category 1 = irreversible effects on the eye/serious damage to the eye: Category 2A = reversible effects on the eye/irritating to the eyes: Category 2B = reversible effects on the eye/mildly irritating to the eyes: Nonirritant = not an eye

[4] EPA=U.S. Environmental Protection Agency (EPA [1996]).

[5] Toxicity Category I for the Primary Eye Irritation Study = Corrosive, or corneal involvement or irritation not reversible within 21 days; Category II = Corneal involvement or irritation clearing in 8-21 days; Category III = Corneal involvement or irritation clearing in 1-7 days]; Category IV: minimal effects clearing in less than 24 hr

[6] EU=European Union (EU [2001]).

[7] Risk phrase R41 = risk of serious damage to the eyes; R36 = irritating to the eyes; nonirritant = not an eye irritant.

Annex III

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications

This page intentionally left blank

Annex III-1

Comparison of *In Vivo* and *In Vitro* Ocular Irritancy Classifications: Sorted by Reference

This page intentionally left blank

Comparison of *In Vivo* and *In Vitro* Ocular Irritancy Classifications: Sorted by Reference

Substance Name	CASRN	*In Vitro* Conc. Tested	*In Vivo* Conc. Tested	Chemical Class	*In Vivo* Physical Form Tested	*In Vivo* (GHS) Classification[1,2]	*In Vivo* (EPA) Classification[3,4]	*In Vivo* (EU) Classification[5,6]	*In Vitro* Classification (IS(A))[7]	*FHSA-20%*[8]	*FHSA-67%*[9]	Reference
Triton X-100	9002-93-1	1%	1%	Ether	Solution	Nonirritant	Category III	Nonirritant	Severe	Irritant	Inconclusive	Bagley et al. (1992)
Oil/Water Emulsion-HZA		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Nonirritant	Irritant	Inconclusive	Gettings et al. (1994)
Oil/Water Emulsion-HZC		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Nonirritant	Irritant	Inconclusive	Gettings et al. (1994)
Oil/Water Emulsion-HZE		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Nonirritant	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZF		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Moderate	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZH		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Severe	Irritant	Irritant	Gettings et al. (1994)
Oil/Water Emulsion-HZI		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Slight	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZJ		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Nonirritant	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZL		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Slight	Irritant	Irritant	Gettings et al. (1994)
Oil/Water Emulsion-HZM		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Moderate	Irritant	Irritant	Gettings et al. (1994)
Oil/Water Emulsion-HZN		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Slight	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZO		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Nonirritant	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZR		Undiluted	Undiluted	Formulation	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Gettings et al. (1994)
Oil/Water Emulsion-HZS		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Severe	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZT		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Slight	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZU		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Nonirritant	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZV		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Nonirritant	Irritant	Inconclusive	Gettings et al. (1994)
Oil/Water Emulsion-HZW		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Nonirritant	Irritant	Irritant	Gettings et al. (1994)
Oil/Water Emulsion-HZY		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Severe	Not labeled	Not labeled	Gettings et al. (1994)
Surfactant Based Formulation 1-HZA		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 10-HZJ		10%	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Slight	Not labeled	Not labeled	Gettings et al. (1996)
Surfactant Based Formulation 11-HZK		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)

Comparison of *In Vivo* and *In Vitro* Ocular Irritancy Classifications: Sorted by Reference

Substance Name	CASRN	*In Vitro* Conc. Tested	*In Vivo* Conc. Tested	Chemical Class	*In Vivo* Physical Form Tested	*In Vivo* (GHS) Classification[1,2]	*In Vivo* (EPA) Classification[3,4]	*In Vivo* (EU) Classification[5,6]	*In Vitro* Classification (IS(A))[7]	*FHSA-20%*[8]	*FHSA-67%*[9]	Reference
Surfactant Based Formulation 12-HZL		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 13-HZM		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Slight	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 14-HZN		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 15-HZP		10%	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Slight	Irritant	Inconclusive	Gettings et al. (1996)
Surfactant Based Formulation 16-HZQ		10%	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Slight	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 17-HZR		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 18-HZS		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 19-HZT		10%	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Nonirritant	Not labeled	Not labeled	Gettings et al. (1996)
Surfactant Based Formulation 2-HZB		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Slight	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 20-HZU		10%	Undiluted	Formulation	Solution	Category 2B	Category III	R36	Slight	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 21-HZV		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 22-HZW		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 23-HZX		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 24-HZY		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 25-HZZ		10%	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Nonirritant	Not labeled	Not labeled	Gettings et al. (1996)
Surfactant Based Formulation 3-HZC		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 4-HZD		10%	Undiluted	Formulation	Solution	Category 2B	Category III	Nonirritant	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 5-HZE		10%	Undiluted	Formulation	Solution	SCNM	Category I	SCNM	Slight	Irritant	Inconclusive	Gettings et al. (1996)
Surfactant Based Formulation 6-HZF		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 7-HZG		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 8-HZH		10%	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Slight	Not labeled	Not labeled	Gettings et al. (1996)
Surfactant Based Formulation 9-HZI		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Gettings et al. (1996)

Comparison of *In Vivo* and *In Vitro* Ocular Irritancy Classifications: Sorted by Reference

Substance Name	CASRN	*In Vitro* Conc. Tested	*In Vivo* Conc. Tested	Chemical Class	*In Vivo* Physical Form Tested	*In Vivo* (GHS) Classification[1,2]	*In Vivo* (EPA) Classification[3,4]	*In Vivo* (EU) Classification[5,6]	*In Vitro* Classification (IS(A))[7]	*FHSA-20%*[8]	*FHSA-67%*[9]	Reference
Acetic acid	64-19-7	10%	10%	Carboxylic acid	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Butanol	71-36-3	10%	10%	Alcohol	Solution	Category 1	SCNM	R41	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Cetyltrimethylammonium bromide	57-09-0	10%	10%	Organic salt, Onium	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Chlorhexidine gluconate Solution (20% Solution)	18472-51-0	10%	10%	Amidine, Ester	Solution	Category 2B	Category II	Nonirritant	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Di(2-ethylhexyl) sodium sulfosuccinate	577-11-7	10%	10%	Organic salt, Sulfur containing compound, Ester	Solution	Category 1	Category I	R36	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Diisopropanolamine	110-97-4	10%	10%	Amine, Alcohol	Solution	Nonirritant	Category III	Nonirritant	Moderate	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Domiphen bromide	538-71-6	10%	10%	Organic salt, Onium, Ether	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	100%	100%	Alcohol	Liquid	SCNM	SCNM	SCNM	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Glycolic acid	79-14-1	10%	10%	Carboxylic acid, Alcohol	Solution	Category 2B	Category III	Nonirritant	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	10%	10%	Carboxylic acid, Alcohol	Solution	SCNM	Category III	SCNM	Severe	Irritant	Inconclusive	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	100%	100%	Carboxylic acid, Alcohol	Liquid	Category 1	Category I	R41	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Monoethanolamine	141-43-5	10%	10%	Amine, Alcohol	Solution	Category 2B	Category III	Nonirritant	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	100%	100%	Amine, Alcohol	Liquid	Nonirritant	Category III	Nonirritant	Moderate	Irritant	Inconclusive	Hagino et al. (1999)/Submitted Y. Ohno Data

Comparison of *In Vivo* and *In Vitro* Ocular Irritancy Classifications: Sorted by Reference

Substance Name	CASRN	*In Vitro* Conc. Tested	*In Vivo* Conc. Tested	Chemical Class	*In Vivo* Physical Form Tested	*In Vivo* (GHS) Classification[1,2]	*In Vivo* (EPA) Classification[3,4]	*In Vivo* (EU) Classification[5,6]	*In Vitro* Classification (IS(A))[7]	*FHSA-20%*[8]	*FHSA-67%*[9]	Reference
Triethanolamine	102-71-6	10%	10%	Amine, Alcohol	Solution	Nonirritant	Category IV	Nonirritant	Severe	Not labeled	Not labeled	Hagino et al. (1999)/Submitted Y. Ohno Data/ Bagley et al. (1992)
Ethanol	64-17-5	10%	10%	Alcohol	Solution	Nonirritant	Category IV	Nonirritant	Slight	Not labeled	Not labeled	Hagino et al. (1999)/Submitted Y. Ohno Data/ Kojima et al. (1995)
Potassium laurate	10124-65-9	10%	10%	Organic salt, Carboxylic acid salt	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data/ Kojima et al. (1995)
Stearyltrimethylammonium chloride	15461-40-2	10%	10%	Organic salt, Onium	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data/ Kojima et al. (1995)
Sodium lauryl sulfate	151-21-3	10%		Organic salt, Carboxylic acid salt	Unknown	Category 1/Category 2A	Category I/Category II	R41/Nonirritant	Moderate	Irritant	Irritant	Kojima et al. (1995)
Triton X-100	9002-93-1	10%		Ether	Unknown	Category 1	Category II	SCNM	Moderate	Irritant	Irritant	Kojima et al. (1995)

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; Conc. = Concentration; EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = Globally Harmonized System; SCNM - Study Criteria Not Met.

[1] GHS=Globally Harmonized System (UN [2003])

[2] Eye Irritant Category 1 = irreversible effects on the eye/serious damage to the eye; Category 2A = reversible effects on the eye/irritating to the eyes; Category 2B = reversible effects on the eye/mildly irritating to the eyes; Nonirritant = not an eye

[3] EPA=U.S. Environmental Protection Agency (EPA [1996]).

[4] Toxicity Category I for the Primary Eye Irritation Study = Corrosive, or corneal involvement or irritation not reversible within 21 days; Category II = Corneal involvement or irritation clearing in 8-21 days; Category III = Corneal involvement or irritation clearing in 1-7 daysl; Category IV: minimal effects clearing in less than 24 hr

[5] EU=European Union (EU [2001]).

[6] Risk phrase R41 = risk of serious damage to the eyes; R36 = irritating to the eyes; nonirritant = not an eye irritant.

[7] IS(A) represents irritation scores that were calculated using the method described in Leupke (1985); classification scheme used as described in Leupke (1985).

[8] FHSA=Federal Hazardous Substance Act (2005). FHSA-20% is based on the proportion of positive animals needed to identify a substance as an irritant using the FHSA sequential testing strategy, where 20% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥1 positive animal in a 3 to 5 animal test or ≥2 positive animals in a 6 animal test.

[9] FHSA=Federal Hazardous Substances Act (2005). FHSA-67% is based on the proportion of positive animals needed to identify a substance as an irritant using the "first test" of the FHSA sequential testing strategy, where 67% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥2/3, 3/4, 4/5, or 4/6 positive animals. If 1/3, 1/4, 2/4, 1/5, 2/5, 3/5, 2/6, or 3/6 animals were positive, further testing would be required.

Annex III-2

Comparison of *In Vivo* and *In Vitro* Ocular Irritancy Classifications: Sorted by Substance

This page intentionally left blank

Comparison of *In Vivo* and *In Vitro* Ocular Irritancy Classifications: Sorted by Substance

Substance Name	CASRN	*In Vitro* Conc. Tested	*In Vivo* Conc. Tested	Chemical Class	*In Vivo* Physical Form Tested	*In Vivo* (GHS) Classification[1,2]	*In Vivo* (EPA) Classification[3,4]	*In Vivo* (EU) Classification[5,6]	*In Vitro* Classification (IS(A))[7]	*FHSA-20%*[8]	*FHSA-67%*[9]	Reference
Acetic acid	64-19-7	10%	10%	Carboxylic acid	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Butanol	71-36-3	10%	10%	Alcohol	Solution	Category 1	SCNM	R41	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Cetyltrimethylammonium bromide	57-09-0	10%	10%	Organic salt, Onium	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Chlorhexidine gluconate Solution (20% Solution)	18472-51-0	10%	10%	Amidine, Ester	Solution	Category 2B	Category II	Nonirritant	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Di(2-ethylhexyl) sodium sulfosuccinate	577-11-7	10%	10%	Organic salt, Sulfur containing compound, Ester	Solution	Category 1	Category I	R36	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Diisopropanolamine	110-97-4	10%	10%	Amine, Alcohol	Solution	Nonirritant	Category III	Nonirritant	Moderate	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Domiphen bromide	538-71-6	10%	10%	Organic salt, Onium, Ether	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	100%	100%	Alcohol	Liquid	SCNM	SCNM	SCNM	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Ethanol	64-17-5	10%	10%	Alcohol	Solution	Nonirritant	Category IV	Nonirritant	Slight	Not labeled	Not labeled	Hagino et al. (1999)/Submitted Y. Ohno Data/ Kojima et al. (1995)
Glycolic acid	79-14-1	10%	10%	Carboxylic acid, Alcohol	Solution	Category 2B	Category III	Nonirritant	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	10%	10%	Carboxylic acid, Alcohol	Solution	SCNM	Category III	SCNM	Severe	Irritant	Inconclusive	Hagino et al. (1999)/Submitted Y. Ohno Data
Lactic acid	50-21-5	100%	100%	Carboxylic acid, Alcohol	Liquid	Category 1	Category I	R41	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Monoethanolamine	141-43-5	10%	10%	Amine, Alcohol	Solution	Category 2B	Category III	Nonirritant	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data
Oil/Water Emulsion-HZA		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Nonirritant	Irritant	Inconclusive	Gettings et al. (1994)

Comparison of *In Vivo* and *In Vitro* Ocular Irritancy Classifications: Sorted by Substance

Substance Name	CASRN	*In Vitro* Conc. Tested	*In Vivo* Conc. Tested	Chemical Class	*In Vivo* Physical Form Tested	*In Vivo* (GHS) Classification[1,2]	*In Vivo* (EPA) Classification[3,4]	*In Vivo* (EU) Classification[5,6]	*In Vitro* Classification (IS(A))[7]	*FHSA-20%*[8]	*FHSA-67%*[9]	Reference
Oil/Water Emulsion-HZC		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Nonirritant	Irritant	Inconclusive	Gettings et al. (1994)
Oil/Water Emulsion-HZE		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Nonirritant	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZF		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Moderate	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZH		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Severe	Irritant	Irritant	Gettings et al. (1994)
Oil/Water Emulsion-HZI		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Slight	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZJ		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Nonirritant	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZL		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Slight	Irritant	Irritant	Gettings et al. (1994)
Oil/Water Emulsion-HZM		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Moderate	Irritant	Irritant	Gettings et al. (1994)
Oil/Water Emulsion-HZN		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Slight	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZO		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Nonirritant	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZR		Undiluted	Undiluted	Formulation	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Gettings et al. (1994)
Oil/Water Emulsion-HZS		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Severe	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZT		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Slight	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZU		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Nonirritant	Not labeled	Not labeled	Gettings et al. (1994)
Oil/Water Emulsion-HZV		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Nonirritant	Irritant	Inconclusive	Gettings et al. (1994)
Oil/Water Emulsion-HZW		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Nonirritant	Irritant	Irritant	Gettings et al. (1994)
Oil/Water Emulsion-HZY		Undiluted	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Severe	Not labeled	Not labeled	Gettings et al. (1994)
Potassium laurate	10124-65-9	10%	10%	Organic salt, Carboxylic acid salt	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data/ Kojima et al. (1995)
Sodium lauryl sulfate	151-21-3	10%		Organic salt, Carboxylic acid salt	Unknown	Category 1/Category 2A	Category I/Category II	R41/Nonirritant	Moderate	Irritant	Irritant	Kojima et al. (1995)

Comparison of *In Vivo* and *In Vitro* Ocular Irritancy Classifications: Sorted by Substance

Substance Name	CASRN	*In Vitro* Conc. Tested	*In Vivo* Conc. Tested	Chemical Class	*In Vivo* Physical Form Tested	*In Vivo* (GHS) Classification[1,2]	*In Vivo* (EPA) Classification[3,4]	*In Vivo* (EU) Classification[5,6]	*In Vitro* Classification (IS(A))[7]	*FHSA-20%*[8]	*FHSA-67%*[9]	Reference
Stearyltrimethylammonium chloride	15461-40-2	10%	10%	Organic salt, Onium	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Hagino et al. (1999)/Submitted Y. Ohno Data/ Kojima et al. (1995)
Surfactant Based Formulation 1-HZA		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 2-HZB		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Slight	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 3-HZC		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 4-HZD		10%	Undiluted	Formulation	Solution	Category 2B	Category III	Nonirritant	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 5-HZE		10%	Undiluted	Formulation	Solution	SCNM	Category I	SCNM	Slight	Irritant	Inconclusive	Gettings et al. (1996)
Surfactant Based Formulation 6-HZF		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 7-HZG		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 8-HZH		10%	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Slight	Not labeled	Not labeled	Gettings et al. (1996)
Surfactant Based Formulation 9-HZI		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 10-HZJ		10%	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Slight	Not labeled	Not labeled	Gettings et al. (1996)
Surfactant Based Formulation 11-HZK		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 12-HZL		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 13-HZM		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Slight	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 14-HZN		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 15-HZP		10%	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Slight	Irritant	Inconclusive	Gettings et al. (1996)
Surfactant Based Formulation 16-HZQ		10%	Undiluted	Formulation	Solution	Nonirritant	Category III	Nonirritant	Slight	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 17-HZR		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 18-HZS		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 19-HZT		10%	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Nonirritant	Not labeled	Not labeled	Gettings et al. (1996)
Surfactant Based Formulation 20-HZU		10%	Undiluted	Formulation	Solution	Category 2B	Category III	R36	Slight	Irritant	Irritant	Gettings et al. (1996)

Comparison of *In Vivo* and *In Vitro* Ocular Irritancy Classifications: Sorted by Substance

Substance Name	CASRN	*In Vitro* Conc. Tested	*In Vivo* Conc. Tested	Chemical Class	*In Vivo* Physical Form Tested	*In Vivo* (GHS) Classification[1,2]	*In Vivo* (EPA) Classification[3,4]	*In Vivo* (EU) Classification[5,6]	*In Vitro* Classification (IS(A))[7]	*FHSA-20%*[8]	*FHSA-67%*[9]	Reference
Surfactant Based Formulation 21-HZV		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 22-HZW		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 23-HZX		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Severe	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 24-HZY		10%	Undiluted	Formulation	Solution	Category 1	Category I	R41	Moderate	Irritant	Irritant	Gettings et al. (1996)
Surfactant Based Formulation 25-HZZ		10%	Undiluted	Formulation	Solution	Nonirritant	Category IV	Nonirritant	Nonirritant	Not labeled	Not labeled	Gettings et al. (1996)
Triethanolamine	102-71-6	100%	100%	Amine, Alcohol	Liquid	Nonirritant	Category III	Nonirritant	Moderate	Irritant	Inconclusive	Hagino et al. (1999)/Submitted Y. Ohno Data
Triethanolamine	102-71-6	10%	10%	Amine, Alcohol	Solution	Nonirritant	Category IV	Nonirritant	Severe	Not labeled	Not labeled	Hagino et al. (1999)/Submitted Y. Ohno Data/ Bagley et al. (1992)
Triton X-100	9002-93-1	1%	1%	Ether	Solution	Nonirritant	Category III	Nonirritant	Severe	Irritant	Inconclusive	Bagley et al. (1992)
Triton X-100	9002-93-1	10%		Ether	Unknown	Category 1	Category II	SCNM	Moderate	Irritant	Irritant	Kojima et al. (1995)

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; Conc. = Concentration; EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = Globally Harmonized System; SCNM - Study Criteria Not Met.

[1] GHS=Globally Harmonized System (UN [2003])

[2] Eye Irritant Category 1 = irreversible effects on the eye/serious damage to the eye; Category 2A = reversible effects on the eye/irritating to the eyes; Category 2B = reversible effects on the eye/mildly irritating to the eyes; Nonirritant = not an eye

[3] EPA=U.S. Environmental Protection Agency (EPA [1996]).

[4] Toxicity Category I for the Primary Eye Irritation Study = Corrosive, or corneal involvement or irritation not reversible within 21 days; Category II = Corneal involvement or irritation clearing in 8-21 days; Category III = Corneal involvement or irritation clearing in 1-7 days; Category IV: minimal effects clearing in less than 24 hr

[5] EU=European Union (EU [2001]).

[6] Risk phrase R41 = risk of serious damage to the eyes; R36 = irritating to the eyes; nonirritant = not an eye irritant.

[7] IS(A) represents irritation scores that were calculated using the method described in Leupke (1985); classification scheme used as described in Leupke (1985).

[8] FHSA=Federal Hazardous Substance Act (2005). FHSA-20% is based on the proportion of positive animals needed to identify a substance as an irritant using the FHSA sequential testing strategy, where 20% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥1 positive animal in a 3 to 5 animal test or ≥2 positive animals in a 6 animal test.

[9] FHSA=Federal Hazardous Substances Act (2005). FHSA-67% is based on the proportion of positive animals needed to identify a substance as an irritant using the "first test" of the FHSA sequential testing strategy, where 67% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥2/3, 3/4, 4/5, or 4/6 positive animals. If 1/3, 1/4, 2/4, 1/5, 2/5, 3/5, 2/6, or 3/6 animals were positive, further testing would be required.

Appendix F

Background Review Document
Current Status of *In Vitro* Test Methods for Identifying
Mild/Moderate Ocular Irritants:
The Isolated Chicken Eye (ICE) Test Method

This page intentionally left blank

Background Review Document
Current Status of *In Vitro* Test Methods for Identifying Mild/Moderate Ocular Irritants:
The Isolated Chicken Eye (ICE) Test Method

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

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

This page intentionally left blank

Table of Contents

List of Tables

List of Figures

List of Abbreviations and Acronyms

BCOP	Bovine corneal opacity and permeability
BRD	Background review document
CASRN	Chemical Abstracts Service Registry number
CEET	Chicken enucleated eye test
CPSC	U.S. Consumer Product Safety Commission
EC	European Commission
EC/HO	European Commission/British Home Office
ECVAM	European Center for the Validation of Alternative Methods
EPA	U.S. Environmental Protection Agency
EU	European Union
FDA	U.S. Food and Drug Administration
FHSA	U.S. Federal Hazardous Substances Act
FR	*Federal Register*
FRAME	Fund for the Replacement of Animals in Medical Experiments
GHS	Globally Harmonized System
GLP	Good Laboratory Practice
HET-CAM	Hen's egg test–chorioallantoic membrane
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Isolated chicken eye
INVITTOX	*In Vitro* Techniques in Toxicology Database
IRE	Isolated rabbit eye
MeSH	U.S. National Library of Medicine Medical Subject Heading
MMAS	Modified maximum average score
NC	Not Classified (as irritant)
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIH	National Institutes of Health
NL	Not Labeled (as irritant)
OECD	Organisation for Economic Co-operation and Development
OPPTS	EPA Office of Prevention, Pesticides and Toxic Substances
OSHA	U.S. Occupational Safety & Hazards Administration
OTWG	Ocular Toxicity Working Group
TNO	TNO Nutrition and Food Research
UN	United Nations
ZEBET	German Center for Documentation and Evaluation of Alternative Methods to Animal Experiments

Interagency Coordinating Committee on the Validation of Alternative Methods: Agency Representatives

Agency for Toxic Substances and Disease Registry
* Moiz Mumtaz, Ph.D.
Bruce Fowler, Ph.D.
Edward Murray, Ph.D.
Eric Sampson, Ph.D.

Consumer Product Safety Commission
* Marilyn L. Wind, Ph.D. (Chair)
+ Kristina Hatlelid, Ph.D.
Joanna Matheson, Ph.D.

Department of Agriculture
* Jodie Kulpa-Eddy, D.V.M. (Vice-Chair)
+ Elizabeth Goldentyer, D.V.M.

Department of Defense
* Robert E. Foster, Ph.D.
+ Patty Decot
Harry Salem, Ph.D.
Peter J. Schultheiss, D.V.M., DACLAM

Department of Energy
* Michael Kuperberg, Ph.D.
+ Marvin Stodolsky, Ph.D.

Department of the Interior
* Barnett A. Rattner, Ph.D.
+ Sarah Gerould, Ph.D. (to Feb. 2009)

Department of Transportation
* George Cushmac, Ph.D.
+ Steve Hwang, Ph.D.

Environmental Protection Agency
Office of Pesticide Programs
* John R. "Jack" Fowle III, Ph.D., DABT
+ Vicki Dellarco, Ph.D.
+ Tina Levine, Ph.D.
Deborah McCall
Christine Augustyniak, Ph.D. *(U.S. Coordinator, OECD Test Guidelines Program)*
Office of Pollution Prevention and Toxics
Jerry Smrchek, Ph.D. *(U.S. Coordinator, OECD Test Guidelines Program,* to July 2009*)*
Office of Research and Development
Suzanne McMaster, Ph.D. (to Dec. 2008)
Julian Preston, Ph.D. (to July 2009)
Stephanie Padilla, Ph.D. (to July 2009)
Office of Science Coordination and Policy
Karen Hamernik, Ph.D. (to July 2009)

Food and Drug Administration
Office of the Commissioner
* Suzanne Fitzpatrick, Ph.D., DABT
Center for Biologics Evaluation and Research
Richard McFarland, Ph.D., M.D.
Ying Huang, Ph.D.
Center for Devices and Radiological Health
Melvin E. Stratmeyer, Ph.D.
Vasant G. Malshet, Ph.D., DABT
Center for Drug Evaluation and Research
+ Abigail C. Jacobs, Ph.D.
Paul C. Brown, Ph.D.
Center for Food Safety and Applied Nutrition
David G. Hattan, Ph.D.
Robert L. Bronaugh, Ph.D.
Center for Veterinary Medicine
Devaraya Jagannath, Ph.D.
M. Cecilia Aguila, D.V.M.
National Center for Toxicological Research
Paul Howard, Ph.D.
Donna Mendrick, Ph.D.
William T. Allaben, Ph.D. (to Jan. 2009)
Office of Regulatory Affairs
Lawrence D'Hoostelaere, Ph.D.

National Cancer Institute
* T. Kevin Howcroft, Ph.D.
Chand Khanna, D.V.M., Ph.D.
Alan Poland, M.D. (to Oct. 2008)

National Institute of Environmental Health Sciences
* William S. Stokes, D.V.M., DACLAM
+ Raymond R. Tice, Ph.D.
Rajendra S. Chhabra, Ph.D., DABT
Jerrold J. Heindel, Ph.D.

National Institute for Occupational Safety and Health
* Paul Nicolaysen, V.M.D.
+ K. Murali Rao, M.D., Ph.D.

National Institutes of Health
* Margaret D. Snyder, Ph.D.

National Library of Medicine
* Pertti (Bert) Hakkinen, Ph.D.
+ Jeanne Goshorn, M.S.

Occupational Safety and Health Administration
* Surender Ahir, Ph.D.

* Principal agency representative
\+ Alternate principal agency representative

Acknowledgements

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Ocular Toxicity Working Group (OTWG)

U.S. Consumer Product Safety Commission
Marilyn L. Wind, Ph.D.
Adrienne Layton, Ph.D.

U.S. Department of Defense
Harry Salem, Ph.D.

U.S. Department of Transportation
Steve Hwang, Ph.D.

U.S. Environmental Protection Agency
Office of Pesticide Programs
Meta Bonner, Ph.D.
Jonathan Chen, Ph.D.
John R. "Jack" Fowle III, Ph.D., DABT
Masih Hashim, D.V.M., Ph.D.
Karen Hicks
Marianne Lewis
Debbie McCall
Timothy McMahon, Ph.D.
Mark Perry
John Redden
Jenny Tao, Ph.D.
Office of Research and Development
Andrew Geller, Ph.D.
Office of Science Coordination and Policy
Karen Hamernik, Ph.D.

U.S. Food and Drug Administration
Center for Drug Evaluation and Research
Paul Brown, Ph.D.
Wiley Chambers, M.D.
Abigail (Abby) Jacobs, Ph.D.
Jill Merrill, Ph.D., DABT (OTWG Chair)
Center for Food Safety and Applied Nutrition
Robert Bronaugh, Ph.D.
Donnie Lowther
Office of the Commissioner
Suzanne Fitzpatrick, Ph.D., DABT

National Institute of Environmental Health Sciences
Warren Casey, Ph.D., DABT
Mark F. Cesta, D.V.M, DACVP
Raymond (Buck) Grissom, Ph.D.
William Stokes, D.V.M., DACLAM

Occupational Safety and Health Administration
Surender Ahir, Ph.D.

European Centre for the Validation of Alternative Methods – Liaison
João Barroso, Ph.D.
Thomas Cole, Ph.D.
Valerie Zuang, Ph.D.

Japanese Center for the Validation of Alternative Methods – Liaison
Hajime Kojima, Ph.D.

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

National Institute of Environmental Health Sciences

William Stokes, D.V.M., DACLAM
Director; Project Officer

Warren Casey, Ph.D., DABT
Deputy Director

Deborah McCarley
Special Assistant; Assistant Project Officer

NICEATM Support Contract Staff (Integrated Laboratory Systems [ILS], Inc.)

David Allen, Ph.D.
Jonathan Hamm, Ph.D.
Nelson Johnson
Brett Jones, Ph.D.
Elizabeth Lipscomb, Ph.D.
Linda Litchfield
Steven Morefield, M.D.
Catherine Sprankle
James Truax, M.A.
Linda Wilson

Statistical Consultant for ILS, Inc.

Joseph Haseman, Ph.D.

Additional Reviewers for the *In Vitro* Ocular Corrosion and Irritation Test Methods Background Review Documents

Chantra Eskes, Eng., Ph.D.
ECVAM
Ispra, Italy

Robert L Guest Bsc, CBiol, MlBiol
SafePharm Laboratories, Ltd.
Derby, United Kingdom

John Harbell, Ph.D.
Institute for In Vitro Sciences
Gaithersburg, Maryland

Joe Haseman, Ph.D.
Consultant
Raleigh, North Carolina

Penny Jones
Unilever Research
Sharnbrook, United Kingdom

Menk Prinsen
TNO Nutrition & Food Research Institute
The Netherlands

Horst Spielmann, Dr.med.
ZEBET
Berlin, Germany

Companies and Individuals that Provided *In Vitro* and/or *In Vivo* Data for the Isolated Chicken Eye Test Method Background Review Document

ICCVAM and NICEATM gratefully acknowledge the following individuals and institutions that submitted data to NICEATM for the evaluation of alternative ocular safety testing methods and approaches

ECVAM
Chantra Eskes, Ph.D.

The Procter and Gamble Company
Dan Marsman, Ph.D.

TNO Nutrition & Food Research Institute
Menk Prinsen

This page intentionally left blank

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 Safety and Health 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. 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. Tests now require only one to three rabbits, compared to six rabbits per test in the original protocol. Provisions have been added that allow for animals with severe lesions or discomfort to be humanely euthanized.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) previously evaluated the validation status of the bovine corneal opacity and permeability (BCOP), isolated chicken eye (ICE), isolated rabbit eye (IRE), and hen's egg test–chorioallantoic membrane (HET-CAM) test methods for the identification of ocular corrosives or severe (irreversible) ocular irritants. ICCVAM used the EPA (2003a), United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UN 2007), and European Union (EU 2001) regulatory hazard classification systems. In ICCVAM's assessment, the performance of the BCOP and ICE test methods substantiated their use in testing some substances for regulatory hazard classification. The IRE and HET-CAM test methods lacked sufficient performance and/or sufficient data to substantiate their use for regulatory hazard classification.

ICCVAM recommended that the BCOP and ICE test methods should be used in a tiered-testing strategy in which positive substances can be classified as ocular corrosives or severe irritants without animal testing. In accordance with the ICCVAM Authorization Act of 2000 (Public Law 106-545), these recommendations were made available to the public and provided to U.S. Federal agencies for consideration in the *ICCVAM Test Method Evaluation Report* – In Vitro *Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives* (ICCVAM 2006b). The ICCVAM recommendations were accepted by U.S. Federal agencies, and *in vitro* test methods may now be used instead of the Draize rabbit eye test for certain regulatory testing purposes.

ICCVAM is now reviewing the validation status of these *in vitro* test methods for identification of nonsevere ocular irritants (that is, those that induce reversible ocular damage [EPA Category II, III; EU Category R36, GHS Category 2A, 2B]) and substances Not Classified as irritant (GHS NC or Not Labeled, EPA Category IV, FHSA Not Labeled, or EU Not Labeled) according to the GHS (UN 2007), EPA (EPA 2003a), FHSA (FHSA 2005), and EU (EU 2001) classification systems. The Federal Hazardous Substances Act (FHSA) classification system (FHSA 2005) as defined in the "Test for Eye Irritants" (i.e., "Irritant" or Not Labeled [as an irritant]) and published in 16 CFR 1500.42 (CPSC 2003) is also provided in the current background review documents. The FHSA classification system was not used in the previous analyses of test methods used for the identification of severe ocular irritants or corrosives because the FHSA classification is limited to irritants and is not intended to identify corrosive substances or to differentiate between severe and nonsevere irritants.

Accordingly, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the ICCVAM Ocular Toxicity Working Group (OTWG) prepared draft background review documents that summarize the current validation status of each test

method based on published studies and other data and information submitted in response to a June 7, 2007, *Federal Register* request (72 FR 31582, available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_10966.pdf). The background review documents (BRDs) form the basis for draft ICCVAM test method recommendations, which are provided in separate documents. Liaisons from the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods will provide input and contribute to the OTWG throughout the evaluation process.

An international independent scientific peer review panel (Panel) met in public session on May 19-21, 2009, to develop conclusions and recommendations on the *in vitro* BCOP, ICE, IRE, and HET-CAM test methods. 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 can use the subsequent independent Panel report (ICCVAM 2009) to deliberate and develop their own test method recommendations. The Panel considered these BRDs and evaluated the extent to which the available information supports the draft ICCVAM test method recommendations.

ICCVAM provided the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) with the draft BRD and draft Test Method Evaluation Report, the Panel's report, and all public comments. SACATM discussed these at their June 25–26, 2009, meeting, where public stakeholders were given another opportunity to comment. After SACATM's meeting, ICCVAM considered the SACATM comments, the Panel report, and all public comments before finalizing the Background Review Document and test method recommendations. These recommendations 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 BRD, including the following staff from the NICEATM support contractor, Integrated Laboratory Systems, Inc.: David Allen, Jon Hamm, Nelson Johnson, Brett Jones, Elizabeth Lipscomb, Linda Litchfield, Steven Morefield, Gregory Moyer, Catherine Sprankle, and Jim Truax. We also thank the members of the ICCVAM Ocular Toxicity Working Group, chaired by Karen Hamernik, Ph.D. (U.S. EPA) and Jill Merrill, Ph.D. (U.S. Food and Drug Administration), and ICCVAM representatives who reviewed and commented on draft versions. We also thank Valerie Zuang, Ph.D., and Dr. Hajime Kojima, Ph.D., the liaisons to the Ocular Toxicity Working Group from the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods, respectively, for their participation.

Marilyn Wind, Ph.D.
Deputy Associate Executive Director
Directorate for Health Sciences
U.S. Consumer Product Safety Commission
Chair, ICCVAM

William S. Stokes, D.V.M., DACLAM
Rear Admiral/Assistant Surgeon General, U.S. Public Health Service
Director, NICEATM
Executive Director, ICCVAM

Executive Summary

In October 2003, the U.S. Environmental Protection Agency (EPA) submitted to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) a nomination requesting the evaluation of several activities related to reducing, refining, and replacing the use of rabbits in the current *in vivo* Draize rabbit eye test (69 FR 13859 [March 24, 2004]). In response to this nomination, ICCVAM evaluated the validation status of the bovine corneal opacity and permeability (BCOP), hen's egg test–chorioallantoic membrane (HET-CAM), isolated chicken eye (ICE), and isolated rabbit eye (IRE) test methods. To evaluate how well these test methods identify ocular corrosives and severe irritants, ICCVAM used the EPA (2003a), European Union (EU 2001), and United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UN 2007) classification systems.

ICCVAM considered the performance of two of these *in vitro* test methods, the BCOP and the ICE, to be sufficient to support their use in testing certain types of substances for regulatory hazard classification. The IRE and HET-CAM test methods lacked sufficient performance and/or sufficient data to support their use for regulatory hazard classification. ICCVAM recommended that the BCOP and ICE test methods should be used in a tiered-testing strategy that would classify positive substances as ocular corrosives or severe irritants without animal testing. These recommendations were accepted by U.S. Federal agencies, and, as a result, *in vitro* test methods may now be used instead of conventional tests for certain regulatory testing purposes.

ICCVAM is now reviewing the validation status of these *in vitro* test methods to identify nonsevere ocular irritants (those that cause reversible ocular damage [EPA Category II and III; EU R36; GHS Category 2A and 2B]) and substances not labeled as irritants (EPA Category IV; EU Not Labeled; GHS Not Classified) according to the EPA (2003a), EU (2001), and GHS (UN 2007) classification systems. The FHSA classification system, which is based on the testing guidelines and associated criteria included in 16 CFR 1500.42 (CPSC 2003), is also included in these evaluations. The FHSA classification system was not used in the original analyses (ability of the test methods to identify ocular corrosives and severe irritants) because the FHSA ocular hazard category that is assigned based on results from the Draize rabbit eye test (Draize et al. 1944) does not distinguish between ocular corrosives and severe irritants and less severe irritants. For this reason, an evaluation to identify ocular corrosives and severe irritants using the FHSA classification system was not possible.

Because the FHSA classification system (2005) is based on a sequential testing strategy that uses up to 18 animals, only a small percentage of the substances in the ICE database would be classifiable if the FHSA criteria were strictly applied. To maximize the number of substances included in these analyses, "proportionality" criteria were applied for the purpose of assigning an FHSA classification to test results that would require additional testing according to the FHSA sequential testing strategy. These "proportionality" criteria (FHSA-20% and FHSA-67%) are as follows:

- FHSA-20% is based on the proportion of positive animals needed to identify a substance as an irritant using the FHSA sequential testing strategy, where 20% of the animals must demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥1 positive animal in a 3- to 5-animal test or ≥2 positive animals in a 6-animal test.
- FHSA-67% is based on the proportion of positive animals needed to identify a substance as an irritant using the "first test" of the FHSA sequential testing strategy, where 67% of the animals must demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled as an irritant if ≤1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an

irritant if there were ≥2/3, 3/4, 4/5, or 4/6 positive animals. If 1/3, 1/4, 2/4, 1/5, 2/5, 3/5, 2/6, or 3/6 animals were positive, further testing would be required.

Together, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the ICCVAM Ocular Toxicity Working Group prepared draft background review documents (BRDs) that summarize the available data and information regarding the validity (usefulness and limitations) of each test method. This BRD summarizes all available information for the ICE test method and its current validation status, including what is known about its reliability and accuracy, and the scope of the substances tested. Original data for the ICE test method will be maintained for future use so that these performance statistics may be updated as additional information becomes available.

ICE Test Method Protocol

The ICE test method is an *in vitro* model that provides short-term maintenance of the chicken eye. Damage caused by a test substance is assessed by determination of corneal swelling, opacity, and fluorescein retention. While the latter two parameters involve a qualitative assessment, analysis of corneal swelling provides for a quantitative assessment. Each measurement is either (1) converted into a quantitative score that is used to calculate an overall irritation index or (2) assigned a qualitative category that is used to assign an *in vitro* ocular irritancy classification. Either outcome can then be used to predict the *in vivo* ocular irritation potential of a test substance.

Validation Database

No new ICE data have been obtained since ICCVAM evaluated the ICE test method for identifying ocular corrosives and severe irritants (ICCVAM 2006a). Therefore, the same database was used in the current evaluation. The ICE validation database contains a total of 175 substances. The most commonly tested chemical classes tested are alcohols, carboxylic acids, esters, and heterocyclics. Of the 175 substances, 48% (85/175) could not be assigned a specific chemical class. The most commonly tested product classes are solvents, soaps/surfactants, industrial chemicals, and pesticides/herbicides. Thirteen percent (23/175) could not be assigned a product class.

In order to calculate the appropriate EPA (2003a), EU (2001), FHSA (2005), and GHS (UN 2007) ocular irritancy hazard classifications, detailed *in vivo* data consisting of cornea, iris, and conjunctiva scores for each animal at 24, 48, and 72 hours following test substance administrations and/or assessment of the presence or absence of lesions at 7, 14, and 21 days are needed. Some of the test substances had only limited *in vivo* data and so could not be used to evaluate test method accuracy and reliability. To maximize the number of substances included in the FHSA analyses, "proportionality" criteria (FHSA-20% and FHSA-67%), as outlined above, were applied for the purpose of assigning a FHSA classification to test results that would require additional testing according to the FHSA sequential testing strategy.

ICE Test Method Accuracy

Identification of All Ocular Hazard Categories

ICCVAM evaluated how well the ICE test method identified all categories of ocular irritation potential as defined by the EPA (2003a), GHS (UN 2007), and EU (2001) classification systems. Because the FHSA classification system does not distinguish between ocular corrosives and severe irritants and less severe irritants, an evaluation for all ocular hazard categories using the FHSA classification system was not possible. Analyses were also performed excluding specific chemical classes and/or physical properties that were previously identified as discordant in the ICE test method (alcohols, surfactants, and solids) relative to the *in vivo* hazard classification (ICCVAM 2006a).

As shown in **Table 1**, overall correct classifications ranged from 59% (83/141) to 77% (118/153) when using the entire database, depending on the hazard classification system used. When discordant

classes are excluded, overall correct classifications improved slightly to a range of 64% (49/77) to 80% (66/82), depending on the classification system used.

Distinguishing Substances Not Labeled as Irritants from All Other Hazard Categories

ICCVAM also evaluated how well the ICE test method distinguished substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other ocular hazard categories (EPA Categories I, II, III; EU R41, R36; FHSA Irritant; GHS Categories 1, 2A, 2B) as defined by the EPA (2003a), GHS (UN 2007), EU (2001), and FHSA (2005) classification systems. Analyses were also performed excluding specific chemical classes and/or physical properties that were previously identified as discordant in the ICE test method (alcohols, surfactants, and solids) relative to the *in vivo* hazard classification (ICCVAM 2006a).

As shown in **Table 2**, overall accuracy ranged from 78% (110/141) to 85% (130/153), depending on the hazard classification system used. The lowest false negative rate (6% [4/62]) was noted for the GHS system, followed by 9% (7/76) for the FHSA-67% criteria, 12% (10/84) for the FHSA-20% criteria, 14% (11/81) for the EPA system, and 22% (13/60) for the EU system. Among these false negatives, at least one substance was classified as an ocular corrosive and severe irritant based on Draize rabbit eye test data (n=1 each for the EPA and GHS systems, and n=6 for the EU system). The lowest false positive rate (11% [10/93]) was noted for the EU system, followed by 22% (13/59) for the EPA system, 24% (15/62) for the FHSA-20% and FHSA-67% criteria, and 34% (27/79) for the GHS system. The exclusion of discordant classes had no affect on accuracy (ranged from 75% [58/77] to 85% [70/82] when discordant classes were removed versus 78% [110/141] to 85% [130/153] for overall accuracy, depending on the hazard classification system used).

ICE Test Method Reliability

Interlaboratory Reproducibility

Previous quantitative and qualitative evaluations of the reliability of the ICE test method have been conducted (ICCVAM 2006a). Because the database used for the current evaluation of the ICE test method has not changed, the quantitative evaluation of test method reliability remains unchanged. Additional qualitative analyses of interlaboratory reproducibility were conducted to evaluate how well the ICE hazard classifications agreed among the four participating laboratories from the interlaboratory validation study (Balls et al. 1995). These evaluations were based on the use of the ICE test method (1) to identify all ocular hazard categories according to the EPA, GHS, or EU systems, and (2) to distinguish substances not labeled as irritants (EPA Category IV, GHS Not Classified, EU Not Labeled) from all other ocular hazard categories (EPA Categories I, II, III; GHS Categories 1, 2A, 2B; EU R41, R36). Because the performance of the ICE test method was similar for the EPA and FHSA hazard classification systems, additional reliability analyses were not conducted for the FHSA hazard classification system.

Table 1 Performance of the ICE Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA, GHS, and EU Classification Systems[1]

Hazard Classification System	Overall Correct Classification	Severe[2]		Moderate[3]			Mild[4]			Not Labeled[5]	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Overall (EPA)	62% (87/140)	48% (13/27)	52% (14/27)	31% (5/16)	50% (8/16)	19% (3/16)	29% (11/38)	53% (20/38)	18% (7/38)	22% (13/59)	78% (46/59)
Without Alcohols, Surfactants, and Solids[6]	67% (52/78)	67% (6/9)	33% (3/9)	20% (2/10)	60% (6/10)	20% (2/10)	17% (1/6)	67% (4/6)	17% (1/6)	21% (8/39)	79% (31/39)
Overall (GHS)	59% (83/141)	52% (15/29)	48% (14/29)	36% (8/22)	36% (8/22)	28% (6/22)	18% (2/11)	73% (8/11)	9% (1/11)	34% (27/79)	66% (52/79)
Without Alcohols, Surfactants, and Solids	64% (49/77)	63% (5/8)	37% (3/8)	23% (3/13)	46% (6/13)	31% (4/13)	17% (1/6)	67% (4/6)	17% (1/6)	32% (16/50)	68% (34/50)
Overall (EU)	77% (118/153)	59% (19/32)	41% (13/32)	18% (5/28)	57% (16/28)	25% (7/28)	NA	NA	NA	11% (10/93)	89% (83/93)
Without Alcohols, Surfactants, and Solids	80% (66/82)	67% (6/9)	33% (3/9)	18% (3/17)	65% (11/17)	18% (3/17)	NA	NA	NA	13% (7/56)	87% (49/56)

Abbreviations: EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = Globally Harmonized System; ICE = isolated chicken eye; NA = not applicable.

[1] EPA classification system (EPA 2003a); GHS classification system (UN 2007); EU classification system (EU 2001). Because the FHSA classification system does not distinguish between ocular corrosives and severe irritants and less severe irritants, an evaluation for all ocular hazard categories using the FHSA classification system was not possible.

[2] Severe = EPA Category I; GHS Category 1, EU R41.

[3] Moderate = EPA Category II; GHS Category 2A; EU R36.

[4] Mild = EPA Category III; GHS Category 2B.

[5] Not Labeled = EPA Category IV; GHS Not Classified; EU Not Labeled.

[6] Alcohols, surfactants, and solids were previously identified as discordant in the ICE test method relative to the *in vivo* hazard classification (ICCVAM 2006a).

Table 2 Accuracy of the ICE Test Method in Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes as Defined by the EPA, GHS, EU, and FHSA Classification Systems

Hazard Classification System	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall (EPA)[1]	140	83	116/140	86	70/81	78	46/59	22	13/59	14	11/81
Without Alcohols, Surfactants, and Solids[2]	78	82	69/78	85	33/39	79	31/39	21	8/39	15	6/39
Overall (GHS)[3]	141	78	110/141	94	58/62	66	52/79	34	27/79	6	4/62
Without Alcohols, Surfactants, and Solids	77	75	58/77	89	24/27	68	34/50	32	16/50	11	3/27
Overall (EU)[4]	153	85	130/153	78	47/60	89	83/93	11	10/93	22	13/60
Without Alcohols, Surfactants, and Solids	82	85	70/82	81	51/26	88	49/56	12	7/56	19	5/26
Overall (FHSA-20%)[5]	146	83	121/146	88	74/84	76	47/62	24	15/62	12	10/84
Without Alcohols, Surfactants, and Solids	76	82	62/76	86	31/36	78	31/40	23	9/40	14	5/36
Overall (FHSA-67%)[5]	138	84	116/138	91	69/76	76	47/62	24	15/62	9	7/76
Without Alcohols, Surfactants, and Solids	72	82	59/72	88	28/32	78	31/40	23	9/40	13	4/32

Abbreviations: EPA = U.S. Environmental Protection Agency; EU = European Union; FHSA = Federal Hazardous Substances Act; GHS = Globally Harmonized System; ICE = isolated chicken eye; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] EPA classification system (EPA 2003a): Category IV vs. Category I/II/III.

[2] Alcohols, surfactants, and solids were previously identified as discordant in the ICE test method relative to the in vivo hazard classification (ICCVAM 2006a).

[3] GHS classification system (UN 2007): Not Classified vs. Category 1/2A/2B.

[4] EU classification system (EU 2001): Not Labeled vs. R41/R36.

[5] FHSA classification system (FHSA 2005): Not Labeled vs. Irritant. To maximize the number of substances included in the FHSA analyses, "proportionality" criteria (FHSA-20% and FHSA-67%) were applied for the purpose of assigning a FHSA classification to test results that would require additional testing according to the FHSA sequential testing strategy.

Using the first approach (identifying all ocular hazard categories), there was 100% agreement among the four laboratories for a majority of the Draize ocular corrosives and severe irritants based on all three classification systems, whether they were correctly identified or underclassified by the ICE test method. For example, for the EPA system, there was 100% agreement for 70% (7/10) of the correctly identified Category I substances. There was also 100% agreement among the four laboratories for at least 50% (3/6 to 3/5) of the correctly identified moderate ocular irritants (EPA Category II, GHS Category 2A, EU R36). For the mild ocular irritants (EPA Category III, GHS Category 2B), there was 100% agreement among the four laboratories for 0% (0/2) to 13% (1/8) of the correctly identified substances. The four laboratories had only 50% agreement for 50% (4/8 or 1/2) of these substances for the EPA and GHS classification systems. A majority of the substances not classified as irritants (EPA Category IV, EU Not Labeled, GHS Not Classified) based on Draize results were overclassified by the ICE test method. The four laboratories had at least 75% agreement for all but two of these substances. For example, there was at least 75% agreement for 85% (11/13) of the GHS Not Labeled substances overclassified by the ICE test method. The four laboratories had at least 75% agreement for 76% (13/17) of the EU Not Labeled substances, whether they were correctly identified or overclassified by the ICE test method. For example, there was at least 75% agreement for 77% (7/9) of the EU Not Labeled substances that were correctly identified and 75% (6/8) of those overclassified by the ICE test method.

Using the second approach (distinguishing substances not labeled as irritants from all other ocular hazard categories), there was 100% agreement among the four laboratories for 61% (36/59) to 75% (44/59) of the substances included in the Balls et al. (1995) study. There was 100% agreement among the four laboratories for 81% (38/47) of the substances correctly identified as irritants according to the EPA system (Category I, II, III). While none of the EPA Category IV substances was correctly identified by the ICE test method, there was 75% agreement among the four laboratories for both of the Category IV substances that were overpredicted by the ICE test method.

The four laboratories had 100% agreement for 87% (33/38) of the substances correctly identified as irritants according to the GHS system (Category 1, 2A, 2B). While only one of the GHS substances not labeled as irritants was correctly identified by the ICE test method (for which there was 75% agreement among the laboratories), there was at least 75% agreement among the four laboratories for 85% (11/13) of the GHS substances not labeled as irritants that were overpredicted by the ICE test method. There was 100% agreement among the four laboratories for 85% (22/26) of the substances correctly identified as irritants according to the EU system (R36 or R41). The laboratories had at least 75% agreement for 77% (7/9) of the substances correctly identified as Not Labeled.

1.0 Introduction

1.1 Background

The current Draize rabbit eye test method identifies both irreversible (i.e., corrosive) and reversible ocular effects. It also provides quantitative scoring with which to categorize the severity of reversible effects such as mild, moderate, or severe irritation. The current U.S. Environmental Protection Agency health effects test guideline for acute eye irritation (EPA 1998) and United Nations Globally Harmonized System (GHS) of Classification and Labelling of Chemicals (UN ocular testing strategy) indicate that if serious ocular damage is anticipated (e.g., a lesion considered to be irreversible or persisting for 21 days), then a test on a single animal may be considered. If serious damage is observed, no further animal testing is necessary (EPA 1998; UN 2007). If no serious damage is observed, additional test animals (1 or 2 rabbits) may be evaluated sequentially until concordant irritant or nonirritant responses are observed based on the GHS (UN 2007) or until unequivocal results are obtained in a minimum of three animals according to the EPA test guideline (EPA 1998). In the FHSA classification system (FHSA 2005), which is based on the testing guidelines and associated criteria included in 16 CFR 1500.42 (CPSC 2003), corrosive substances are identified by other test methods (e.g., Draize skin test or human accidental exposure data) and excluded from further irritant testing.

In 2006, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) completed an evaluation of the isolated chicken eye (ICE) test method for its ability to identify ocular corrosives and severe irritants (ICCVAM 2006a). ICCVAM concluded that the ICE test method could be used, in appropriate circumstances and with certain limitations, as a screening test to identify substances as ocular corrosives and severe irritants (i.e., EPA Category I, GHS Category 1, European Union [EU] R41) (ICCVAM 2006b). While it was not considered valid as a complete replacement for the *in vivo* rabbit eye test, the ICE test method was recommended for use as part of a tiered-testing strategy for regulatory classification and labeling within a specific applicability domain. Accordingly, substances that are positive in this test method can be classified as ocular corrosives or severe irritants without further testing in rabbits, while a substance that tests negative would need additional testing in rabbits using a sequential testing strategy as outlined in Organisation for Economic Co-operation and Development Test Guideline 405 (OECD 2002).

ICCVAM is now evaluating the usefulness and limitations of the ICE test method for identifying nonsevere irritants (i.e., those that induce reversible ocular damage [EPA Category II and III; EU R36; GHS Category 2A and 2B]) and substances not labeled as irritants (i.e., EPA Category IV; EU Not Labeled; FHSA Not Labeled; GHS Not Classified) according to the EPA, EU, FHSA, and GHS classification systems (EPA 2003a; EU 2001; FHSA 2005; UN 2007). However because the FHSA classification system (2005) is based on a sequential testing strategy, which uses up to 18 animals, only a small percentage of the substances in the ICE database would be classifiable if the FHSA criteria were strictly applied. In order to maximize the number of substances included in these analyses, “proportionality” criteria (i.e., FHSA-20% and FHSA-67%) were applied for the purpose of assigning an FHSA classification for test results that would require additional testing according to the FHSA sequential testing strategy (see **Section 4.1**).

As part of the evaluation process, this background review document (BRD) has been prepared to describe the current validation status of the ICE test method, including what is known about its reliability and accuracy, its applicability domain, the numbers and types of substances tested, and the availability of a standardized protocol. An ICCVAM expert panel used this BRD when reviewing the ICE test method to identify all categories of ocular irritants and substances not labeled as irritants.

Parallel review of the ICE, isolated rabbit eye (IRE), hen’s egg test–chorioallantoic membrane (HET-CAM), and bovine corneal opacity and permeability (BCOP) test methods were conducted. The expert panel report and the analyses presented in the BRDs were used to support ICCVAM

recommendations on the proposed standardized test method protocols, proposed list of recommended reference substances, and additional optimization and/or validation studies that may be necessary to further develop and characterize the usefulness and limitations of these methods.

For a more detailed discussion on the background of the ICE test method, including its scientific basis and regulatory rationale and applicability, see the *Background Review Document—Current Status of* In Vitro *Test Methods for Identifying Ocular Corrosives and Severe Irritants: Isolated Chicken Eye Test Method* (ICCVAM 2006a).

1.2 Use of the ICE Test Method in Overall Strategy of Hazard or Safety Assessment

As shown in **Figure 1-1**, the GHS allows for use of validated and accepted *in vitro* methods to identify ocular corrosives/severe irritants without further testing. The GHS currently recommends the ICE test method for use in identifying ocular corrosives and severe irritants in a tiered-testing strategy for regulatory classification and labeling (UN 2007).

1.3 Validation of the ICE Test Method

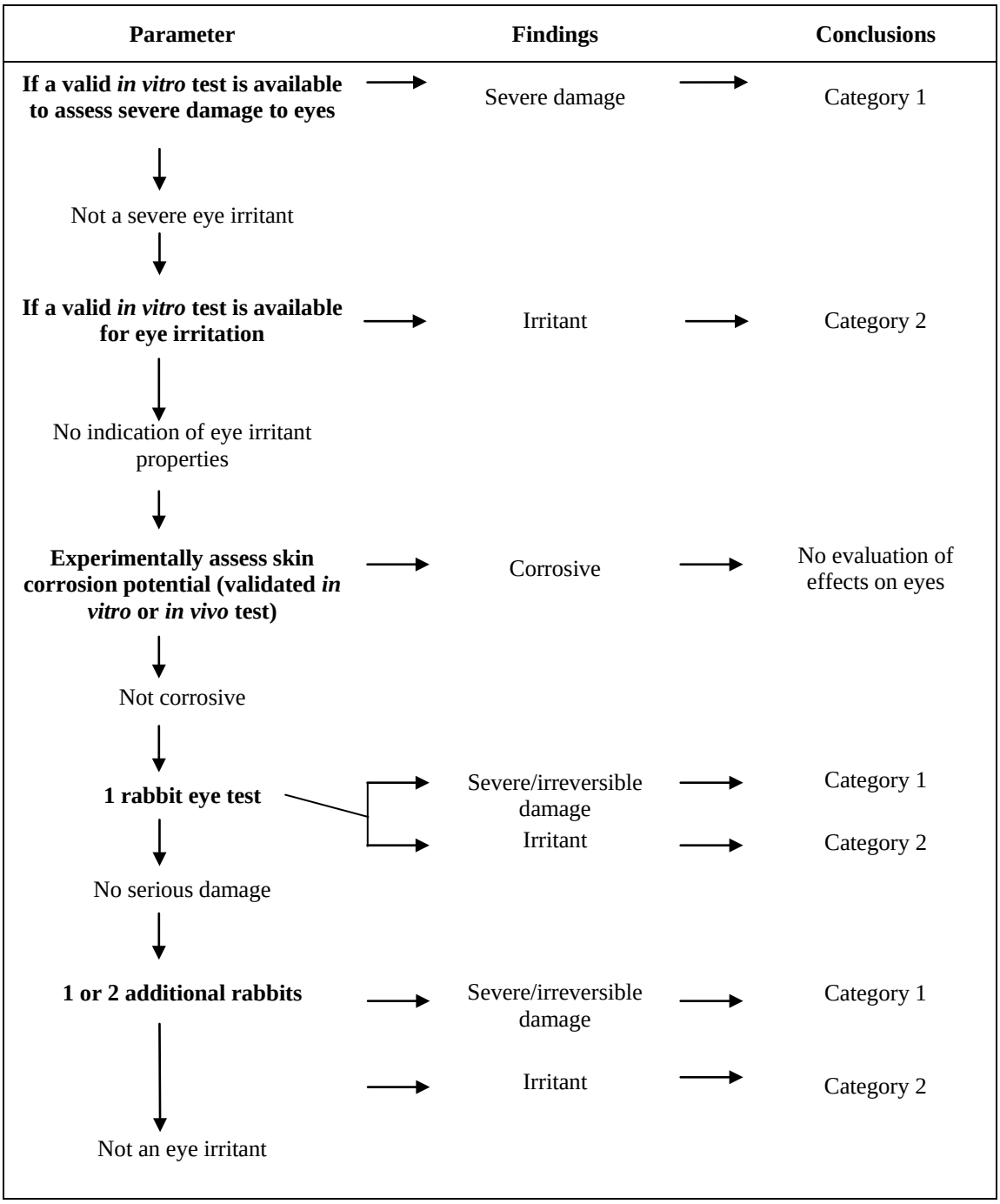
The ICCVAM Authorization Act of 2000 (Sec. 4([c]) mandates that "each Federal Agency … shall ensure that any new or revised … test method … is determined to be valid for its proposed use prior to requiring, recommending, or encouraging [its use]" (Public Law 106-545).

Validation is the process that establishes the reliability and relevance of a test method for a specific purpose (ICCVAM 1997). *Relevance* is defined as the extent to which a test method will correctly predict or measure the biological effect of interest (ICCVAM 1997). For the ICE test method described in this BRD, relevance is restricted to how well the test method identifies (1) substances that are capable of producing nonsevere ocular irritation or (2) substances not labeled as irritants.

Reliability is defined as the reproducibility of a test method within and among laboratories. Reliability should be based on performance with a diverse set of substances that (1) represent the types of chemical and product classes likely to be tested and (2) cover the range of responses that need to be identified. The validation process will provide data and information to allow U.S. Federal agencies to develop guidance on the development and use of the ICE test method as part of a tiered-testing approach to evaluating substances' eye irritation potential.

The first stage in this evaluation is the preparation of a BRD that presents and evaluates the relevant data and information about the test method, including its mechanistic basis, proposed uses, reliability, and performance characteristics (ICCVAM 1997). This BRD summarizes the available information on the ICE test method. Where adequate data are available, the qualitative and quantitative performance of the test method is evaluated.

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



Abbreviation: GHS = United Nations Globally Harmonized System for Classification and Labelling of Chemicals

[1] Adapted from UN (2007).

1.4 Search Strategies and Selection of Citations for the ICE BRD

The ICE test method data summarized in this BRD are derived from peer-reviewed scientific literature detail in the *Background Review Document, Current Status of In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants: Isolated Chicken Eye Test Method* (ICCVAM 2006a). A subsequent literature search conducted in January 2009 revealed no new articles containing

results from an ICE test method. Therefore, the database used in this analysis is the same as the database previously used (ICCVAM 2006a).

2.0 Isolated Chicken Eye Test Method Protocol Components

The ICE test method is an *in vitro* model that provides short-term maintenance of the chicken eye. Damage caused by the test substance is assessed by determination of corneal swelling, opacity, and fluorescein retention. While the latter two parameters involve a qualitative assessment, analysis of corneal swelling provides for a quantitative assessment. Each measurement is either (1) converted into a quantitative score that is used to calculate an overall irritation index or (2) assigned a qualitative categorization that is used to assign an *in vitro* ocular irritancy classification. Either outcome can then be used to predict the *in vivo* ocular irritation potential of a test substance.

For a detailed description of how the ICE test method is conducted, see the *Background Review Document—Current Status of In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants: Isolated Chicken Eye Test Method* (ICCVAM 2006a). Briefly, during an ICE study, a test substance is applied to the corneas of enucleated chicken eyes, isolated from chickens processed for human consumption. Chicken heads are transported from the slaughterhouse to the laboratory, and eyes are dissected within 2 hours after death. After dissection, the eyes are placed in a superfusion apparatus, where isotonic saline is applied to the cornea at a rate of 2 to 3 drops per minute through a steel tube attached to a peristaltic pump. Substances are applied as a single dose (30 µL for liquids, 30 mg for solids) for 10 seconds.

Corneal swelling and opacity are measured at regular intervals for up to 4 hours after treatment. Fluorescein retention is evaluated 30 minutes after treatment only. Mean values for each parameter (corneal swelling, corneal opacity, and fluorescein retention) are determined. The maximum mean value for each parameter is classified in one of four irritancy categories as shown in **Tables 2-1**, **2-2**, and **2-3**.

Table 2-1 Categorization of Corneal Thickness Measurements

Mean Corneal Swelling (%)	Category
0 to 5	I
>5 to 12	II
>12 to 18 (>75 min after treatment)	II
>12 to 18 (<75 min after treatment)	III
>18 to 26	III
>26 to 32 (>75 min after treatment)	III
>26 to 32 (<75 min after treatment)	IV
>32	IV

Table 2-2 Categorization of Corneal Opacity Scores

Mean Maximum Opacity Score	Category
0.0–0.5	I
0.6–1.5	II
1.6–2.5	III
2.6–4.0	IV

Table 2-3 Categorization of Fluorescein Retention Scores

Mean Fluorescein Retention Score 30 Minutes After Treatment	Category
0.0–0.5	I
0.6–1.5	II
1.6–2.5	III
2.6–3.0	IV

The categories for each individual endpoint are then combined into an overall *in vitro* ocular irritancy classification for comparison to the *in vivo* ocular irritancy classification according to the following scheme (**Table 2-4**) (INVITTOX 1994).

Table 2-4 *In Vitro* Ocular Irritancy Classification Scheme for the ICE Test Method

Overall *In Vitro* Classification	Combinations of the Three Endpoints
Nonirritant	3 x I
	2 x 1, 1 x II
Mild Irritant	3 x II
	2 x II, 1 x I
	2 x 11, 1 x III
Moderate Irritant	3 x III
	2 x III, 1 x II
	2 x III, 1 x IV
	2 x III, 1 x I[1]
	2 x II, 1 x IV[1]
	I x II, 1 x III, 1 x IV[1]
Severe Irritant	3 x IV
	2 x IV, 1 x III
	2 x IV, 1 x II[1]
	2 x IV, 1 x I[1]

[1] Combinations less likely to occur.

For the purposes of this evaluation, Nonirritant = EPA Category IV, GHS Not Classified, EU Not Labeled, FHSA Not Labeled; Mild Irritant = EPA Category III, GHS Category 2B; Moderate Irritant = EPA Category II, GHS Category 2A; Severe Irritant = EPA Category I, GHS Category 1, EU Category R41. The Mild and Moderate Irritant categories were combined to generate EU Category R36. The Mild, Moderate, and Severe Irritant categories were combined to generate FHSA Irritant.

To date, this scheme has been published only as an application to the EU classification system (EU 2001). However, using this same scheme, ICE results have also reportedly been used to predict the *in vivo* classification of substances according to the GHS classification system (Prinsen M, personal communication). For this BRD, the *in vitro* classification was compared to the corresponding *in vivo* classification for each of the EPA, GHS, and EU classification systems (EPA 2003a; EU 2001; UN 2007). For the FHSA classification system, the *in vivo* classification was compared to the *in vitro*

classification based on the EPA classification system. *In vitro* classifications of Mild, Moderate, and Severe Irritant were classified as FHSA Irritant; and Nonirritant was classified as FHSA Not Labeled.

3.0 Substances Used for Validation of the ICE Test Method

Validation studies for *in vitro* ocular test methods should, ideally, evaluate an adequate sample of test substances and products from chemical and product classes that would be evaluated using the *in vivo* rabbit eye test method. Test substances with a wide range of *in vivo* ocular responses (e.g., corrosive/severe irritant to not labeled) also should be assessed to determine limits to the range of responses that can be evaluated by the *in vitro* test method.

No new ICE test method data have been obtained since ICCVAM originally evaluated the ICE test method for identification of ocular corrosives and severe irritants (ICCVAM 2006a). Therefore, the same database (n=175 substances) (derived from Balls et al. 1995; Prinsen 1996, 2000, 2005; Prinsen and Koëter 1993) was used in the current evaluation.

Tables 3-1 and **3-2** show the chemical and product classes of the test substances in the database used in this assessment. Information, including substance name, Chemical Abstracts Service Registry Number (CASRN), chemical and/or product class, concentration(s) tested, purity, supplier or source, and literature reference for the test substances are provided in **Annex I**. If not assigned in the study report, the product class was sought from other sources, including the National Library of Medicine's ChemIDplus® database. Chemical classes were assigned to each test substance using a standard classification scheme based on the National Library of Medicine Medical Subject Headings (MeSH®) classification system (available at http//www.nlm.nih.gov/mesh), which ensures consistency in classifying substances among all *in vitro* ocular test methods under consideration. A substance could be classified in more than one chemical or product class.

Table 3-1 Chemical Classes Tested in the ICE Test Method

Chemical Class	# of Substances	Chemical Class	# of Substances
Acetate	1	Inorganic chloride compound	1
Acid	5	Inorganic salt	3
Acyl halide	1	Inorganic silver/ Nitrogen compound	1
Alcohol	15	Ketone	4
Aldehyde	2	Lactone	1
Alkali	3	Lipid	1
Amide/Amidine	7	Nitrile	1
Amino acid	1	Nitro compound	1
Boron compound	1	Not classified	85
Carbohydrate	2	Onium compound	8
Carboxylic acid	12	Organic silicon compound	2
Ester	10	Organic sulfur compound	3
Ether	1	Organometallic	2
Heterocyclic	9	Organophosphrous compound	1
Hydrocarbon	5	Polycyclic	4
Imide	2	Polyether	3
Inorganic chemical	1	Urea compound	1

Abbreviation: ICE = isolated chicken eye

As shown in **Table 3-1**, the chemical classes tested most often in the ICE test method are alcohols, carboxylic acids, esters, and heterocyclics. Of the 175 substances included in the database used for this assessment (see **Annex I)**, 85 (including formulations of unidentified composition) could not be assigned a specific chemical class.

As shown in **Table 3-2**, the product classes tested most in the ICE test method are solvents, soaps/surfactants, industrial chemicals, and pesticides/herbicides. Of the 175 substances (see **Annex I)**, 23 could not be assigned a product class.

Table 3-2 Product Classes Tested in the ICE Test Method

Product Class	# of Substances	Product Class	# of Substances
Adhesive	2	Fertilizer	1
Antifungal	2	Food additive	1
Antihistamine	1	Fungicide/Germicide	1
Anti-infective	3	Industrial chemical, intermediate or formulation	20
Antiseptic	2	Not classified	23
Caustic agent	4	Optical resolution agent	1
Chlorination byproduct	1	Paint	4
Cleaner	8	Pesticide/Herbicide	15
Copolymer	3	Pharmaceutical compound	5
Cosmetic ingredient	1	Preservative	6
Detergent	8	Raw material	9
Developer	1	Reagent	4
Disinfectant	5	Resin	2
Dyes and stains	10	Silicone resin	1
Elastomer	2	Soap	9
Enzyme inhibitor	1	Solvent	37
Enzyme solution	3	Surfactant	25

4.0 *In Vivo* Reference Data Used to Assess Isolated Chicken Eye Test Method Accuracy

A detailed description of the test method protocol used to generate the *in vivo* reference data (i.e., the Draize rabbit eye test) is provided in the *Background Review Document—Current Status of* In Vitro *Test Methods for Identifying Ocular Corrosives and Severe Irritants: Isolated Chicken Eye Test Method* (ICCVAM 2006a). A number of national and international test guidelines also describe this procedure (EPA 1998; OECD 2002; CPSC 2003; EU 2004). The subjective scoring system used to assign an ocular hazard classification is based on a discrete scale for grading the severity of ocular lesions on the cornea, iris, and conjunctiva.

Most of the ICE studies evaluated in this BRD include *in vivo* reference data generated using the basic procedures for the *in vivo* rabbit eye test method described above. These data were used by NICEATM to assign an ocular hazard classification according to the EPA (2003a), EU (2001), FHSA (2005), and the GHS (UN 2007) ocular irritancy classification systems (**Annex III**). Exceptions include the following:

- For Prinsen (2000), no original *in vivo* data were provided. The irritancy classification, based on the EU system (1992) only, was provided for the four substances tested.
- For Prinsen (1996), summary data and the irritancy classification, based on the EU system (1992) only, were provided. Individual animal *in vivo* data were not provided, which precluded assigning a precise classification according to the EPA (2003a), GHS (UN 2007), and FHSA (2005) classification systems for most test substances. However, for some test substances, adequate information was provided such that they could be included in the evaluation.
- For Prinsen and Koëter (1993), no original *in vivo* data were provided. The published report provides the irritancy classification, based on the EU system (1992) only, for 19 of 21 chemicals, as assigned by Botham et al. (1989). The remaining two chemicals were classified based on *in vivo* studies conducted in the author's laboratory (Prinsen 1991a, 1991b, data requested but not provided). Botham et al. (1989) includes toxicological summaries that provide a recommended EU classification for each of the chemicals. In three cases, there were adequate summary *in vivo* data with which to also generate irritancy classifications for the EPA (2003a) and GHS (UN 2007) classification systems. *In vivo* rabbit eye test results were available from other sources for eight substances. Therefore, *in vivo* data were obtained for 11 of 21 chemicals tested in this study.

4.1 *In Vivo* Classification Criteria Used for BRD Analysis

As described in the *Background Review Document—Current Status of* In Vitro *Test Methods for Identifying Ocular Corrosives and Severe Irritants: Isolated Chicken Eye Test Method* (ICCVAM 2006a), the *in vivo* rabbit eye test database that was used to analyze the accuracy of the ICE test method includes studies conducted using from one to six rabbits. However, some of the *in vivo* classification systems considered for the accuracy analyses are designed for application to studies using no more than three rabbits. Thus, to maximize the amount of data used to evaluate the ICE test method, the decision criteria for each classification system were expanded to include studies that used more than three rabbits in their evaluation. The criteria used for classification according to the EPA (2003a), EU (2001), or GHS (UN 2007), classification systems were detailed in the 2006 ICCVAM BRD (ICCVAM 2006a). Each of these classification systems requires that the Draize scoring system be used. For these classification systems, scoring continues until the effect is cleared, but usually not beyond 21 days after the substance is applied to the eye of the rabbit. In order for a substance to be included in the accuracy evaluations in the 2006 ICCVAM BRD, the following four criteria must have been met.

- At least three rabbits were tested in the study unless a severe effect (e.g., corrosion of the cornea) was noted in a single rabbit. In such cases, substance classification could proceed based on the effects observed in fewer than three rabbits.
- A volume of 0.1 mL or 100 mg was tested in each rabbit. A study in which a lower quantity was applied to the eye could be accepted for substance classification provided that a severe effect (e.g., corrosion of the cornea, lesion persistence) was observed in a rabbit.
- Observations of the eye were made at least 24, 48, and 72 hours after test substance application if no severe effect was observed.
- Observations of the eye were made until reversibility was assessed, typically meaning that all endpoint scores were cleared. Results from a study terminated early were not used unless the reason for the early termination was documented.

If any of the above criteria were not fulfilled, then the data for that substance were not used for the accuracy analyses.

For the FHSA classification system (FHSA 2005), the testing guidelines and associated criteria are included in 16 CFR 1500.42 (CPSC 2003). The FHSA classification system is based on using up to three sequential tests for each test substance with six animals used per test (**Table 4-1**). Decisions on further sequential testing are based on the number of positive responses in each test. The severity of effects for each endpoint (i.e., corneal ulceration and opacity, conjunctival redness and/or swelling, and iritis) is measured at 24, 48, and 72 hours after test substance administration. Positive responses include corneal ulceration (other than a fine stippling), corneal opacity or iritis ≥1, and conjunctival swelling and/or redness ≥2. In the first test, six animals are tested. If ≥4 animals are positive, the test is positive. If ≤1 animal tests positive, the test is negative. If 2/6 or 3/6 animals are positive, then a second test is performed with six additional animals. A third test is needed if 1/6 or 2/6 animals are positive with the second test.

Table 4-1 FHSA Classification System (16 CFR 1500.42)[1,2]

Positive Response for a Single Rabbit[3] ≥1 of the following at 24, 48, and/or 72 hours	***In Vivo* Effect**
Corneal ulceration (other than a fine stippling) Corneal opacity (CO) ≥1 Iritis (IR) ≥1 Conjuctival redness (CR) and/or chemosis (CC) ≥2	First Test – If ≥4/6 animals are positive, the test is positive. If ≤1 animal is positive, the test is negative. If 2/6 or 3/6 animals are positive, the test is repeated using a different group of six animals. Second Test – If ≥3/6 animals are positive, the test is positive. If 0/6 animals are positive, the test is negative. If 1/6 or 2/6 animals are positive, the test is repeated using a different group of six animals. Third Test – Should a third test be needed, the test is positive if ≥1/6 animals are positive. If 0/6 animals are positive, the test is negative.

Abbreviations: CC = conjunctival chemosis; CFR = Code of Federal Regulations; CO = corneal opacity; CR = conjunctival redness; FHSA = Federal Hazardous Substances Act; IR = iritis.

[1] For the FHSA Classification System (2005), the testing guidelines and associated criteria are included in 16 CFR 1500.42 (CPSC 2003).

[2] At least three animals per test (one animal screen for corrosive/severe irritants permitted). Maximum score in any animal used for classification.

[3] The following scores are considered positive: CO or IR ≥1 or CR or CC ≥2. Therefore, CO and IR scores of 0 or CR and CC scores ≤1 are considered negative.

The FHSA classification system (FHSA 2005) is a binary system, which classifies substances that test positive (according to the criteria provided in **Table 4-1)** as irritants and substances that test negative

as not requiring labeling (i.e. FHSA Not Labeled). Based on the FHSA sequential testing strategy, a substance can be classified as an eye irritant hazard with as few as 22% of the animals having a positive response (i.e., 2/6 [first test] +1/6 [second test] +1/6 [third test] = 4/18 or 22%).

Because the FHSA classification system is based on a sequential testing strategy, which uses up to 18 animals, only a small percentage of the substances in ICE database would be classifiable if the FHSA criteria were strictly applied. In order to maximize the number of substances include in these analyses, "proportionality" criteria were developed by NICEATM for the purpose of assigning an FHSA classification for test results that would require additional testing according to the FHSA sequential testing strategy (**Table 4-2**).

Table 4-2 Proposed FHSA "Proportionality" Criteria

No. of Animals in Test	FHSA-20%[1]		FHSA-67%[1]		
	NL	Irritant	NL	Irritant	Further Testing Required[2]
3	0/3	≥1 (≥33%)	0/3	≥2 (≥67%)	1/3
4	0/4	≥1 (≥25%)	0/4	≥3 (≥75%)	1/4, 2/4
5	0/5	≥1 (≥20%)	0/5	≥4 (≥80%)	1/5, 2/5, 3/5
6	0/6, 1/6	≥2 (≥33%)	0/6, 1/6	≥4 (≥67%)	2/6, 3/6

Abbreviations: CPSC = U.S. Consumer Product Safety Commission; FHSA = Federal Hazardous Substances Act; NL = Not Labeled (as irritant); No. = number.

[1] FHSA-20% and FHSA-67% analysis methods are based on the proportionality of positive animals needed to identify a substance as an irritant.

[2] For FHSA-67%, Further Testing Required refers to substances that do not meet adequate positive or negative criteria to be classified.

These "proportionality" criteria (i.e., FHSA-20% and FHSA-67%) are as follows:

- (FHSA-20%) – FHSA-20% is based on the proportion of positive animals needed to identify a substance as an irritant using the FHSA sequential testing strategy, where 20% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥1 positive animal in a 3 to 5 animal test or ≥2 positive animals in a 6 animal test.
- (FHSA-67%) – FHSA-67% is based on the proportion of positive animals needed to identify a substance as an irritant using the "first test" of the FHSA sequential testing strategy, where 67% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥2/3, 3/4, 4/5, or 4/6 positive animals. If 1/3, 1/4, 2/4, 1/5, 2/5, 3/5, 2/6, or 3/6 animals were positive, further testing would be required.

4.2 *In Vivo* Data Quality

Ideally, all data supporting the validity of a test method should be obtained and reported in accordance with Good Laboratory Practice (GLP) guidelines. GLP guidelines are nationally and

internationally recognized rules designed to produce high-quality laboratory records (OECD 1998; EPA 2003b, 2003c; FDA 2003). These guidelines provide an internationally standardized approach for the conduct of studies, reporting requirements, archival of study data and records, and information about the test protocol, thereby ensuring the integrity, reliability, and accountability of a study.

The extent to which the *in vivo* rabbit eye studies that were used to provide the comparative data in the published ICE validation studies complied with GLP guidelines is based on the information provided in the reports. Based on the available information, all of the reports included *in vivo* data obtained according to GLP guidelines.

5.0 Isolated Chicken Eye Test Method Data and Results

A total of five reports, three published (Balls et al. 1995; Prinsen 1996; Prinsen and Koëter 1993) and two unpublished (Prinsen 2000, 2005), included sufficient data for an accuracy analysis of the ICE test method for the identification of all categories of ocular irritation. **Section 6.0** details how these data were evaluated collectively (i.e., data from all studies combined) and on a per-study basis.[1]

5.1 Availability of Copies of Original Data Used to Evaluate the Accuracy and Reliability

Original study records containing data for the substances screened with the ICE test method in Prinsen (1996), Prinsen (2000), and Prinsen (2005) were kindly provided by Mr. Menk Prinsen of TNO Nutrition and Food Research. Summaries of ICE results (i.e., total scores) but no original data were obtained for the 60 substances evaluated by Balls et al. (1995). No other ICE test method data have been obtained by NICEATM.

5.2 Description of the Statistical Approaches Used to Evaluate the Resulting Data

Statistical analyses to compare ICE test method results to those from the *in vivo* reference test method have been done predominantly by comparing the ICE irritation index and the maximum mean scores of its individual components (i.e., corneal swelling, corneal opacity, fluorescein retention) to a numerical *in vivo* rabbit eye score (e.g., modified maximum average score [MMAS]). However, because the current evaluation focuses on the regulatory applicability of the ICE test method, and MMAS scores are not used for regulatory classification, this BRD did not use this approach. Rather, an *in vitro* classification system was used to assign an ocular irritation classification for each test substance (see **Section 2.0**).

5.3 Summary of Results

The information extracted for the database used in this assessment includes, when provided, the following specifics:

- Name
- CASRN (if available)
- Chemical class and/or product class
- Concentration(s) tested
- Purity
- Form tested
- ICE test method endpoint values (maximum mean)
- *In vitro* classification
- Supplier or source
- Literature reference

If not provided, the CASRN was obtained from various sources, including the National Library of Medicine's ChemIDplus® database (available at http://chem2.sis.nlm.nih.gov/chemidplus). All substances with the same CASRN were listed under the same name regardless of the synonym used in the original report. Chemical and product classes were assigned to each test substance based on the MeSH® classification system (available at http://www.nlm.nih.gov/mesh). **Annex I** provides information on the names, synonyms, CASRNs, and chemical/product classes, where available, for

[1] Because Prinsen (2000) includes only four test substances, data from this study were included only in the overall analysis and were not evaluated separately.

each substance. **Annex II** provides the *in vitro* ICE test method data sorted by reference and alphabetically by substance name.

5.4 Use of Coded Chemicals and Compliance with GLP Guidelines

Ideally, all data supporting the validity of a test method should be obtained and reported in accordance with GLP guidelines and with the use of coded chemicals (EPA 2003b, 2003c; FDA 2003; OECD 1998). The data quality was evaluated by reviewing the methods section in literature references and the submitted reports. The data quality presented in the reviewed literature references can only be evaluated to the extent such information was provided in the published reports. Based on the available information, all ICE test method studies evaluated were conducted according to GLP guidelines.

Based on the information in the five studies evaluated, Balls et al. (1995) was the only study that employed specific mechanisms to code the chemicals that were tested (see Section 3.4.2 in ICCVAM 2006a).

6.0 Isolated Chicken Eye Test Method Accuracy

A critical component of an ICCVAM evaluation of a test method's validation status is an assessment of the proposed test method's accuracy when compared to that of the current reference test method (ICCVAM 2003). This aspect of test method performance is typically evaluated by calculating:

- *Accuracy* (concordance): the proportion of correct outcomes (positive and negative) of a test method
- *Sensitivity*: the proportion of all positive substances that are classified as positive
- *Specificity*: the proportion of all negative substances that are classified as negative
- *Positive predictivity*: the proportion of correct positive responses among substances testing positive
- *Negative predictivity*: the proportion of correct negative responses among substances testing negative
- *False positive rate*: the proportion of all negative substances that are falsely identified as positive
- *False negative rate*: the proportion of all positive substances that are falsely identified as negative

ICCVAM evaluated the ability of the ICE test method to identify all categories of ocular irritation potential as defined by the EPA (EPA 2003a), GHS (UN 2007), and EU (EU 2001) classification systems. Given that the FHSA classification system is used to identify eye irritants based on incidence and does not differentiate between irreversible (i.e., corrosive or severe) and reversible (i.e., nonsevere) ocular effects based on Draize rabbit eye test results, an evaluation for all ocular hazard categories using the FHSA classification system was not possible.

Analyses were also performed with specific chemical classes and/or physical properties excluded based on their previous identification as discordant in the ICE test method (ICCVAM 2006a). These evaluations were conducted on the overall data set created by combining results from the reports discussed in **Section 5.0** (Balls et al. 1995; Prinsen 1996, 2005; Prinsen and Koëter 1993) then assigning an overall ocular irritancy classification for each substance. (See **Annexes II** and **III**). When the same substance was evaluated in multiple laboratories, an overall ICE classification was based on the majority classification among all of the studies. When there were an equal number of different irritancy classifications for substances (e.g., two tests classified a substance as Not Labeled, and two tests classified a substance as a mild irritant), the more severe irritancy classification was used for the overall classification for the substance (i.e., mild irritant, in this case).

6.1 GHS Classification System: ICE Test Method Accuracy

The four studies (Balls et al. 1995; Prinsen 1996, 2005; Prinsen and Koëter 1993) contained ICE test method data on 174 substances, 141 of which had sufficient *in vivo* data to be assigned an ocular irritancy classification according to the GHS classification system (UN 2007) (see **Annex III**). Based on results from *in vivo* rabbit eye experiments, 20% (29/141[2]) were classified as Category 1, 16% (22/141[3]) were classified as Category 2A, 8% (11/141) were classified as Category 2B, and 56%

---

[2] One chemical (benzalkonium chloride, 1%) was tested *in vivo* twice in the same laboratory. The results were discordant with respect to GHS classification. According to one test, the classification was Category 1, while results from the other test yielded a Category 2B classification. The accuracy analysis was performed with the substance classified as Category 1. Another chemical (1% sodium hydroxide) was duplicated in the database. Sodium hydroxide (Prinsen and Koëter 1993) was removed because the *in vivo* classification corresponded to a 10% solution.

[3] Triton X-100 (10%) and dibenzyl phosphate were excluded because they were classified *in vitro* as Category 2A/2B.

(79/141) were classified as Not Labeled as Irritant. The remaining 33 substances could not be classified according to the GHS classification system due to the lack of adequate animal data and are so noted in **Annex III**.

6.1.1 Identification of Category 1 Substances (Ocular Corrosives/Severe Irritants)

The ICE test method correctly identified 52% (15/29) of the Category 1 substances (**Table 6-1**). Among the remaining 48% (14/29) Category 1 substances that were underpredicted by ICE, 10% (3/29) were classified as Category 2A, 35% (10/29) were classified as Category 2B, and 3% (1/29) were classified as Not Classified as Irritant.

6.1.2 Identification of Category 2A Substances (Moderate Ocular Irritants)

For the 22 substances that could be evaluated, the ICE test method correctly identified 36% (8/22) as moderate irritants, while 36% (8/22) were overpredicted and 28% (6/22) were underpredicted (**Table 6-1**).

6.1.3 Identification of Category 2B Substances (Mild Ocular Irritants)

For the 11 substances that could be evaluated, the ICE test method correctly identified 73% (8/11) as mild irritants, while 18% (2/22) were overpredicted and 9% (1/11) were underpredicted (**Table 6-1**).

6.1.4 Identification of Not Classified Substances

For the 79 substances that could be evaluated, the ICE test method correctly identified 66% (52/79) as substances not classified as irritants, while 34% (27/79) were overpredicted (**Table 6-1**).

6.1.5 Ability to Distinguish Substances Not Classified as Irritants from All Other Classes

In addition to evaluating the ability of the ICE test method to identify each individual ocular hazard category according to the GHS classification system, ICCVAM also evaluated the ability of the ICE test method to distinguish substances not classified as irritants from all irritant classes.[4] Using this approach for the 141 substances, the ICE test method has an overall accuracy of 78% (110/141), a sensitivity of 94% (58/62), a specificity of 66% (52/79), a false positive rate of 34% (27/79), and a false negative rate of 6% (4/62) (**Table 6-2**). One (25%) of the 4 false negative substances (4-carboxybenzaldehyde) was from one of the discordant classes (solids).

As detailed below, the results from each individual study were also evaluated separately.

Prinsen and Koëter (1993): Based upon the *in vivo* rabbit data, eight substances could be assigned a GHS classification. Among these eight substances, the ICE test method has an accuracy of 75% (6/8), sensitivity of 75% (3/4), specificity of 75% (3/4), false positive rate of 25% (1/4), and a false negative rate of 25% (1/4) (**Table 6-2**).

---

[4] The 2006 ICCVAM BRD provides an evaluation of the ICE test method for distinguishing ocular corrosives and severe irritants from all other classes. Because the database of ICE test method results has not changed, this analysis has not been repeated here.

Table 6-1 Performance of the ICE Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the GHS Classification System,[1] by Study and Overall

Data Source	Overall Correct Classification	Severe (Category 1)		Moderate (Category 2A)			Mild (Category 2B)			Not Classified as Irritant	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Prinsen and Koëter (1993)	63% (5/8)	100% (2/2)	0% (0/2)	100% (1/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	100% (1/1)	25% (1/4)	75% (3/4)
Balls et al. (1995)	38% (19/50)	55% (11/20)	45% (9/20)	46% (6/13)	38% (5/13)	16% (2/13)	50% (2/4)	50% (2/4)	0% (0/4)	92% (12/13)	8% (1/13)
Prinsen (1996)	81% (29/36)	50% (1/2)	50% (1/2)	0% (0/3)	33% (1/3)	67% (2/3)	0% (0/2)	100% (2/2)	0% (0/2)	14% (4/29)	86% (25/29)
Prinsen (2005)	63% (29/46)	0% (0/4)	100% (4/4)	20% (1/5)	40% (2/5)	40% (2/5)	0% (0/4)	100% (4/4)	0% (0/4)	30% (10/33)	70% (23/33)
Overall[2]	59% (83/141)	52% (15/29)	48% (14/29)	36% (8/22)	36% (8/22)	28% (6/22)	18% (2/11)	73% (8/11)	9% (1/11)	34% (27/79)	66% (52/79)

Abbreviations: GHS = Globally Harmonized System; ICE = isolated chicken eye.

[1] GHS classification system (UN 2007).

[2] Because Prinsen (2000) includes only four test substances, data from this study were included only in the overall analysis and were not evaluated separately.

Table 6-2 Accuracy of the ICE Test Method in Distinguishing Substances Not Classified as Irritants from All Other Irritant Classes as Defined by the GHS Classification System,[1] by Study and Overall

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Prinsen and Koëter (1993)	8	75	6/8	75	3/4	75	3/4	25	1/4	25	1/4
Balls et al. (1995)	50	72	36/50	95	35/37	8	1/13	92	12/13	5	2/37
Prinsen (1996)	36	89	32/36	100	7/7	86	25/29	14	4/29	0	0/7
Prinsen (2005)	46	76	35/46	92	12/13	70	23/33	30	10/33	8	1/13
Overall[2]	141	78	110/141	94	58/62	66	52/79	34	27/79	6	4/62

Abbreviations: GHS = Globally Harmonized System; ICE = isolated chicken eye; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] GHS classification system (UN 2007): Not Classified as Irritant vs. Category 1/2A/2B.

[2] Because Prinsen (2000) includes only four test substances, data from this study were included only in the overall analysis and were not evaluated separately.

Balls et al. (1995): Based upon the *in vivo* rabbit data, 50 substances could be assigned a GHS classification. Among these 50 substances, the ICE test method has an accuracy of 72% (36/50), sensitivity of 95% (35/37), specificity of 8% (1/13), false positive rate of 92% (12/13), and a false negative rate of 5% (2/37) (**Table 6-2**). One of the two false negative substances (4-carboxybenzaldehyde) was from one of the discordant classes (solids).

Prinsen (1996): Based upon the *in vivo* rabbit data, 36 substances could be assigned a GHS classification. Among these 36 substances, the ICE test method has an accuracy of 89% (32/36), sensitivity of 100% (7/7), specificity of 86% (25/29), false positive rate of 14% (4/29), and a false negative rate of 0% (0/7) (**Table 6-2**).

Prinsen (2005): Based upon the *in vivo* rabbit data, 46 substances could be assigned a GHS classification. Among these 46 substances, the ICE test method has an accuracy of 76% (35/46), sensitivity of 92% (12/13), specificity of 70% (22/33), false positive rate of 30% (10/33), and a false negative rate of 8% (1/13) (**Table 6-2**).

6.1.6 Performance of the ICE Test Method with Discordant Classes Excluded

The previous ICCVAM BRD identified limitations of the ICE test method based upon the false positive rate for alcohols and the false negative rates for solids and surfactants when the ICE is used to identify ocular corrosives and severe irritants (ICCVAM 2006a). For this reason, the performance of the ICE test method in identifying all ocular irritant classes was evaluated with these substances excluded from the database. The overall performance statistics improved slightly (e.g., overall correct classification increased from 59% to 64%) when these substances were excluded (**Table 6-3**).

When the ability of the ICE test method to distinguish substances not labeled as irritants from all irritant classes was evaluated with the discordant classes removed, overall accuracy of the ICE method was actually slightly reduced from 78% (110/141) to 75% (58/77), false negative rates increased from 6% (4/62) to 11% (3/27), and false positive rates decreased from 34% (27/79) to 32% (16/50) (**Table 6-4**). Following the removal of substances belonging to discordant classes (i.e., alcohols, surfactants and solids; see also ICCVAM 2006a), there were three GHS ocular irritants classified as Not Classified as Irritant using the ICE test method (i.e., false negatives; see **Table 6-5**). Among the three false negatives for the GHS system, 33% (1/3) were GHS Category 2B substances, 33% (1/3) were GHS Category 2A substances, and 33% (1/3) were GHS Category 1 substances.

Table 6-3 Performance of the ICE Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the GHS Classification System,[1] with Discordant Chemical and Physical Classes Excluded

ICE	Overall Correct Classification	Severe (Category 1)		Moderate (Category 2A)			Mild (Category 2B)			Not Classified as Irritant	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Overall	59% (83/141)	52% (15/29)	48% (14/29)	36% (8/22)	36% (8/22)	28% (6/22)	18% (2/11)	73% (8/11)	9% (1/11)	34% (27/79)	66% (52/79)
Without Alcohols	62% (80/130)	52% (14/27)	48% (13/27)	19% (3/16)	44% (7/16)	38% (6/16)	10% (1/10)	80% (8/10)	10% (1/10)	34% (26/77)	66% (51/77)
Without Surfactants	61% (74/121)	52% (11/21)	48% (10/21)	40% (8/20)	35% (7/20)	25% (5/20)	20% (2/10)	70% (7/10)	10% (1/10)	30% (21/70)	70% (49/70)
Without Solids	57% (57/107)	59% (10/17)	41% (7/17)	38% (8/21)	38% (8/21)	24% 5/21)	25% (2/8)	63% (5/8)	12% (1/8)	38% (23/61)	62% (38/61)
Without Alcohols and Surfactants	64% (70/110)	53% (10/19)	47% (9/19)	21% (3/14)	43% (6/14)	36% (5/14)	11% (1/9)	78% (7/9)	11% (1/9)	29% (20/68)	71% (48/68)
Without Alcohols, Surfactants, and Solids	64% (49/77)	63% (5/8)	37% (3/8)	23% (3/13)	46% (6/13)	31% (4/13)	17% (1/6)	67% (4/6)	17% (1/6)	32% (16/50)	68% (34/50)

Abbreviations: GHS = Globally Harmonized System; ICE = isolated chicken eye.

[1] GHS classification system (UN 2007).

Table 6-4 Accuracy of the ICE Test Method in Distinguishing Substances Not Classified as Irritants from All Other Irritant Classes as Defined by the GHS Classification System,[1] with Discordant Chemical and Physical Classes Excluded

ICE	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall	141	78	110/141	94	58/62	66	52/79	34	27/79	6	4/62
Without Alcohols	129	78	100/129	92	49/53	67	51/76	33	25/76	8	4/53
Without Surfactants	122	79	96/122	92	47/51	69	49/71	31	22/71	8	4/51
Without Solids	107	76	81/107	93	43/46	62	38/61	38	23/61	7	3/46
Without Alcohols and Surfactants	109	78	85/109	90	37/41	71	48/68	29	20/68	10	4/41
Without Alcohols, Surfactants, and Solids	77	75	58/77	89	24/27	68	34/50	32	16/50	11	3/27

Abbreviations: GHS = Globally Harmonized System; ICE = isolated chicken eye; N = number of substances included in this analysis/the total number of substances in the study; NC = Not Classified (as an irritant); No. = data used to calculate the percentage.

[1] GHS classification system (UN 2007): NC vs. Category 1/2A/2B.

Table 6-5 ICE False Negative Substances[1]

Substance	*In Vivo* Classification					*In Vivo* Scores		
	EPA	GHS	EU	FHSA-20%	FHSA-67%	N	Corneal Opacity: Score (Day Cleared)	Conjunctival Redness: Score (Day Cleared)
TNO-94[2]	I	1	R41	Irr	Irr	3	N=1 2(7)	N=2 3(14)
TNO-28[3] (toilet bowl cleaner-1)	I	1	R41	Irr	Irr	3	None	N=1 2(7) N=1 3(28)
Methyl cyanoacetate	II	2A	R36	Irr	Irr	3	N=1 1(2) N=1 1(7)	N=1 3(7) N=2 3(14)
TNO-9 (paint)	II	NC	NL	Irr	Irr	3	N=1 2(14)	N=1 2(2) N=1 3(3)
DMSO	III	2B	NL	Irr	FTR	3	None	N=1 2(3) N=1 2(4)
Methyl cyclopentane	III	NC	NL	NL	NL	6	None	N=1 2(2)
TNO-3 (pesticide)	III	NC	NL	Irr	Irr	3	None	N=1 2(2) N=1 2(3)
TNO-29 (toilet bowl cleaner-2)	III	2A	R36	Irr	Irr	3	N=1 1(2) N=1 1(3)	N=1 3(7) N=1 2(14) N=1 3(14)
TNO-52	III	2A	R36	Irr	Irr	3	N=3 1(7)	N=3 3(14)

Abbreviations: DMSO = dimethyl sulfoxide; EPA = U.S. Environmental Protection Agency; EU = European Union; FHSA = U.S. Federal Hazardous Substances Act; FTR = further testing required; GHS = Globally Harmonized System; ICE = isolated chicken eye; Irr = irritant; N = number of animals; NC = Not Classified (as irritant); NL = Not Labeled (as irritant); TNO = TNO Nutrition and Food Research Institute, Netherlands.

For the purposes of this evaluation, *clearing* is defined in the EPA hazard classification system as corneal opacity or iritis scores = 0 or redness or chemosis scores = 1; in the GHS and EU hazard classification systems as corneal opacity, iritis, redness, or chemosis scores = 0.

[1] False negative compounds (shaded here) are those that test as nonirritants *in vitro* but are mild, moderate, or severe ocular irritants/corrosive *in vivo*, i.e., EPA Categories I, II, and III; GHS Categories 1, 2A, and 2B; and EU R41 and R36.

[2] One animal with ischemic necrosis of conjunctiva; study terminated.

[3] One animal with ischemic necrosis of conjunctiva.

Further analysis of substances according to chemical class for which hazard classification was underpredicted by the ICE test method indicated that carboxylic acids had the highest proportion of underpredicted substances (19% [4/21]). Among the underpredicted substances, 12 were liquids and 8 were solids. Six surfactants were underpredicted by the ICE test method (**Table 6-6**).

According to the GHS classification system, the most overpredicted substances (false positives) were alcohols, which accounted for 24% (9/37) of the overpredicted substances. Among the overpredicted substances, 73% (27/37) were liquids, 4 were solids, and six were surfactants (**Table 6-6**).

6.2 EPA Classification System: ICE Test Method Accuracy

The four studies (Balls et al. 1995; Prinsen 1996, 2005; Prinsen and Koëter 1993) contained ICE test method data on 174 substances, 140 of which had sufficient *in vivo* data to be assigned an ocular

irritancy classification according to the EPA classification system (EPA 2003a) (see **Annex III**). Based on results from *in vivo* rabbit eye experiments, 19% (27/140[5]) were classified as Category 1, 11% (16/140[6]) were classified as Category II, 27% (38/140) were classified as Category III, and 42% (59/140) were classified as Category IV. The remaining 34 substances could not be classified according to the EPA classification system due to the lack of adequate animal data and are so noted in **Annex III**.

6.2.1 Identification of Category I Substances (Ocular Corrosives/Severe Irritants)

The ICE test method correctly identified 48% (13/27) of the Category I substances (**Table 6-7**). Among the remaining 52% (14/27) of the Category I substances underpredicted by the ICE test method, 11% (3/27) were classified as Category II, 37% (10/27) were classified as Category III, and 4% (1/27) were classified as Category IV.

[5] One substance (1% sodium hydroxide) was duplicated in the database. Sodium hydroxide (Prinsen and Koëter 1993) was removed because the *in vivo* classification corresponded to a 10% solution.

[6] Triton X-100 (10%) and dibenzyl phosphate were removed because they were classified as Category II/III.

Table 6-6 Under- and Overprediction of the ICE Test Method Using the GHS Classification System[1] in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property

Category	N	Underprediction (*In Vivo/In Vitro*)						Overprediction (*In Vivo/In Vitro*)					
		Severe (Category 1)			Moderate (Category 2A)		Mild (Cat 2B)	Moderate (Cat 2A)	Mild (Category 2B)		Not Classified (NC)		
		NC	2B	2A	NC	2B	NC	1	2A	1	2B	2A	1
Overall	141	3% (1/29)	34% (10/29)	10% (3/29)	9% (2/22)	18% (4/22)	9% (1/11)	36% (8/22)	18% (2/11)	0% (0/11)	27% (21/79)	8% (6/79)	0% (0/79)
Chemical Class[2]													
Alcohol	12	0% (0/2)	50% (1/2)	0% (0/2)	0% (0/6)	0% (0/6)	-	83% (5/6)	100% (1/1)	-	67% (2/3)	33% (1/3)	0% (0/3)
Carboxylic Acid	10	0% (0/7)	43% (3/7)	0% (0/7)	100% (1/1)	-	-	-	-	-	50% (1/2)	0% (0/2)	0% (0/2)
Ester	9	0% (0/1)	0% (0/1)	0% (0/1)	33% (1/3)	0% (0/3)	0% (0/1)	33% (1/3)	0% (0/1)	0% (0/1)	50% (2/4)	50% (2/4)	0% (0/4)
Heterocyclic	9	0% (0/6)	11% (1/6)	11% (1/6)	0% (0/1)	0% (0/1)	-	0% (0/1)	-	-	50% (1/2)	0% (0/2)	0% (0/2)
Onium Compound	8	0% (0/6)	0% (0/6)	33% (2/6)	-	-	0% (0/1)	-	0% (0/1)	0% (0/1)	100% (1/1)	-	-
Properties of Interest													
Liquids[3]	100	6% (1/18)	17% (3/18)	11% (2/18)	5% (1/19)	21% (4/19)	13% (1/8)	37% (7/19)	-	-	27% (15/55)	9% (5/55)	0% (0/55)
Pesticide	10	0% (0/4)	50% (2/4)	0% (0/4)	0% (0/1)	100% (1/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	50% (2/4)	0% (0/4)	0% (0/4)
Solids[3]	35	0% (0/12)	58% (7/12)	0% (0/12)	50% (1/2)	0% (0/2)	0% (0/3)	0% (0/2)	0% (0/3)	0% (0/3)	22% (4/18)	0% (0/18)	0% (0/18)

continued

Table 6-6 Under- and Overprediction of the ICE Test Method Using the GHS Classification System[1] in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property (continued)

Category	N	Underprediction (*In Vivo/In Vitro)*						Overprediction (*In Vivo/In Vitro)*					
		Severe (Category 1)			Moderate (Category 2A)		Mild (Cat 2B)	Moderate (Cat 2A)	Mild (Category 2B)		Not Classified (NC)		
		NC	2B	2A	NC	2B	NC	1	2A	1	2B	2A	1
Overall	141	3% (1/29)	34% (10/29)	10% (3/29)	9% (2/22)	18% (4/22)	9% (1/11)	36% (8/22)	18% (2/11)	0% (0/11)	27% (21/79)	8% (6/79)	0% (0/79)
Properties of Interest (continued)													
Surfactant—Total	21	0% (0/9)	22% (2/9)	22% (2/9)	-	100% (2/2)	0% (0/1)	-	0% (0/1)	0% (0/1)	67% (6/9)	0% (0/9)	0% (0/9)
-nonionic	4	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	100% (2/2)	-	-
Anionic	2	-	100% (1/1)	-	-	-	-	-	-	-	100% (1/1)	-	-
Cationic	7	0% (0/6)	0% (0/6)	33% (2/6)	-	-	-	-	-	-	100% (1/1)	-	-
pH-Total	22	0% (0/20)	30% (6/20)	10% (2/20)	-	-	-	-	-	-	100% (2/2)	-	-
-acidic (pH < 7.0)	14	0% (0/20	25% (3/12)	8% (1/12)	-	-	-	-	-	-	100% (2/2)	-	-
-basic (pH > 7.0)	8	0% (0/20	38% (3/8)	13% (1/8)	-	-	-	-	-	-	-	-	-

Abbreviations: GHS = Globally Harmonized System; ICE = isolated chicken eye; N = number of substances; NC = Not Classified/not labeled as irritant.

[1] GHS classification system (UN 2007).

[2] Chemical classes included in this table are represented by at least five substances tested in the ICE test method and assignments are based upon National Library of Medicine medical subject heading (MeSH) categories (www.nlm.nih.gov/mesh) as defined in Annex I.

[3] Physical form (i.e., solid and liquid) not known for some substances; therefore, the overall number does not equal the sum of the solid and liquid substances.

Table 6-7 Performance of the ICE Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA Classification System,[1] by Study and Overall

Data Source	Overall Correct Classification	Severe (Category I)		Moderate (Category II)			Mild (Category III)			Not Labeled (Category IV)	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Prinsen and Koëter (1993)	75% (6/8)	100% (2/2)	0% (0/2)	100% (1/1)	0% (0/1)	0% (0/1)	0% (0/2)	50% (1/2)	50% (1/2)	0% (0/3)	100% (3/3)
Balls et al. (1995)	46% (23/50)	53% (10/19)	47% (9/19)	30% (3/10)	50% (5/10)	20% (2/10)	50% (10/20)	40% (8/20)	10% (2/20)	100% (1/1)	0% (0/1)
Prinsen (1996)	81% (29/36)	50% (1/2)	50% (1/2)	0% (0/3)	67% (2/3)	33% (1/3)	0% (0/6)	67% (4/6)	33% (2/6)	12% (3/25)	88% (22/25)
Prinsen (2005)	63% (29/46)	0% (0/4)	100% (4/4)	50% (1/2)	50% (1/2)	0% (0/2)	10% (1/10)	70% (7/10)	20% (2/10)	30% (9/30)	70% (21/30)
Overall[2]	62% (87/140)	48% (13/27)	52% (14/27)	31% (5/16)	50% (8/16)	19% (3/16)	29% (11/38)	53% (20/38)	18% (7/38)	22% (13/59)	78% (46/59)

Abbreviations: EPA = U.S. Environmental Protection Agency; ICE = isolated chicken eye.

[1] EPA classification system (EPA 2003a).

[2] Because Prinsen (2000) includes only four test substances, data from this study were included only in the overall analysis and were not evaluated separately.

6.2.2 Identification of Category II Substances (Moderate Ocular Irritants)

For the 16 substances that could be evaluated, the ICE test method correctly identified 50% (8/16) as Category II irritants, while 31% (5/16) were overpredicted and 19% (3/16) were underpredicted (**Table 6-7**).

6.2.3 Identification of Category III (Mild Ocular Irritants)

For the 38 substances that could be evaluated, the ICE test method correctly identified 53% (20/38) as mild irritants, while 29% (11/38) were overpredicted and 18% (7/38) were underpredicted (**Table 6-7**).

6.2.4 Identification of Category IV Substances (Not Labeled)

For the 59 substances that could be evaluated, the ICE test method correctly identified 78% (46/59) as substances not labeled as irritants, while 22% (46/59) were overpredicted (**Table 6-7**).

6.2.5 Ability to Distinguish Category IV Substances from All Other Classes

Using this approach for the 140 substances, the ICE test method had an overall accuracy of 83% (116/140), a sensitivity of 86% (70/81), a specificity of 78% (46/59), a false positive rate of 22% (13/59), and a false negative rate of 14% (11/81) (**Table 6-8**).

As detailed below, the results from each individual study were also evaluated separately.

Prinsen and Koëter (1993): Based upon the *in vivo* rabbit data, eight substances could be assigned an EPA classification. Among these eight substances, the ICE test method had an accuracy of 88% (7/8), sensitivity of 80% (4/5), specificity of 100% (3/3), false positive rate of 0% (0/3), and a false negative rate of 20% (1/5) (**Table 6-8**).

Balls et al. (1995): Based upon the *in vivo* rabbit data, 50 substances could be assigned an EPA classification. Among these 50 substances, the ICE test method has an accuracy of 90% (45/50), sensitivity of 92% (45/49), specificity of 0% (0/1), false positive rate of 100% (1/1), and a false negative rate of 8% (4/49) (**Table 6-8**). Two (4-carboxybenzaldehyde and maneb) of the four false negative substances were from the discordant classes (both solids).

Prinsen (1996): Based upon the *in vivo* rabbit data, 36 substances could be assigned an EPA classification. Among these 36 substances, the ICE test method had an accuracy of 83% (30/36), sensitivity of 73% (8/11), specificity of 88% (22/25), false positive rate of 12% (3/25), and a false negative rate of 27% (3/11) (**Table 6-8**).

Prinsen (2005): Based upon the *in vivo* rabbit data, 46 substances could be assigned an EPA classification. Among these 46 substances, the ICE test method had an accuracy of 74% (34/46), sensitivity of 81% (13/16), specificity of 70% (21/30), a false positive rate of 30% (9/30), and a false negative rate of 19% (3/16) (**Table 6-8**).

Table 6-8 Accuracy of the ICE Test Method in Distinguishing Category IV Substances from All Other Irritant Classes as Defined by the EPA Classification System,[1] by Study and Overall

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Prinsen and Koëter (1993)	8	88	7/8	80	4/5	100	3/3	0	0/3	20	1/5
Balls et al. (1995)	50	90	45/50	92	45/49	0	0/1	100	1/1	8	4/49
Prinsen (1996)	36	83	30/36	73	8/11	88	22/25	12	3/25	27	3/11
Prinsen (2005)	46	74	34/46	81	13/16	70	21/30	30	9/30	19	3/16
Overall[2]	140	83	116/140	86	70/81	78	46/59	22	13/59	14	11/81

Abbreviations: EPA = U.S. Environmental Protection Agency; ICE = isolated chicken eye; N = number of substances included in this analysis/the total number of substances in the study; No. = data used to calculate the percentage.

[1] EPA classification system (EPA 2003a): Category IV vs. Category I/II/III.

[2] Because Prinsen (2000) includes only four test substances, data from this study were included only in the overall analysis and were not evaluated separately.

6.2.6 Performance of the ICE Test Method with Discordant Classes Excluded

The ICE test method limitations identified in the 2006 ICCVAM BRD were based upon the false positive rate for alcohols and the false negative rates for solids and surfactants when the ICE test method is used to identify ocular corrosives and severe irritants (ICCVAM 2006a). For this reason, the performance of the ICE test method in identifying all ocular irritant classes was evaluated with these substances excluded from the database. The overall performance statistics improved slightly (e.g., overall correct classification increased from 59% to 64%) when these substances were excluded (**Table 6-9**).

When the ability of the ICE test method to distinguish Category IV substances from all other irritant classes was evaluated with the discordant classes removed, the overall accuracy was generally unchanged (e.g., overall accuracy decreased from 83% to 82%) when these substances were excluded. False negative rates changed from 14% (11/81) to 15% (6/39) and false positive rates changed from 22% (13/59) to 21% (8/39) when the discordant classes were removed (**Table 6-10**).

Following the removal of substances belonging to discordant classes (i.e. alcohols, surfactants and solids, see also ICCVAM [2006a]), there were six EPA ocular irritants classified as Category IV using the ICE test method (i.e. were false negatives, see **Table 6-5**). Among the six false negatives for the EPA system, 50% (3/6) were EPA Category III substances, 33% (2/6) were EPA Category II substances, and 17% (1/6) were EPA Category I substances.

Further analysis of substances for which hazard classification was underpredicted by the ICE test method according to chemical class indicated that carboxylic acids had the highest proportion of underpredicted substances (17% [4/24]). Of the underpredicted substances, 11 were liquids and 12 were solids. Two surfactants were underpredicted by the ICE test method (**Table 6-11**).

According to the EPA classification system, the most overpredicted substances (false positives) were alcohols, which accounted for 21% (6/29) of the overpredicted substances. Of the overpredicted substances, 79% (23/29) were liquids, 2 were solids, and 1 was a surfactant (**Table 6-11**).

Table 6-9 Performance of the ICE Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA Classification System,[1] with Discordant Chemical and Physical Classes Excluded

ICE	Overall Correct Classification	Severe (Category I)		Moderate (Category II)			Mild (Category III)			Not Labeled (Category IV)	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Overall	62% (87/140)	48% (13/27)	52% (14/27)	31% (5/16)	50% (8/16)	19% (3/16)	29% (11/38)	53% (20/38)	18% (7/38)	22% (13/59)	78% (46/59)
Without Alcohols	64% (82/128)	48% (12/25)	52% (13/25)	18% (2/11)	55% (6/11)	27% (3/11)	26% (9/35)	54% (19/35)	20% (7/35)	21% (12/57)	79% (45/57)
Without Surfactants	62% (76/122)	50% (10/20)	50% (10/20)	31% (5/16)	50% (8/16)	19% (3/16)	31% (10/32)	47% (15/32)	22% (7/32)	19% (10/53)	81% (43/53)
Without Solids	64% (68/107)	59% (10/17)	41% (7/17)	33% (5/15)	53% (8/15)	13% (2/15)	38% (11/29)	52% (15/29)	10% (3/29)	24% (11/46)	76% (35/46)
Without Alcohols and Surfactants	65% (71/110)	50% (9/18)	50% (9/18)	18% (2/11)	55% (6/11)	27% (3/11)	28% (8/29)	48% (14/29)	24% (7/29)	19% (10/52)	81% (42/52)
Without Alcohols, Surfactants, and Solids	67% (52/78)	67% (6/9)	33% (3/9)	20% (2/10)	60% (6/10)	20% (2/10)	17% (1/6)	67% (4/6)	17% (1/6)	21% (8/39)	79% (31/39)

Abbreviations: EPA = U.S. Environmental Protection Agency; ICE = isolated chicken eye.

[1] EPA classification system (EPA 2003a).

Table 6-10 Accuracy of the ICE Test Method in Distinguishing Category IV Substances from All Other Irritant Classes as Defined by the EPA Classification System,[1] with Discordant Chemical and Physical Classes Excluded

ICE	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall	140	83	116/140	86	70/81	78	46/59	22	13/59	14	11/81
Without Alcohols	128	82	105/128	85	60/71	79	45/57	21	12/57	15	11/71
Without Surfactants	122	82	100/122	84	57/68	80	43/54	20	11/54	16	11/68
Without Solids	107	84	90/107	90	55/61	76	35/46	24	11/46	10	6/61
Without Alcohols and Surfactants	110	81	89/110	81	47/58	81	42/52	19	10/52	19	11/58
Without Alcohols, Surfactants, and Solids	78	82	69/78	85	33/39	79	31/39	21	8/39	15	6/39

Abbreviations: EPA = U.S. Environmental Protection Agency; ICE = isolated chicken eye; N = number of substances included in this analysis/the total number of substances in the study; No. = data used to calculate the percentage.

[1] EPA classification system (EPA 2003a): Category IV vs. Category I/II/III.

Table 6-11 Under- and Overprediction of the ICE Test Method Using the EPA Classification System[1] in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property

Category	N	Underprediction (*In Vivo/In Vitro*)						Overprediction (*In Vivo/In Vitro*)					
		Severe (Category I)			Moderate (Category II)		Mild (Cat III)	Moderate (Cat II)	Mild (Cat III)		Not Labeled (Category IV)		
		IV	III	II	IV	III	IV	I	II	I	III	II	I
Overall	140	4% (1/27)	37% (10/27)	11% (3/27)	19% (3/16)	0% (0/16)	18% (7/38)	31% (5/16)	21% (8/38)	8% (3/38)	22% (13/59)	0% (0/59)	0% (0/50)
Chemical Class[2]													
Alcohol	12	0% (0/2)	50% (1/2)	0% (0/2)	0% (0/5)	0% (0/5)	-0% (0/3)	60% (3/5)	0% (0/3)	67% (2/3)	50% (1/2)	0% (0/2)	0% (0/2)
Carboxylic Acid	10	0% (0/7)	43% (3/7)	0% (0/7)	100% (1/1)	-	0% (0/2)	-	50% (1/2)	0% (0/2)	-	-	-
Ester	9	-	-	-	25% (1/4)	0% (0/4)	0% (0/5)	25% (1/4)	40% (2/5)	0% (0/5)	-	-	-
Heterocyclic	8	0% (0/5)	0% (0/5)	20% (1/5)	0% (0/1)	0% (0/1)	0% (0/2)	0% (0/1)	0% (0/2)	0% (0/2)	-	-	-
Onium Compound	7	0% (0/5)	0% (0/5)	40% (2/5)	-	-	0% (0/2)	-	0% (0/2)	0% (0/2)	-	-	-
Properties of Interest													
Liquids[3]	101	6% (1/17)	18% (3/17)	12% (2/17)	13% (2/15)	0% (0/15)	11% (3/28)	27% (4/15)	25% (7/28)	11% (3/28)	22% (9/41)	0% (0/41)	0% (0/41)
Solids[3]	34	0% (0/10)	70% (7/10)	0% (0/10)	50% (1/2)	0% (0/2)	44% (4/9)	0% (0/2)	0% (0/9)	0% (0/9)	15% (2/13)	0% (0/13)	0% (0/13)
Pesticide	10	0% (0/4)	75% (3/4)	0% (0/4)	0% (0/1)	0% (0/1)	50% (2/5)	0% (0/1)	0% (0/5)	0% (0/5)	50% (1/2)	0% (0/2)	0% (0/2)

continued

Table 6-11 Under- and Overprediction of the ICE Test Method Using the EPA Classification System[1] in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property (continued)

Category	N	Underprediction (*In Vivo/In Vitro*)						Overprediction (*In Vivo/In Vitro*)					
		Severe (Category I)			Moderate (Category II)		Mild (Cat III)	Moderate (Cat II)	Mild (Cat III)		Not Labeled (Category IV)		
		IV	III	II	IV	III	IV	I	II	I	III	II	I
Overall	140	4% (1/27)	37% (10/27)	11% (3/27)	19% (3/16)	0% (0/16)	18% (7/38)	31% (5/16)	21% (8/38)	8% (3/38)	22% (13/59)	0% (0/59)	0% (0/50)
Properties of Interest (continued)													
Surfactant-—Total	20	0% (0/7)	29% (2/7)	0% (0/7)	-	0% (0/1)	0% (0/6)	-	17% (1/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)
-nonionic	4	-	-	-	-	0% (0/1)	-	-	100% (1/1)	-	-	-	-
Anionic	2	-	100% (1/1)	-	-	-	-	-	-	-	-	-	-
Cationic	6	0% (0/5)	0% (0/5)	40% (2/5)	-	-	-	-	-	-	-	-	-
pH-Total	19	0% (0/16)	25% (4/16)	6% (1/16)	0% (0/1)	0% (0/1)	0% (0/2)	0% (0/1)	0% (0/2)	0% (0/2)	-	-	-
-acidic (pH < 7.0)	12	0% (0/10)	30% (3/10)	10% (1/10)	-	-	0% (0/2)	-	0% (0/2)	0% (0/2)	-	-	-
-basic (pH > 7.0)	7	0% (0/6)	17% (1/6)	0% (0/6)	0% (0/1)	0% (0/1)	-	0% (0/1)	-	-	-	-	-

Abbreviations: EPA = U.S. Environmental Protection Agency; ICE = isolated chicken eye; N = number of substances included in this analysis/the total number of substances in the study.

[1] EPA classification system (EPA 2003a).

[2] Chemical classes included in this table are represented by at least five substances tested in the ICE test method and assignments are based upon National Library of Medicine medical subject heading (MeSH) categories (www.nlm.nih.gov/mesh) as defined in Annex I.

[3] Physical form (i.e., solid and liquid) not known for some substances, and therefore the overall number does not equal the sum of the solid and liquid substances.

6.3 EU Classification System: ICE Test Method Accuracy

The four studies (Balls et al. 1995; Prinsen 1996, 2005; Prinsen and Koëter 1993) contained ICE test method data on 174 substances, 153 of which had sufficient *in vivo* data to be assigned an EU ocular irritancy classification, duplicates removed (EU 2001) (see **Annex III**). Based on results from *in vivo* rabbit eye experiments, 21% (32/153[7]) were classified as severe irritants (R41), 18% (28/153) were classified as moderate irritants (R36), and 61% (93/153) were classified as Not Labeled. The remaining 21 substances could not be classified according to the EU classification system due to the lack of adequate animal data and are so noted in **Annex III**.

6.3.1 Identification of R41 Substances (Ocular Corrosives/Severe Irritants)

The ICE test method correctly identified 59% (19/32) of the R41 substances (**Table 6-12**). Among the remaining 41% (13/32) R41 substances that were underpredicted by the ICE test method, 22% (7/32) were classified as R36, and 19% (6/32) were classified as Not Labeled.

6.3.2 Identification of R36 Substances (Moderate Ocular Irritants)

Of the 28 substances that could be evaluated, the ICE test method correctly identified 57% (16/28) as moderate irritants, while 18% (5/28) were overpredicted and 25% (7/28) were underpredicted (**Table 6-12**).

6.3.3 Identification of Not Labeled Substances

Of the 93 substances that could be evaluated, the ICE test method correctly identified 89% (83/93) as substances not labeled as irritants, while 11% (10/93) were overpredicted (**Table 6-12**).

Table 6-12 Performance of the ICE Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EU Classification System,[1] by Study and Overall

Data Source	Overall Correct Classification	Severe (R41)		Moderate (R36)			Not Labeled	
		Actual	Under	Over	Actual	Under	Over	Actual
Prinsen and Koëter (1993)	100% (19/19)	100% (7/7)	0% (0/7)	0% (0/3)	100% (3/3)	0% (0/3)	0% (0/9)	100% (9/9)
Balls et al. (1995)	52% (25/48)	56% (10/18)	44% (8/18)	29% (4/14)	50% (7/14)	31% (3/14)	50% (8/16)	50% (8/16)
Prinsen (1996)	94% (34/36)	50% (1/2)	50% (1/2)	0% (0/3)	67% (2/3)	33% (1/3)	8% (3/36)	92% (33/36)
Prinsen (2005)	80% (37/46)	0% (0/4)	100% (4/4)	17% (1/6)	50% (3/6)	33% (2/6)	6% (2/36)	94% (34/36)
Overall[2]	77% (118/153)	59% (19/32)	41% (13/32)	18% (5/28)	57% (16/28)	25% (7/28)	11% (10/93)	89% (83/93)

Abbreviations: EU = European Union; ICE = isolated chicken eye.

[1] EU classification system (EU 2001).

[2] Because Prinsen (2000) includes only four test substances, data from this study were included only in the overall analysis and were not evaluated separately.

[7] 1% sodium hydroxide was duplicated in the database. Sodium hydroxide (Prinsen and Koëter, 1993) was removed because the *in vivo* classification corresponded to a 10% solution.

6.3.4 Ability to Distinguish Not Labeled Substances from All Other Classes

In addition to evaluating the ability of the ICE test method to identify each individual ocular hazard category according to the EU classification system, ICCVAM evaluated the ability of the ICE test method to distinguish substances not labeled as irritants from all irritant classes.[8] Using this approach for the 153 substances considered, the ICE test method has an overall accuracy of 85% (130/153), a sensitivity of 78% (47/60), a specificity of 89% (83/93), a false positive rate of 11% (10/93), and a false negative rate of 22% (13/60) (**Table 6-13**).

As detailed below, the results from each individual study were also evaluated separately.

Prinsen and Koëter (1993): Based upon the *in vivo* rabbit data, 19 substances could be assigned an EU classification. Among these 19 substances, the ICE test method has an accuracy of 100% (19/19), sensitivity of 100% (10/10), specificity of 100% (9/9), false positive rate of 0% (0/9), and a false negative rate of 0% (0/10) (**Table 6-13**).

Balls et al. (1995): Based upon the *in vivo* rabbit data, 48 substances could be assigned an EU classification. Among these 48 substances, the ICE test method has an accuracy of 69% (33/48), sensitivity of 78% (25/32), specificity of 50% (8/16), false positive rate of 50% (8/16), and a false negative rate of 32% (7/32) (**Table 6-13**). Six of the 7 substances identified as false negatives were from the discordant classes (alcohol, solids, surfactants).

Prinsen (1996): Based upon the *in vivo* rabbit data, 36 substances could be assigned an EU classification. Among these 36 substances, the ICE test method has an accuracy of 94% (34/36), sensitivity of 60% (3/5), specificity of 100% (31/31), false positive rate of 0% (0/31), and a false negative rate of 40% (2/5) (**Table 6-13**).

Prinsen (2005): Based upon the *in vivo* rabbit data 46 substances could be assigned an EU classification. Among these 46 substances, the ICE test method has an accuracy of 89% (41/46), sensitivity of 70% (7/10), specificity of 94% (34/36), a false positive rate of 6% (2/36), and a false negative rate of 30% (3/10) (**Table 6-13**).

[8] The 2006 ICCVAM BRD (2006a) provides an evaluation of the ICE test method for distinguishing ocular corrosives and severe irritants from all other classes. Because the database of ICE test method results has not changed, this analysis has not been repeated here.

Table 6-13 Accuracy of the ICE Test Method in Distinguishing Not Labeled Substances from All Other Irritant Classes as Defined by the EU Classification System,[1] by Study and Overall

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Prinsen and Koëter (1993)	19	100	19/19	100	10/10	100	9/9	0	0/9	0%	0/10
Balls et al. (1995)	48	69	33/48	78	25/32	50	8/16	50	8/16	32	7/32
Prinsen (1996)	36	94	34/36	60	3/5	100	31/31	0	0/31	40	2/5
Prinsen (2005)	46	89	41/46	70	7/10	94	34/36	6	2/36	30	3/10
Overall[2]	153	85	130/153	78	47/60	89	83/93	11	10/93	22	13/60

Abbreviations: EU = European Union; ICE = isolated chicken eye; N = number of substances included in this analysis/the total number of substances in the study; No. = data used to calculate the percentage.

[1] EU classification system (EU 2001): Not Labeled vs. R41/R36.

[2] Because Prinsen (2000) includes only four test substances, data from this study were included only in the overall analysis and were not evaluated separately.

6.3.5 Performance of the ICE Test Method with Discordant Classes Excluded

The ICE test method limitations identified in the 2006 ICCVAM BRD were based upon the false positive rate for alcohols and the false negative rates for solids and surfactants when the ICE test method is used to identify ocular corrosives and severe irritants (ICCVAM 2006a). For this reason, the performance of the ICE test method for identifying all ocular irritant classes was evaluated with these substances excluded from the database. However, the performance statistics were slightly improved (77% versus 80%) when these substances were excluded relative to the performance with the entire database (**Table 6-14**).

When the evaluation was broadened to the ability of the ICE test method to distinguish Not Labeled substances from all other irritant classes, and the discordant classes were removed, overall accuracy of the ICE method was unchanged at 85% (130/153 and 70/82). False positive and false negative rates also were generally comparable when the discordant classes were removed. False negative rates changed from 22% (13/60) to 19% (5/26), and false positive rates changed from 11% (10/93) to 12% (7/56) when the discordant classes were removed (**Table 6-15**).

Following the removal of substances belonging to discordant classes (i.e. alcohols, surfactants, and solids, see also ICCVAM [2006a]), there were five EU ocular irritants classified as Not Labeled using the ICE test method (i.e., they were false negatives, see **Table 6-5**). Among the five false negatives for the EU system, 60% (3/5) were EU Category R36 substances, and 40% (2/5) were EU Category R41 substances.

Further analysis of underpredicted (false negative) results by chemical class indicated that onium compounds were the most underpredicted, with 3 of the 20 substances underpredicted. Six *in vivo* severe substances (carboxylic acid, heterocyclic, and an inorganic) were underclassified as Not Labeled. One of these substances had a pH <7, while 3 had a pH >7. Regarding the physical form of underpredicted substances, 12 were liquids, 8 were solids, and 6 were surfactants (**Table 6-16**).

According to the EU classification system, the most overpredicted substances (false positives) were alcohols, which accounted for 4 of the 15 substances overpredicted overall. Regarding the physical form of overpredicted substances, 14 were liquids and 2 were surfactants (**Table 6-16**).

Table 6-14 Performance of the ICE Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EU Classification System,[1] with Discordant Chemical and Physical Classes Excluded

ICE	Overall Correct Classification	Severe (R41)		Moderate (R36)			Not Labeled	
		Actual	Under	Over	Actual	Under	Over	Actual
Overall	77% (118/153)	59% (19/32)	41% (13/32)	18% (5/28)	57% (16/28)	25% (7/28)	11% (10/93)	89% (83/93)
Without Alcohols	78% (109/139)	59% (17/29)	41% (12/29)	13% (3/23)	57% (13/23)	30% (7/23)	9% (8/87)	91% (79/87)
Without Surfactants	79% (104/132)	63% (15/24)	37% (9/24)	20% (5/25)	60% (15/25)	20% (5/25)	11% (9/83)	89% (74/83)
Without Solids	77% (89/116)	63% (12/19)	37% (7/19)	20% (5/25)	60% (15/25)	20% (5/25)	14% (10/72)	86% (62/72)
Without Alcohols and Surfactants	81% (95/118)	62% (13/21)	38% (8/21)	15% (3/20)	60% (12/20)	25% (5/20)	9% (7/77)	91% (70/77)
Without Alcohols, Surfactants, and Solids	80% (66/82)	67% (6/9)	33% (3/9)	18% (3/17)	65% (11/17)	18% (3/17)	13% (7/56)	87% (49/56)

Abbreviations: EU = European Union; ICE = isolated chicken eye.

[1] EU classification system (EU 2001).

Table 6-15 Accuracy of the ICE Test Method in Distinguishing Not Labeled Substances from All Other Irritant Classes as Defined by the EU Classification System,[1] with Discordant Chemical and Physical Classes Excluded

ICE	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall	153	85	130/153	78	47/60	89	83/93	11	10/93	22	13/60
Without Alcohols	139	85	118/139	75	39/52	91	79/87	9	8/87	25	13/52
Without Surfactants	132	85	112/132	78	38/49	89	74/83	11	9/83	22	11/49
Without Solids	116	85	99/116	84	37/44	86	62/72	14	10/72	16	7/44
Without Alcohols and Surfactants	118	85	100/118	73	30/41	91	70/77	9	7/77	27	11/41
Without Alcohols, Surfactants, and Solids	82	85	70/82	81	51/26	88	49/56	12	7/56	19	5/26

Abbreviations: EU = European Union; ICE = isolated chicken eye; N = number of substances included in this analysis/the total number of substances in the study; No. = data used to calculate the percentage.

[1] EU classification system (EU 2001): Not Labeled vs. R41/R36.

Table 6-16 Under- and Overprediction of the ICE Test Method Using the EU Classification System[1] in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property

Category	N	Underprediction (*In Vivo/In Vitro*)			Overprediction (*In Vivo/In Vitro*)		
		Severe (R41)		Mild/Mod (R36)	Mild/Mod (R36)	Not Labeled (NL)	
		NL	R36	NL	R41	R36	R41
Overall	153	18% (6/32)	22% (7/32)	25% (7/28)	18% (5/28)	10% (9/93)	1% (1/93)
Chemical Class[2]							
Alcohol	14	0% (0/3)	33% (1/3)	0% (0/5)	40% (2/5)	17% (1/6)	17% (1/6)
Carboxylic Acid	10	17% (1/6)	0% (0/6)	50% (1/2)	0% (0/2)	0% (0/2)	0% (0/2)
Ester	9	0% (0/1)	0% (0/1)	33% (1/3)	33% (1/3)	40% (2/5)	0% (0/5)
Heterocyclic	9	17% (1/6)	17% (1/6)	0% (0/1)	0% (0/1)	0% (0/2)	0% (0/2)
Inorganic	5	50% (1/2)	0% (0/2)	0% (0/1)	0% (0/1)	0% (0/2)	0% (0/2)
Onium Compound	8	0% (0/6)	33% (2/6)	100% (1/1)	-	0% (0/1)	0% (0/1)
Polyether	5	-	100% (1/1)	100% (1/1)	-	0% (0/3)	0% (0/3)
Properties of Interest							
Liquids[3]	112	8% (2/24)	21% (5/24)	23% (5/22)	18% (4/22)	14% 9/66	2% (1/66)
Solids[3]	39	27% (4/15)	13% (2/15)	66% (2/3	0% (0/3)	0% (0/21)	0% (0/21)

continued

Table 6-16 Under- and Overprediction of the ICE Test Method Using the EU Classification System[1] in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property (continued)

Category	N	Underprediction (*In Vivo/In Vitro*)			Overprediction (*In Vivo/In Vitro*)		
		Severe (R41)		Mild/Mod (R36)	Mild/Mod (R36)	Not Labeled (NL)	
		NL	R36	NL	R41	R36	R41
Overall	153	18% (6/32)	22% (7/32)	25% (7/28)	18% (5/28)	10% (9/93)	1% (1/93)
Properties of Interest (continued)							
Pesticide	11	20% (1/5)	20% (1/5)	1% (1/1)	-	0% (0/5)	0% (0/5)
Surfactant—Total	24	0% (0/9)	44% (4/9)	67% (2/3)	0% (0/3)	17% (2/12)	0% (0/12)
-nonionic	5	-	100% (1/1)	100% (1/1)	-	67% (2/3)	0% (0/3)
Anionic	3	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)
Cationic	7	0% (0/6)	33% (2/6)	-	-	0% (0/1)	0% (0/1)
pH-Total	20	22% (4/18)	17% (3/18)	-	-	0% (0/2)	0% (0/2)
-acidic (pH < 7.0)	13	9% (1/11)	18% (2/11)	-	-	0% (0/2)	0% (0/2)
-basic (pH > 7.0)	7	43% (3/7)	14% (1/7)	-	-	-	-

Abbreviations: EU = European Union; ICE = isolated chicken eye; N = number of substances included in this analysis/the total number of substances in the study; NL = Not Labeled (as irritant).

[1] EU classification system (EU 2001).

[2] Chemical classes included in this table are represented by at least five substances tested in the ICE test method, and assignments are based upon National Library of Medicine medical subject heading (MeSH) categories (www.nlm.nih.gov/mesh) as defined in Annex I.

[3] Physical form (i.e., solid and liquid) not known for some substances; therefore, the overall number does not equal the sum of the solid and liquid substances.

6.4 FHSA Classification System: ICE Test Method Accuracy

The four studies (Prinsen and Köeter 1993; Balls et al. 1995; Prinsen 1996; Prinsen 2005) contained ICE test method data on 174 substances, 146 and 138 of which had sufficient *in vivo* data to be assigned an ocular irritancy classification according to the FHSA-20% and FHSA-67% criteria, respectively. Based on results from *in vivo* rabbit eye experiments using the FHSA-20% criteria, 58% (84/146) were classified as irritants and 42% (62/146) were classified as Not Labeled. The remaining 28 substances could not be classified according to the FHSA-20% criteria due to lack of adequate data and are so noted in **Annex III**.

Based on results from *in vivo* rabbit eye experiments using the FHSA-67% criteria, 55% (76/138) were classified as irritants and 45% (62/138) were classified as Not Labeled. The remaining 36 substances could not be classified according to the FHSA-67% criteria due to lack of adequate data and are so noted in **Annex III**.

6.4.1 Ability to Distinguish Not Labeled Substances from Irritants

ICCVAM evaluated the ability of the ICE test method to distinguish substances not labeled as irritants from irritants. Using this approach for the 146 substances classified according to the FHSA-20% criteria, the ICE test method has an overall accuracy of 83% (121/146), a sensitivity of 88% (74/84), a specificity of 76% (47/62), a false positive rate of 24% (15/62), and a false negative rate of 12% (10/84) (**Table 6-17**).

Using this approach for the 138 substances classified according to the FHSA-67% criteria, the ICE test method has an overall accuracy of 84% (116/138), a sensitivity of 91% (69/76), a specificity of 76% (47/62), a false positive rate of 24% (15/62), and a false negative rate of 9% (7/76) (**Table 6-18**).

As detailed below, the results from each individual study were evaluated separately.

Prinsen and Köeter (1993): Based upon *in vivo* rabbit data using the FHSA-20% criteria (**Table 6-17**), ten substances could be assigned a classification. Among these ten substances, the ICE test method has an accuracy of 80% (8/10), sensitivity of 83% (5/6), specificity of 75% (3/4), a false positive rate of 25% (1/4), and a false negative rate of 17% (1/6).

Based upon *in vivo* rabbit data using the FHSA-67% analysis method (**Table 6-18**), nine substances could be assigned a classification. Among these nine substances, the ICE test method has an accuracy of 89% (8/9), sensitivity of 100% (5/5), specificity of 75% (3/4), a false positive rate of 25% (1/4), and a false negative rate of 0% (0/5).

Balls et al. (1995): Based upon *in vivo* rabbit data using the FHSA-20% criteria (**Table 6-17**), 53 substances could be assigned a classification. Among these 53 substances, the ICE test method has an accuracy of 91% (48/53), sensitivity of 94% (47/50), specificity of 33% (1/3), a false positive rate of 67% (2/3), and a false negative rate of 6% (3/50).

Based upon *in vivo* rabbit data using the FHSA-67% criteria (**Table 6-18**), 48 substances could be assigned a classification. Among these 48 substances, the ICE test method has an accuracy of 90% (43/48), sensitivity of 93% (42/45), specificity of 33% (1/3), a false positive rate of 67% (2/3), and a false negative rate of 7% (3/45).

Prinsen (1996): Based upon *in vivo* rabbit data using the FHSA-20% criteria (**Table 6-17**), 38 substances could be assigned a classification. Among these 38 substances, the ICE test method has an accuracy of 84% (32/38), sensitivity of 77% (10/13), specificity of 88% (22/25), a false positive rate of 12% (3/25), and a false negative rate of 23% (3/13).

Based upon *in vivo* rabbit data using the FHSA-67% criteria (**Table 6-18**), 37 substances could be assigned a classification. Among these 37 substances, the ICE test method has an accuracy of 86%

(32/37), sensitivity of 83% (10/12), specificity of 88% (22/25), a false positive rate of 12% (3/25), and a false negative rate of 17% (2/12).

Prinsen (2005): Based upon *in vivo* rabbit data using the FHSA-20% criteria (**Table 6-17**), 44 substances could be assigned a classification. Among these 44 substances, the ICE test method has an accuracy of 73% (32/44), sensitivity of 79% (11/14), specificity of 70% (21/30), a false positive rate of 30% (9/30), and a false negative rate of 21% (3/14).

Based upon *in vivo* rabbit data using the FHSA-67% criteria (**Table 6-18**), 43 substances could be assigned a classification. Among these 43 substances, the ICE test method has an accuracy of 74% (32/43), sensitivity of 85% (11/13), specificity of 70% (21/30), a false positive rate of 30% (9/30), and a false negative rate of 15% (2/13).

Table 6-17 Accuracy of the ICE Test Method in Distinguishing Not Labeled Substances from Irritants as Defined by the FHSA-20% Criteria,[1] by Study and Overall

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Prinsen and Koëter (1993)	10	80	8/10	83	5/6	75	3/4	25	1/4	17	1/6
Balls et al. (1995)	53	91	48/53	94	47/50	33	1/3	67	2/3	6	3/50
Prinsen (1996)	38	84	32/38	77	10/13	88	22/25	12	3/25	23	3/13
Prinsen (2005)	44	73	32/44	79	11/14	70	21/30	30	9/30	21	3/14
Overall[2]	146	83	121/146	88	74/84	76	47/62	24	15/62	12	10/84

Abbreviations: FHSA = Federal Hazardous Substances Act; ICE = isolated chicken eye; N = number of substances; No. = data used to calculate the percentage.

[1] For the FHSA classification system (FHSA 2005), "proportionality" criteria (i.e., FHSA-20%) were applied for the purpose of assigning an FHSA classification for test results that would require additional testing according to the FHSA sequential testing strategy in order to maximize the number of substances included in these analyses.

[2] Because Prinsen (2000) includes only one test substance that could be classified by FHSA-20%, data from this study were included only in the overall analysis and were not evaluated separately.

Table 6-18 Accuracy of the ICE Test Method in Distinguishing Not Labeled Substances from Irritants as Defined by the FHSA-67% Criteria,[1] by Study and Overall

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Prinsen and Koëter (1993)	9	89	8/9	100	5/5	75	3/4	25	1/4	0	0/5
Balls et al. (1995)	48	90	43/48	93	42/45	33	1/3	67	2/3	7	3/45
Prinsen (1996)	37	86	32/37	83	10/12	88	22/25	12	3/25	17	2/12
Prinsen (2005)	43	74	32/43	85	11/13	70	21/30	30	9/30	15	2/13
Overall[2]	138	84	116/138	91	69/76	76	47/62	24	15/62	9	7/76

Abbreviations: FHSA = Federal Hazardous Substances Act; ICE = isolated chicken eye; N = number of substances; No. = data used to calculate the percentage.

[1] For the FHSA classification system (FHSA (2005), "proportionality" criteria (i.e., FHSA-67%) were applied for the purpose of assigning an FHSA classification for test results that would require additional testing according to the FHSA sequential testing strategy in order to maximize the number of substances included in these analyses.

[2] Because Prinsen (2000) includes only one test substance that could be classified by FHSA-67%, data from this study were included only in the overall analysis and were not evaluated separately.

6.4.2 Performance of the ICE Test Method with Discordant Classes Excluded

The previous ICCVAM BRD identified limitations of the ICE test method based upon the false positive rate for alcohols and the false negative rates for solids and surfactants when the ICE test method is used to identify ocular corrosives and severe irritants (ICCVAM 2006a). For this reason, the performance of the ICE test method in identifying FHSA irritants using the FHSA-20% and FHSA-67% criteria was evaluated with these substances excluded from the database. The overall performance statistics using the FHSA-20% criteria (**Table 6-19**) or the FHSA-67% criteria (**Table 6-20**) were not affected by the exclusion of substances belonging to any of the three discordant classes or by any combinations of them**.**

The ability of the ICE test method to distinguish substances not labeled as irritants from irritants as defined by the FHSA-20% criteria was evaluated with the discordant classes removed separately and in combination (**Table 6-19**). The overall accuracy of the ICE database was 83% (121/146) compared to 82% (62/76) with all previously discordant alcohols, surfactants, and solids removed. The overall false negative rate of 12% (10/84) ranged from a low of 8% (5/60) with solids removed to a high of 17% (10/59) with alcohols and surfactants removed. However, the overall false positive rate increased from 24% (47/62) to 27% (13/49) when solids were removed and decreased marginally to 21% (11/53) when alcohols and surfactants were removed.

The ability of the ICE test method to distinguish substances not labeled as irritants from irritants as defined by the FHSA-67% criteria was evaluated with the discordant classes removed separately and in combination (**Table 6-20**). The overall accuracy of the ICE database was 84% (116/138) compared to 82% (59/72) with all previously discordant alcohols, surfactants, and solids removed. The overall false negative rate of 9% (7/76) ranged from a low of 7% (4/54) with solids removed to a high of 13% with alcohols and surfactants removed (10/59) or alcohols, surfactants, and solids (9/40) removed. However, the overall false positive rate increased marginally from 24% (15/62) to 27% (13/49) when solids were removed and decreased slightly to 21% (11/53) when alcohols and surfactants were removed.

Following the removal of substances belonging to the discordant classes (i.e., alcohols, surfactants and solids; see ICCVAM 2006a), there were five FHSA-20% criteria ocular irritants and four FHSA-67% criteria ocular irritants classified as Not Labeled as Irritant by the ICE test method (i.e., false negatives; see **Table 6-5**).

Table 6-19 Accuracy of the ICE Test Method in Distinguishing Not Labeled Substances from Irritants as Defined by the FHSA-20% Criteria,[1] with Discordant Chemical and Physical Classes Excluded

ICE	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall	146	83	121/146	88	74/84	76	47/62	24	15/62	12	10/84
Without Alcohols	132	83	109/132	78	64/74	78	45/58	22	13/58	14	10/74
Without Surfactants	124	82	102/124	86	59/69	78	43/55	22	12/55	14	10/69
Without Solids	109	83	91/109	92	55/60	73	36/49	27	13/49	8	5/60
Without Alcohols and Surfactants	112	81	91/112	83	49/59	79	42/53	21	11/53	17	10/59
Without Alcohols, Surfactants, and Solids	76	82	62/76	86	31/36	78	31/40	23	9/40	14	5/36

Abbreviations: FHSA = U.S. Federal Hazardous Substances Act; ICE = isolated chicken eye; N = number of substances included in this analysis/the total number of substances in the study; No. = data used to calculate the percentage.

[1] For the FHSA classification system (FHSA 2005), "proportionality" criteria (i.e., FHSA-20%) were applied for the purpose of assigning an FHSA classification for test results that would require additional testing according to the FHSA sequential testing strategy in order to maximize the number of substances included in these analyses.

Table 6-20 Accuracy of the ICE Test Method in Distinguishing Not Labeled Substances from Irritants as Defined by the FHSA-67% Criteria,[1] with Discordant Chemical and Physical Classes Excluded

ICE	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall	138	84	116/138	91	69/76	76	47/62	24	15/62	9	7/76
Without Alcohols	124	84	104/124	89	59/66	78	45/58	22	13/58	11	7/66
Without Surfactants	116	84	99/118	89	56/63	78	43/55	22	12/55	11	7/63
Without Solids	103	83	86/103	93	50/54	73	36/49	27	13/49	7	4/54
Without Alcohols and Surfactants	106	83	88/106	87	46/53	79	42/53	21	11/53	13	7/53
Without Alcohols, Surfactants, and Solids	72	82	59/72	88	28/32	78	31/40	23	9/40	13	4/32

Abbreviations: FHSA = U.S. Federal Hazardous Substances Act; ICE = isolated chicken eye; N = number of substances included in this analysis/the total number of substances in the study; No. = data used to calculate the percentage.

[1] For the FHSA classification system (FHSA (2005), "proportionality" criteria (i.e., FHSA-67%) were applied for the purpose of assigning an FHSA classification for test results that would require additional testing according to the FHSA sequential testing strategy in order to maximize the number of substances included in these analyses.

7.0 Isolated Chicken Egg Test Method Reliability

Assessment of test method reliability (intralaboratory repeatability and intra- and interlaboratory reproducibility) is essential to any evaluation of the performance of an alternative test method (ICCVAM 2003). Quantitative and qualitative evaluations of ICE test method reliability have been conducted previously (ICCVAM 2006a). Because the database used for the current evaluation of the ICE test method has not changed, the quantitative evaluation of test method reliability remains unchanged.

However, ICCVAM conducted additional qualitative analyses of interlaboratory reproducibility to evaluate the extent to which the four laboratories participating in the interlaboratory validation study (Balls et al. 1995) agreed on ICE hazard classifications. As was done for the accuracy evaluation, these qualitative evaluations of reproducibility were conducted based on (1) the use of the ICE test method to identify all ocular hazard categories according to the EPA, GHS, and EU systems; and (2) the use of the ICE test method to distinguish substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, GHS Not Classified as Irritant) from all other ocular hazard categories (i.e., EPA Categories I, II, and III; EU R41 and R36; GHS Categories 1, 2A, and 2B). Given that the performance of the ICE test method was similar for the EPA and FHSA classification systems, additional reliability analyses were not conducted for the FHSA classification system.

7.1 Interlaboratory Reproducibility of Hazard Classification Category Using the GHS Classification System

Of 14 substances classified by the GHS as Not Classified, 7% (1/14) were correctly identified, while 50% (2/4) of GHS Category 2B substances were correctly identified, 43% (6/14) of substances classified as GHS Category 2A were correctly identified, and 50% (11/22) of GHS Category 1 substances were correctly identified (**Table 7-1)**.

The four participating laboratories were in 100%, 75%, and 50% agreement on the ocular irritancy classification when distinguishing Not Classified substances from all other classes of 75% (44/59), 14% (8/59), and 12% (7/59), respectively (**Table 7-2**).[9]

All four participating laboratories agreed on the classification of 64% (7/11) of substances that were correctly identified as GHS Category 1,[10] 50% (3/6) of substances correctly classified as GHS Category 2A, 0% (0/2) of substances correctly classified as GHS Category 2B, and 0% (0/1) of substances correctly classified as GHS Not Classified (**Table 7-1**).

Three of the four laboratories were in agreement for 27% (3/11) of the correctly identified GHS Category 1 substances, 0% (0/6) of GHS Category 2A substances, 50% (1/2) of GHS Category 2B substances, and 100% (1/1) of the Not Classified substances **(Table 7-1)**.

---

[9] Because the database of ICE test method results has not changed, the qualitative evaluation of reproducibility presented in ICCVAM (2006a) is not repeated here.

[10] As described in **Section 6.1**, the overall *in vitro* classification for each substance was determined based on the most frequent individual laboratory classification or, in the case of an even number of discordant responses, the most severe classification. For one chemical (trichloroacetic acid, 30%), scores for fluorescein retention and corneal swelling were not provided from one laboratory. Therefore, this chemical was classified based on the results from only three laboratories.

Table 7-1 Interlaboratory Variability of Balls et al. (1995) in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method as Defined by the GHS Classification System[1]

In Vivo **Classification (No.)[2]**	**Classification (*In Vitro*)**	**Number of Substances (%)**	**Number of Testing Laboratories**	**Substances with 100% Agreement Among Laboratories (%)**	**Substances with 75% Agreement Among Laboratories (%)**	**Substances with 50% Agreement Among Laboratories (%)**
NC (14)	Actual	1 (7)	4	0	1 (100)	0
	Over	13 (93)	4	7 (54)	4 (31)	2 (15)
2B (4)	Under	0	4	0	0	0
	Actual	2 (50)	4	0	1 (50)	1 (50)
	Over	2 (50)	4	0	2 (100)	0
2A (14)	Under	2 (14)	4	0	0	2 (100)
	Actual	6 (43)	4	3 (50)	0	3 (50)
	Over	6 (43)	4	1 (17)	0	5 (83)
1 (22)	Under	11 (50)	4	9 (82)	2 (18)	0
	Actual	11 (50)	4[3]	7 (64)	3 (27)	1(9)

Abbreviation: GHS = Globally Harmonized System; NC = Not Classified; No. = number of substances included in this analysis/the total number of substances in the study.

[1] GHS classification system (UN 2007); Mild, Moderate, or Corrosive/Severe irritant (2B, 2A, or 1, respectively).

[2] Due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects), a GHS classification could not be made for 5 substances. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro*.

[3] Scores for fluorescein retention and corneal swelling were not provided by one laboratory for one substance (trichloroacetic acid, 30%); therefore, this substance was classified based on results from only three laboratories.

Table 7-2 Interlaboratory Variability of Balls et al. (1995) for Substances Classified as Not Classified or Category 1/2A/2B Using the GHS Classification System[1]

Classification (*In Vivo/In Vitro*)[2]	Number of Substances	Number of Testing Laboratories	Substances with 100% Agreement Among Laboratories (%)	Substances with 75% Agreement Among Laboratories (%)	Substances with 50% Agreement Among Laboratories (%)
+/+	38	4[3]	33 (87)	3 (8)	2 (5)
+/-	2	4	0	0	2 (100)
-/+	13	4	7 (54)	4 (31)	2 (15)
-/-	1	4	0	1 (100)	0
?/-	1	4	0	0	1 (100)
?/+	4	4	4 (100)	0	0
TOTAL	59	4[3]	44 (75)	8 (14)	7 (12)

Abbreviation: GHS = Globally Harmonized System.

[1] GHS classification system (UN 2007).

[2] A "+" indicates that the substance was assigned an overall classification of Mild, Moderate, or Corrosive/Severe irritant (2B, 2A, or 1, respectively). A "-" indicates that the substance was assigned a classification of Not Classified. A "?" indicates that, due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects), a GHS classification could not be made. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro.*

[3] Scores for fluorescein retention and corneal swelling were not provided by one laboratory for one substance (trichloroacetic acid, 30%); therefore, this substance was classified based on results from only three laboratories.

Two of the four laboratories were in agreement for 9% (1/11) of the GHS Category 1 substances identified correctly, 50% (3/6) of GHS Category 2A substances, 50% (1/2) of GHS Category 2B substances, and 0% (0/1) of the GHS Not Classified substances (**Table 7-1**). The labs with discordant data were not consistent within or across the irritant classes.

Of 14 substances classified by the GHS as Not Classified, 93% (13/14) were incorrectly identified, while 50% (2/4) of GHS Category 2B substances were incorrectly identified, 57% (8/14) of Category 2A substances were incorrectly identified, and 50% (11/22) of GHS Category 1 substances were incorrectly identified (**Table 7-1**).

All four participating laboratories (100%) incorrectly classified 82% (9/11) of the GHS Category 1 substances, 13% (1/8) of the GHS Category 2A substances, 0% (0/2) of the GHS Category 2B substances, and 54% (7/13) of the GHS Not Classified substances (**Table 7-1**).

Three of the four laboratories (75%) were in agreement in incorrectly classifying 18% (2/11) of the GHS Category 1 substances, 0% (0/8) of the GHS Category 2A substances, 100% (2/2) of Category 2B substances, and 31% (4/13) of the GHS Not Classified substances (**Table 7-1**).

Two of the four laboratories (50%) were in agreement in incorrectly classifying 0% (0/11) of the GHS Category 1 substances, 88% (7/8) of the GHS Category 2A substances, 0% (0/2) of the GHS Category 2B substances, and 15% (2/13) of the GHS Not Classified substances (**Table 7-1**).

7.2 Interlaboratory Reproducibility of Hazard Classification Category Using the EPA Classification System

Of two substances classified by the EPA as Category IV, 0% (0/2) were correctly identified, while 40% (8/20) EPA Category III substances were correctly identified, 50% (5/10) of the EPA Category

II substances were correctly identified, and 53% (10/19) of the EPA Category I substances were correctly identified (**Table 7-3).**

The four participating laboratories were in 100%, 75%, and 50% agreement in regard to the ocular irritancy classification when distinguishing Category IV substances from all other classes of 75% (44/59), 14% (8/59), and 12% (7/59), respectively (**Table 7-4**).[11]

All four participating laboratories (100%) agreed on the classification of 70% (7/10) of substances that were correctly identified as EPA Category I,[12] 60% (3/5) of substances correctly classified as EPA Category II, 13% (1/8) of substances correctly classified as EPA Category III, and 0 substances classified as Category IV (**Table 7-3**).

Three of the four laboratories (75%) were in agreement for 20% (2/10) of the correctly identified EPA Category I substances, 20% (1/5) of the EPA Category II substances, 38% (3/8) of the EPA Category III substances, and 0 of the substances classified as Category IV (**Table 7-3).** The discordant laboratory was not consistent among these substances.

Two of the four laboratories (50%) were in agreement for 10% (1/10) of the EPA Category I substances identified correctly, 20% (1/5) of the EPA Category II substances, 50% (4/8) of the EPA Category III substances correctly identified, and 0 of the substances classified as Category IV (**Table 7-3**).

Of two substances classified by the EPA as Category IV, 100% (2/2) were incorrectly identified, while 60% (12/20) of substances classified as EPA Category III were incorrectly identified, 50% (5/10) of EPA Category II substances were incorrectly identified, and 47% (9/19) of EPA Category I substances were incorrectly identified (**Table 7-3**).

The four participating laboratories (100%) were in 100% agreement in incorrectly classifying 78% (7/9) of the EPA Category I substances, 20% (1/5) of the EPA Category II substances, 50% (6/12) of the EPA Category III substances, and 0% (0/2) of the EPA Category IV substances (**Table 7-3**).

Three of the four laboratories (75%) were in agreement in incorrectly classifying 22% (2/9) of the EPA Category I substances, 20% (1/5) of the EPA Category II substances, 33% (4/12) of the Category III substances, and 100% (2/2) of the EPA Category IV substances (**Table 7-3).** The lab with the discordant results was not consistent within and across the irritant classes.

Two of the four laboratories were in agreement of incorrectly classifying 0% (0/9) of the EPA Category I substances, 60% (3/5) of the EPA Category II substances, 17% (2/12) of the EPA Category III substances, and 0% (0/2) of the EPA Category IV substances (**Table 7-3**).

---

[11] Because the database of ICE test method results has not changed, the qualitative evaluation of reproducibility presented in ICCVAM (2006a) is not repeated here.

[12] As described in **Section 6.1**, the overall *in vitro* classification for each substance was determined based on the most frequent individual laboratory classification or, in the case of an even number of discordant responses, the most severe classification. For one chemical (trichloroacetic acid, 30%), scores for fluorescein retention and corneal swelling were not provided by one laboratory. Therefore, this chemical was classified based on the results from only three laboratories.

Table 7-3 Interlaboratory Variability of Balls et al. (1995) in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method as Defined by the EPA Classification System[1]

***In vivo* Classification (No.)[2]**	**Classification (*In vitro*)**	**Number of Substances (%)**	**Number of Testing Laboratories**	**Substances with 100% Agreement Among Laboratories (%)**	**Substances with 75% Agreement Among Laboratories (%)**	**Substances with 50% Agreement Among Laboratories (%)**
IV (2)	Actual	0	4	0	0	0
	Over	2 (100)	4	0	2 (100)	0
III (20)	Under	2 (10)	4	0	1 (50)	1 (50)
	Actual	8 (40)	4	1 (13)	3 (38)	4 (50)
	Over	10 (50)	4	6 (60)	3 (30)	1 (10)
II (10)	Under	2 (20)	4	0	1 (50)	1 (50)
	Actual	5 (50)	4	3 (60)	1 (20)	1 (20)
	Over	3 (30)	4	1 (33)	0	2 (67)
1 (19)	Under	9 (47)	4	7 (78)	2 (22)	0
	Actual	10 (53)	4[3]	7 (70)	2 (20)	1 (10)

Abbreviation: EPA = U.S. Environmental Protection Agency; No. = number of substances included in this analysis/the total number of substances in the study

[1] EPA classification system (EPA 2003a).

[2] Due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects), an EPA classification could not be made for 6 substances. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro.*

[3] Scores for fluorescein retention and corneal swelling were not provided from one laboratory for one substance (trichloroacetic acid, 30%); therefore, this substance was classified based on results from only three laboratories.

Table 7-4 Interlaboratory Variability of Balls et al. (1995) for Substances Classified as Category IV or Category I/ II/III Using the EPA Classification System[1]

Classification (*In vivo/In vitro*)[2]	Number of Substances	Number of Testing Laboratories	Substances with 100% Agreement Among Laboratories (%)	Substances with 75% Agreement Among Laboratories (%)	Substances with 50% Agreement Among Laboratories (%)
+/+	47	4[3]	38 (81)	5 (11)	4 (9)
+/-	4	4	0	1 (25)	3 (75)
-/+	2	4	0	2 (100)	0
-/-	0	4	0	0	0
?/-	0	4	0	0	0
?/+	6	4	6 (100)	0	0
TOTAL	59	4[3]	44 (75)	8 (14)	7 (12)

Abbreviation: EPA = U.S. Environmental Protection Agency

[1] EPA classification system (2003a).

[2] A "+" indicates that the substance was assigned an overall classification of Severe, Moderate, or Mild irritant (I, II, or III, respectively). A "-" indicates that the substance was assigned a classification of not classified as an irritant (Category IV). A "?" indicates that, due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects), an EPA classification could not be made. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro.*

[3] Scores for fluorescein retention and corneal swelling were not provided from one laboratory for one substance (trichloroacetic acid, 30%); therefore, this substance was classified based on results from only three laboratories.

7.3 Interlaboratory Reproducibility of Hazard Classification Category Using the EU Classification System

Of 17 substances classified by the EU as Not Labeled, 53% (9/17) were correctly identified, while 50% (7/14) of substances classified as EU moderate irritants (R36) were correctly identified, and 53% (10/19) substances classified by the EU as corrosive/severe irritants (R41) were correctly identified (**Table 7-5**).

The four participating laboratories were in 100%, 75%, and 50% agreement in regard to the ocular irritancy classification when distinguishing Not Labeled substances from all other classes of 61% (36/59), 25% (15/59), and 14% (8/59), respectively (**Table 7-6**).[13]

All four participating laboratories (100%) agreed on the classification of 70% (7/10) of the substances that were correctly identified as R41, 57% (4/7) of substances correctly classified as EU R36, and 33% (3/9) of those correctly classified as EU Not Labeled (**Table 7-5**).

Three of the four laboratories (75%) were in agreement on 20% (2/10) of the correctly identified R41 substances, 29% (2/7) of the R36 substances, and 44% (4/9) of the substances classified as EU Not Labeled (**Table 7-5**). The discordant laboratory was not consistent among these substances.

Two of the four laboratories (50%) were in agreement for 10% (1/10) of the R41 substances correctly identified, 14% (1/7) of the R36 substances, and 22% (2/9) of the substances classified as EU Not Labeled (**Table 7-5**).

[13] Because the database of ICE test method results has not changed, the qualitative evaluation of reproducibility presented in ICCVAM (2006) is not repeated here.

Of 17 substances classified by the EU as Not Labeled, 47% (8/17) were incorrectly identified, while 50% (7/14) of substances classified as R36 substances were incorrectly identified, and 47% (9/19) of substances classified as R41 were incorrectly identified (**Table 7-5**).

The four participating laboratories (100%) were in 100% agreement in incorrectly classifying 78% (7/9) of the R41 substances, 14% (1/7) of the R36 substances, and 63% (5/8) of the EU Not Labeled substances (**Table 7-5**).

Three of the four laboratories (75%) were in agreement in incorrectly classifying 22% (2/9) of the R41 substances, 29% (2/7) of the R36 substances, and 13% (1/8) of the EU Not Labeled substances (**Table 7-5**).

Two of the four laboratories (50%) were in agreement in incorrectly classifying 0% (0/9) of the R41 substances, 57% (4/7) of the R36 substances, and 25% (2/8) of the EU Not Labeled substances (**Table 7-5**).

Table 7-5 Interlaboratory Variability of Balls et al. (1995) in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method as Defined by the EU Classification System[1]

***In vivo* Classification (No.)[2]**	**Classification (*in vitro*)**	**Number of Substances (%)**	**Number of Testing Laboratories**	**Substances with 100% Agreement Among Laboratories (%)**	**Substances with 75% Agreement Among Laboratories (%)**	**Substances with 50% Agreement Among Laboratories (%)**
NL (17)	Actual	9 (53)	4	3 (33)	4 (44)	2 (22)
	Over	8 (47)	4	5 (63)	1 (13)	2 (25)
R36 (14)	Under	3 (21)	4	0	2 (67)	1 (33)
	Actual	7 (50)	4	4 (57)	2 (29)	1 (14)
	Over	4 (29)	4	1 (25)	0	3 (75)
R41 (19)	Under	9 (47)	4	7 (78)	2 (22)	0
	Actual	10 (53)	4[3]	7 (70)	2 (20)	1 (10)

Abbreviation: EU = European Union; NL = Not Labeled (as an irritant); No. = number of substances included in this analysis

[1] EU classification system (2001).

[2] Due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects), a EU classification could not be made for 9 substances. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro.*

[3] Scores for fluorescein retention and corneal swelling were not provided from one laboratory for one substance (trichloroacetic acid, 30%); therefore, this substance was classified based on results from only three laboratories.

Table 7-6 Interlaboratory Variability of Balls et al. (1995) for Substances Classified as Not Labeled or R36/R41 Using the EU Classification System[1]

Classification (*In vivo/In vitro*)[2]	Number of Substances	Number of Testing Laboratories	Substances with 100% Agreement Among Laboratories (%)	Substances with 75% Agreement Among Laboratories (%)	Substances with 50% Agreement Among Laboratories (%)
+/+	26	4[3]	22 (85)	3 (12)	1 (4)
+/-	7	4	2 (29)	3 (42)	2 (29)
-/+	8	4	5 (63)	1 (13)	2 (25)
-/-	9	4	3 (33)	4 (44)	2 (22)
?/-	1	4	0	1 (100)	0
?/+	8	4	4 (50)	3 (38)	1 (13)
TOTAL	59	4[3]	36 (61)	15 (25)	8 (14)

Abbreviation: EU = European Union.

[1] EU classification system (2001).

[2] A "+" indicates that the substance was assigned an overall classification of Severe or Nonsevere irritant (Category R41 or R36). A "-" indicates that the substance was assigned a classification of Not Labeled (as an irritant) (Category NL). A "?" indicates that, due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects), a EU classification could not be made. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro.*

[3] Scores for fluorescein retention and corneal swelling were not provided by one laboratory for one substance (trichloroacetic acid, 30%); therefore, this substance was classified based on results from only three laboratories.

8.0 Isolated Chicken Egg Test Method Data Quality

The database used in this assessment did not change from that used in the previous assessment of the ability of the ICE method to identify ocular corrosives and severe irritants. The evaluation of ICE test method data quality is detailed in the *Background Review Document: Current Status of In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants: Isolated Chicken Eye Test Method* (ICCVAM 2006a).

9.0 Other Scientific Reports and Reviews

No new data, nor published or unpublished studies, have been located since the previous evaluation of the ICE test method for identification of ocular corrosives and severe irritants (ICCVAM 2006a).

10.0 Animal Welfare Considerations (Refinement, Reduction, and Replacement)

10.1 How the ICE Test Method Will Refine, Reduce, or Replace Animal Use

ICCVAM promotes the scientific validation and regulatory acceptance of new methods that refine, reduce, or replace animal use where scientifically feasible. Refinement, reduction, and replacement are known as the "three Rs" of animal protection. These principles of humane treatment of laboratory animals are described as:

- Refining experimental procedures such that animal suffering is minimized
- Reducing animal use through improved science and experimental design
- Replacing animal models with non-animal procedures (e.g., *in vitro* technologies), where possible (Russell and Burch 1992)

The ICE test method refines animal use. Because these animals are being humanely killed for nonlaboratory purposes, the testing procedure inflicts no additional pain or distress on animals. Substances that are identified as corrosive or severe irritants *in vitro* are excluded from *in vivo* testing. Furthermore, the ability to identify mild and moderate ocular irritants would eliminate the need for *in vivo* testing, thus sparing rabbits from the pain associated with these types of substances.

The ICE test method can also reduce animal use because the test method was adapted from the IRE test method, which replaces laboratory animals with animal species routinely raised in large numbers as a food source. Additionally, with the ability to identify ocular corrosives and severe irritants as well as mild and moderate ocular irritants from the *in vitro* method, the animals that would have been used in the *in vivo* rabbit eye test would be spared.

10.2 Requirement for the Use of Animals

Although chickens are required as a source of corneas for this *in vitro* test method, only chickens humanely killed for food or other nonlaboratory purposes are used as eye donors (i.e., no live animals are used in this test method).

11.0 Practical Considerations

Practical considerations for the ICE test method are detailed in the *Background Review Document: Current Status of In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants: Isolated Chicken Eye Test Method* (ICCVAM 2006a).

12.0 References

Balls M, Botham PA, Bruner LH, Spielmann H. 1995. The EC/HO international validation study on alternatives to the Draize eye irritation test. Toxicol In Vitro 9:871-929.

Botham JW, McKillop CM, Purchase IFH. 1989. Acute and topical toxicity profiles for substances involved in the *in vitro* validation of the *in vivo* ocular irritation model. In: Collaborative Study on the Evaluation of Alternative Methods to the Eye Irritation Test. EC Document XI/632/91, V/E/1/131/91. Commission of the European Communities.

CPSC. 2003. Commercial practices. Hazardous Substances and Articles; Administration and Enforcement Regulations. 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 Pharmacol Exper Ther 82:377-390.

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. 2003a. Label Review Manual: 3rd ed. EPA737-B-96-001. Washington, DC: U.S. Environmental Protection Agency.

EPA. 2003b. Good Laboratory Practice Standards. Toxic Substances Control Act. 40 CFR 792.

EPA. 2003c. Good Laboratory Practice Standards. Toxic Substances Control Act. 40 CFR 160.

EU. 1992. Commission Directive 92/69/EEC of 31 July 1992 adapting to technical progress for the seventeenth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L383:113-115.

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.

EU. 2004. Commission Directive 2004/73/EC of 29 April 2004 adapting to technical progress for the twenty-ninth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Union L152:1-311.

FDA. 2003. Good Laboratory Practice for Nonclinical Laboratory Studies. 21 CFR 58.

Federal Hazardous Substances Act (FHSA). 2005. Public Law 86-613.

ICCVAM. 1997. Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 97-3981. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

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. 2006a. Background Review Document: Current Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants: Isolated Chicken Eye Test Method. NIH Publication No. 06-4513. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

ICCVAM. 2006b. ICCVAM Test Method Evaluation Report: *In Vitro* Ocular Toxicity Methods for Identifying Severe Irritants and Corrosives. NIH Publication No. 07-4517. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

ICCVAM. 2009. Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches. Research Triangle Park, NC: National Institute of Environmental Health Sciences.

ICCVAM Authorization Act of 2000. 2000. Public Law 106-545. Available: http://iccvam.niehs.nih.gov/about/overview.htm [accessed 21 October 2004].

INVITTOX. 1994. Protocol No. 80: Chicken enucleated eye test (CEET). Available: http://ecvam-sis.jrc.it/invittox/static/index.html [accessed 18 February 2004].

MeSH Medical Subject Heading. Available: http://www.nlm.nih.gov/mesh [accessed 12 July 2005].

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

OECD. 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring Number 1: OECD Principles on Good Laboratory Practice (as revised in 1997). ENV/MC/CHEM(98)17. Paris:OECD.

OECD. 2002. Test guideline 405. Acute eye irritation/corrosion, adopted April 24, 2002. In: OECD Guidelines for Testing of Chemicals. Paris:OECD.

Prinsen MK. 1991a. Acute eye irritation/corrosion study with 1-butanol in albino rabbits. TNO-report V91.187/210069, November 1991. Dept. of Biological Toxicology, TNO Toxicology and Nutrition Institute, Zeist, The Netherlands. (Available on request.)

Prinsen MK. 1991b. Acute eye irritation/corrosion study with 2-methoxyethanol in albino rabbits. TNO-report V91.188/210069, November 1991. Dept. of Biological Toxicology, TNO Toxicology and Nutrition Institute, Zeist, The Netherlands. (Available on request.)

Prinsen MK. 1996. The chicken enucleated eye test (CEET): A practical (pre)screen for the assessment of eye irritation/corrosion potential of test materials. Food Chem Toxicol 34:291-296.

Prinsen MK. 2000. Chicken encleated eye test with reference surfactants and siloxane polymers in Phase II of the reference standard validation project; an *ex vivo* alternative to the Draize eye irritation test with rabbits. TNO Report V99.521b. Unpublished report provided directly to NICEATM by MK Prinsen, TNO Nutrition and Food Research Institute.

Prinsen MK. 2005. *In vitro* and *in vivo* data for 94 substances tested in the isolated chicken eye test. Unpublished data provided directly to NICEATM by MK Prinsen, TNO Nutrition and Food Research Institute.

Prinsen MK, Koëter BWM. 1993. Justification of the enucleated eye test with eyes of slaughterhouse animals as an alternative to the Draize eye irritation test with rabbits. Food Chem Toxicol 31:69-76.

Russell WMS, Burch RL. 1992. The Principles of Humane Experimental Technique. 14th ed. South Mimms, Potters Bar, Herts:Universities Federation for Animal Welfare.

United Nations. 2007. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). 2d rev ed. New York:UN. Available: http://www.unece.org/trans/danger/publi/ghs/ghs_rev02/02files_e.html.

13.0 Glossary[14]

Accuracy:[15] (a) The closeness of agreement between a test method result and an accepted reference value. (b) The proportion of correct outcomes of a test method. It is a measure of test method performance and one aspect of "relevance." The term is often used interchangeably with *concordance* (see also *two-by-two table*). Accuracy is highly dependent on the prevalence of positives in the population being examined.

Assay:[15] The experimental system used. Often used interchangeably with *test* and *test method.*

Benchmark control: A sample containing all components of a test system and treated with a known substance (i.e., the benchmark substance) to induce a known response. The sample is processed with test substance-treated and other control samples to compare the response produced by the test substance to the benchmark substance to allow for an assessment of the sensitivity of the test method to assess a specific chemical class or product class.

Benchmark substance: A substance used as a standard for comparison to a test substance. A benchmark substance should have the following properties:

- a consistent and reliable source(s)
- structural and functional similarity to the class of substances being tested
- known physical/chemical characteristics
- supporting data on known effects
- known potency in the range of the desired response

Blepharitis: Inflammation of the eyelids.

Bulbar conjunctiva: The portion of the conjunctiva that covers the outer surface of the eye.

CEET: Chicken Enucleated Eye Test; the original name of the test method referred to in this BRD as ICE.

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.

Coded substances: Substances labeled by code rather than name so that they can be tested and evaluated without knowledge of their identity or anticipation of test results. Coded substances are used to avoid intentional or unintentional bias when evaluating laboratory or test method performance.

Coefficient of variation: A statistical representation of the precision of a test. It is expressed as a percentage and is calculated as follows:

$$\left(\frac{standard\ deviation}{mean}\right) \times 100\%$$

[14] The definitions in this Glossary are restricted to their uses with respect to the Draize rabbit eye test method and the ICE test method.

[15] Definition used by the 2003 Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods (NIH Publication No. 03-4508).

Concordance:[15] The proportion of all substances tested that are correctly classified as positive or negative. It is a measure of test method performance and one aspect of relevance. The term is often used interchangeably with *accuracy* (see also *two-by-two table*). Concordance is highly dependent on the prevalence of positives in the population being examined.

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 swelling: An objective measurement in the ICE test of the extent of distention of the cornea following exposure to a test substance. It is expressed as a percentage and is calculated from corneal thickness measurements that are recorded at regular intervals during the ICE test. Increased corneal swelling is indicative of damage to the corneal epithelium.

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.

Endpoint:[15] The biological process, response, or effect assessed by a test method.

Enucleate: To remove without cutting into.

***Ex vivo*:** Outside of the living organism. Refers to assays conducted on a component(s) of a living organism in an artificial environment outside of the living organism (e.g., an enucleated eye).

False negative:[15] A substance incorrectly identified as negative by a test method.

False negative rate:[15] The proportion of all positive substances falsely identified by a test method as negative (see *two-by-two table*). It is one indicator of test method accuracy.

False positive:[15] A substance incorrectly identified as positive by a test method.

False positive rate:[15] The proportion of all negative substances that are falsely identified by a test method as positive (see *two-by-two table*). It is one indicator of test method accuracy.

Fibrous tunic: The outer of the three membranes of the eye, comprising the cornea and the sclera; called also *tunica fibrosa oculi*.

Fluorescein retention: A subjective measurement in the ICE test of the extent of fluorescein sodium that is retained by epithelial cells in the cornea following exposure to a test substance. Increased fluorescein retention is indicative of damage to the corneal epithelium.

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.

Good Laboratory Practices (GLP):[15] Regulations promulgated by the U.S. Food and Drug Administration and the U.S. Environmental Protection Agency, and principles and procedures adopted by the Organization for Economic Cooperation and Development and Japanese authorities

that describe record keeping and quality assurance procedures for laboratory records that will be the basis for data submissions to national regulatory agencies.

Hazard:[15] 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.

Interlaboratory reproducibility:[15] A measure of whether different qualified laboratories using the same protocol and test substances can produce qualitatively and quantitatively similar results. Interlaboratory reproducibility is determined during the prevalidation and validation processes and indicates the extent to which a test method can be transferred successfully among laboratories.

Intralaboratory repeatability:[15] The closeness of agreement between test results obtained within a single laboratory, when the procedure is performed on the same substance under identical conditions within a given time period.

Intralaboratory reproducibility:[15] The first stage of validation; a determination of whether qualified people within the same laboratory can successfully replicate results using a specific test protocol at different times.

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.

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.

Irritation index: A value calculated by summing the maximum mean scores of each of the ICE test method endpoints (corneal opacity, corneal swelling, and fluorescein retention). In order to increase their weighting relative to the corneal swelling value, the maximum corneal opacity and fluorescein retention scores obtained are multiplied by a factor of 20. Therefore, the irritation index has a possible range of 0 to 200.

Negative control: An untreated sample containing all components of a test system except the test substance solvent, which is replaced with a known nonreactive material, such as water. This sample is processed with test substance-treated samples and other control samples to determine whether the solvent interacts with the test system.

Negative predictivity:[15] The proportion of correct negative responses among substances testing negative by a test method (see *two-by-two table*). It is one indicator of test method accuracy. Negative predictivity is a function of the sensitivity of the test method and the prevalence of negatives among the substances tested.

Neuroectodermal tunic: The innermost of three membranes of the eye, comprising the retina.

Nicititating membrane: The membrane that moves horizontally across the eye in some animal species (e.g., rabbit, cat) to provide additional protection in particular circumstances. It may be referred to as the *third eyelid*.

Nonirritant: (a) A substance that 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.

Nonsevere irritant: (a) A substance that causes tissue damage in the eye following application to the anterior surface of the eye; the tissue damage is reversible within 21 days of application and the observed adverse effects in the eye are less severe than observed for a severe irritant. (b) Substances that are classified as GHS Category 2A or 2B; EPA Category II, III, or IV; EU R36.

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.

Palpebral conjunctiva: The part of the conjunctiva that covers the inner surface of the eyelids.

Pannus: A specific type of corneal inflammation that begins within the conjunctiva, and with time spreads to the cornea. Also referred to as *chronic superficial keratitis*.

Performance:[15] The accuracy and reliability characteristics of a test method (see *accuracy*, *reliability*).

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.

Positive control: A sample containing all components of a test system and treated with a substance known to induce a positive response, which is processed with the test substance-treated and other control samples to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay over time.

Positive predictivity:[15] The proportion of correct positive responses among substances testing positive by a test method (see *two-by-two* table). It is one indicator of test method accuracy. Positive predictivity is a function of the sensitivity of the test method and the prevalence of positives among the substances tested.

Prevalence:[15] The proportion of positives in the population of substances tested (see *two-by-two* table).

Protocol:[15] 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.

Quality assurance:[15] A management process by which adherence to laboratory testing standards, requirements, and record keeping procedures is assessed independently by individuals other than those performing the testing.

Reduction alternative:[15] A new or modified test method that reduces the number of animals required.

Reference test method:[15] The accepted *in vivo* test method used for regulatory purposes to evaluate the potential of a test substance to be hazardous to the species of interest.

Refinement alternative:[15] A new or modified test method that refines procedures to lessen or eliminate pain or distress in animals or enhances animal wellbeing.

Relevance:[15] The extent to which a test method correctly predicts or measures the biological effect of interest in humans or another species of interest. Relevance incorporates consideration of the *accuracy* or *concordance* of a test method.

Reliability:[15] A measure of the degree to which a test method can be performed reproducibly within and among laboratories over time. It is assessed by calculating intra- and interlaboratory reproducibility and intralaboratory repeatability.

Replacement alternative:[15] A new or modified test method that replaces animals with nonanimal systems or one animal species with a phylogenetically lower one (e.g., a mammal with an invertebrate).

Reproducibility:[15] The consistency of individual test results obtained in a single laboratory (intralaboratory reproducibility) or in different laboratories (interlaboratory reproducibility) using the same protocol and test substances (see intra- and interlaboratory reproducibility).

Sclera: The tough, fibrous tissue that extends from the cornea to the optic nerve at the back of the eye.

Secondary bacterial keratitis: Inflammation of the cornea that occurs secondary to another insult that compromised the integrity of the eye.

Sensitivity:[15] The proportion of all positive substances that are classified correctly as positive in a test method. It is a measure of test method accuracy (see *two-by-two* table).

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) A substance classified as GHS Category 1, EPA Category I, or EU R41 ocular irritants.

Slit-lamp microscope: An instrument used to directly examine the eye under the magnification of a binocular microscope by creating a stereoscopic, erect image. In the ICE test method, this instrument is used to view the anterior structures of the chicken eye as well as to objectively measure corneal thickness with a depth-measuring device attachment.

Solvent control: An untreated sample containing all components of a test system, including the solvent that is processed with the test substance-treated and other control samples to establish the baseline response for the samples treated with the test substance dissolved in the same solvent. When tested with a concurrent negative control, this sample also demonstrates whether the solvent interacts with the test system.

Specificity:[15] The proportion of all negative substances that are classified correctly as negative in a test method. It is a measure of test method accuracy (see *two-by-two* table).

Superfusion apparatus: A custom-built experimental setup for the ICE test that provides a controlled environment for short-term maintenance of the metabolic and physiological activity of the isolated chicken eye and a continuous flow of isotonic saline over the ocular surface.

Test:[15] The experimental system used; used interchangeably with *test method* and *assay*.

Test method:[15] 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*.

Test method component: Structural, functional, and procedural elements of a test method that are used to develop the test method protocol. These components include unique characteristics of the test method, critical procedural details, and quality control measures.

Tiered testing: A testing strategy where all existing information on a test substance is reviewed, in a specified order, prior to *in vivo* testing. If the irritancy potential of a test substance can be assigned, based on the existing information, no additional testing is required. If the irritancy potential of a test substance cannot be assigned, based on the existing information, a step-wise animal testing procedure is performed until an unequivocal classification can be made.

Toxic keratoconjunctivitis: Inflammation of the cornea and conjunctiva due to contact with an exogenous agent. Used interchangeably with *contact keratoconjunctivitis*, *irritative keratoconjunctivitis* and *chemical keratoconjunctivitis*.

Transferability:[15] The ability of a test method or procedure to be accurately and reliably performed in different, competent laboratories.

Two-by-two table:[15] The two-by-two table can be used for calculating accuracy (concordance) ([a+d]/[a+b+c+d]), negative predictivity (d/[c+d]), positive predictivity (a/[a+b]), prevalence ([a+c]/[a+b+c+d]), sensitivity (a/[a+c]), specificity (d/[b+d]), false positive rate (b/[b+d]), and false negative rate (c/[a+c]).

		New Test Outcome		
		Positive	**Negative**	**Total**
Reference Test Outcome	**Positive**	a	c	a + c
	Negative	b	d	b + d
	Total	a + b	c + d	a + b + c + d

Uvea tract: The middle of three membranes of the eye, comprising the iris, ciliary body, and choroid. Also referred to as the *vascular tunic*.

Validated test method:[15] 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:[15] The process by which the reliability and relevance of a procedure are established for a specific purpose.

Vascular tunic: The middle of three membranes of the eye, comprising the iris, ciliary body, and choroid. Also referred to as the *uvea*.

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.

Annex I

Chemical and Product Class Information for the Substances Tested in the ICE Test Method

Reprinted from **Appendix B** of the *Background Review Document: Current Status of In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants: Isolated Chicken Eye Test Method* (ICCVAM 2006a).

The 2006 ICCVAM Background Review Document is available on request from NICEATM.

This page intentionally left blank

Annex II

In Vitro Data for Substances Tested in the ICE Test Method

This page intentionally left blank

Annex II-1

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Reference

This page intentionally left blank

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Reference

Substance/Product Name	CASRN	Form Tested	Water Solubility[1]	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
Acetone	67-64-1	liquid	S	undiluted	99%	Fisher	1	1.4	0.4	9.6	2B	2A	III	II	NI	R36	Balls et al. (1995)
Acetone	67-64-1	liquid	S	undiluted	99%	Fisher	2	1	1.7	49	2A		II		R36		Balls et al. (1995)
Acetone	67-64-1	liquid	S	undiluted	99%	Fisher	3	1.83	1.17	7.64	2B		III		R36		Balls et al. (1995)
Acetone	67-64-1	liquid	S	undiluted	99%	Fisher	4	3	1	13.8	2A		II		R36		Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	S	undiluted	>99.9%	Aldrich	1	1.2	0.9	6.7	2B	2B	III	III	NI	NI	Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	S	undiluted	>99.9%	Aldrich	2	2	1.3	42	2A		II		R36		Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	S	undiluted	>99.9%	Aldrich	3	1.33	1.5	12.33	2B		III		NI		Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	S	undiluted	>99.9%	Aldrich	4	2	0.5	6	2B		III		NI		Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	S	neat	100%	Degussa	1	1	0.7	3.2	2B	2A	III	II	NI	R36	Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	S	neat	100%	Degussa	2	2	2	56	2A		II		R36		Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	S	neat	100%	Degussa	3	1.83	1.67	14.67	2A		II		R36		Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	S	neat	100%	Degussa	4	2	1	10	2B		III		R36		Balls et al. (1995)
Benzalkonium chloride (1%)	8001-54-5	liquid	S	1%	98%	Sigma	1	1.8	0.6	18	2A/2B	2A	II/III	II	R36	R36	Balls et al. (1995)
Benzalkonium chloride (1%)	8001-54-5	liquid	S	1%	98%	Sigma	2	1.3	2.3	47	2A		II		R36		Balls et al. (1995)
Benzalkonium chloride (1%)	8001-54-5	liquid	S	1%	98%	Sigma	3	2.67	1.5	12.66	2A		II		R36		Balls et al. (1995)
Benzalkonium chloride (1%)	8001-54-5	liquid	S	1%	98%	Sigma	4	2	3	8.8	2A		II		R36		Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	S	10%	98%	Sigma	1	3	3	37.7	1	1	I	I	R41	R41	Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	S	10%	98%	Sigma	2	3	2.3	95	1		I		R41		Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	S	10%	98%	Sigma	3	3	2.33	40.72	1		I		R41		Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	S	10%	98%	Sigma	4	3	2	41.1	1		I		R41		Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	S	5%	98%	Sigma	1	3	2.6	36.3	1	1	I	I	R41	R41	Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	S	5%	98%	Sigma	2	1	2	42	2A		II		R36		Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	S	5%	98%	Sigma	3	3	2	33.77	1		I		R41		Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	S	5%	98%	Sigma	4	3	3	68.9	1		I		R41		Balls et al. (1995)
n-Butyl acetate	123-86-4	liquid	I*	undiluted	99%	Fisher	1	1.8	1.8	13.9	2A	2A	II	II	R36	R36	Balls et al. (1995)
n-Butyl acetate	123-86-4	liquid	I*	undiluted	99%	Fisher	2	0.5	2.7	42	1		I		R41		Balls et al. (1995)
n-Butyl acetate	123-86-4	liquid	I*	undiluted	99%	Fisher	3	1	2	14.67	2A/2B		II/III		R36		Balls et al. (1995)
n-Butyl acetate	123-86-4	liquid	I*	undiluted	99%	Fisher	4	1	2	32.2	2A		II		R36		Balls et al. (1995)
Gammabutyrolactone	96-48-0	liquid	S	undiluted	>99%	Aldrich	1	2.6	1.4	15.8	2A	2A	II	II	R36	R36	Balls et al. (1995)
Gammabutyrolactone	96-48-0	liquid	S	undiluted	>99%	Aldrich	2	1.3	2	47	2A		II		R36		Balls et al. (1995)
Gammabutyrolactone	96-48-0	liquid	S	undiluted	>99%	Aldrich	3	1.67	1.5	13.1	2A/2B		II/III		R36		Balls et al. (1995)
Gammabutyrolactone	96-48-0	liquid	S	undiluted	>99%	Aldrich	4	1	2	13	2A/2B		II/III		R36		Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	S	neat	90%	EPA	1	0	0.4	1.7	NI	2B	IV	III	NI	NI	Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	S	neat	90%	EPA	2	0.2	1	27	2B		III		NI/R36		Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	S	neat	90%	EPA	3	0	1.33	19.17	2B		III		NI		Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	S	neat	90%	EPA	4	1	1	20	2B		III		R36		Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	I*	neat	95%	Sigma	1	1	0.5	5.4	NI	NI	IV	IV	NI	NI	Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	I*	neat	95%	Sigma	2	1.3	3	89	1		I		R41		Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	I*	neat	95%	Sigma	3	0.67	0.5	-1.4	NI		IV		NI		Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	I*	neat	95%	Sigma	4	2	1	12.7	2A/2B		II/III		R36		Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	Sf	0.1%	98%	Sigma	1	1	0	2.2	NI	2B	IV	III	NI	NI	Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	Sf	0.1%	98%	Sigma	2	0.7	0	21	2B		III		NI		Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	Sf	0.1%	98%	Sigma	3	0.67	1	10.29	2B		III		NI		Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	Sf	0.1%	98%	Sigma	4	1	1	14.6	2B		III		NI/R36		Balls et al. (1995)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Reference

Substance/Product Name	CASRN	Form Tested	Water Solubility[1]	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
Cetylpyridinium bromide (10%)	140-72-7	liquid	Sf	10%	98%	Sigma	1	2.6	1	25.8	2A		II		R36		Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	Sf	10%	98%	Sigma	2	1.7	2	41	2A	2A	II	II	R36	R36	Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	Sf	10%	98%	Sigma	3	2	1.67	27.2	2A		II		R36		Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	Sf	10%	98%	Sigma	4	3	3	17.8	1		I		R41		Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	Sf	6%	98%	Sigma	1	2	1.2	27.2	2A		II		R36		Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	Sf	6%	98%	Sigma	2	2	0.5	49	2A	2A	II	II	R36	R36	Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	Sf	6%	98%	Sigma	3	3	1.83	24.55	2A		II		R36		Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	Sf	6%	98%	Sigma	4	2.7	1.7	13.5	2A		II		R36		Balls et al. (1995)
Chlorhexidine	55-56-1	solid	I*	neat	n.p.	Degussa	1	3	4	32	1		I		R41		Balls et al. (1995)
Chlorhexidine	55-56-1	solid	I*	neat	n.p.	Degussa	2	3	4	150	1	1	I	I	R41	R41	Balls et al. (1995)
Chlorhexidine	55-56-1	solid	I*	neat	n.p.	Degussa	3	3	3	53.13	1		I		R41		Balls et al. (1995)
Chlorhexidine	55-56-1	solid	I*	neat	n.p.	Degussa	4	3	4	-	1		I		R41		Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	S	undiluted	97%	Fisher	1	2.2	2.2	24.7	2A		II		R36		Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	S	undiluted	97%	Fisher	2	3	2	103	1	1	I	I	R41	R41	Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	S	undiluted	97%	Fisher	3	3	2.5	35.74	1		I		R41		Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	S	undiluted	97%	Fisher	4	3	2.5	45.3	1		I		R41		Balls et al. (1995)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	I	neat	98%	Aldrich	1	2.8	3	12.8	1		I		R41		Balls et al. (1995)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	I	neat	98%	Aldrich	2	1	2.7	75	1	1	I	I	R41	R41	Balls et al. (1995)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	I	neat	98%	Aldrich	3	2	1.5	6.36	2B		III		R36		Balls et al. (1995)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	I	neat	98%	Aldrich	4	1	2	6.7	2B		III		R36		Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	I*	neat	99%	Aldrich	1	2.6	2	12.2	2A		II		R36		Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	I*	neat	99%	Aldrich	2	1	0	22	2B	2A/2B	III	II/III	R36	R36	Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	I*	neat	99%	Aldrich	3	2	1.5	17.07	2A/2B		II/III		R36		Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	I*	neat	99%	Aldrich	4	2	2	40.9	1		I		R36		Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	I*	undiluted	99%	Aldrich	1	2.3	0.8	12.7	2A/2B		II/III		R36		Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	I*	undiluted	99%	Aldrich	2	2	1.3	26	2A	2A	II	II	R36	R36	Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	I*	undiluted	99%	Aldrich	3	1.83	1.67	17.15	2A		II		R36		Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	I*	undiluted	99%	Aldrich	4	1.8	0.8	16.8	2A/2B		II/III		R36		Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	I	undiluted	96%	Aldrich	1	3	2.4	43.8	1		I		R41		Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	I	undiluted	96%	Aldrich	2	3	2.7	74	1	1	I	I	R41	R41	Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	I	undiluted	96%	Aldrich	3	3	2.5	35.9	1		I		R41		Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	I	undiluted	96%	Aldrich	4	3	3	62.7	1		I		R41		Balls et al. (1995)
2,5-Dimethylohexanediol	110-03-2	solid	I	neat	99.5%	BASF	1	2	1	11.9	2B		III		R36		Balls et al. (1995)
2,5-Dimethylohexanediol	110-03-2	solid	I	neat	99.5%	BASF	2	3	3	64	1	2B	I	III	R41	R36	Balls et al. (1995)
2,5-Dimethylohexanediol	110-03-2	solid	I	neat	99.5%	BASF	3	1.33	1.67	11.57	2B		III		R36		Balls et al. (1995)
2,5-Dimethylohexanediol	110-03-2	solid	I	neat	99.5%	BASF	4	2	1	6.7	2B		III		R36		Balls et al. (1995)
Ethanol	64-17-5	liquid	S	undiluted	n.p.	Local vendor	1	2.8	2.8	30.7	1		I		R41		Balls et al. (1995)
Ethanol	64-17-5	liquid	S	undiluted	n.p.	Local vendor	2	2	3	74	1	1	I	I	R41	R41	Balls et al. (1995)
Ethanol	64-17-5	liquid	S	undiluted	n.p.	Local vendor	3	2.5	2.33	35.88	2A		II		R36		Balls et al. (1995)
Ethanol	64-17-5	liquid	S	undiluted	n.p.	Local vendor	4	2	2.3	34.6	2A		II		R36		Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	S	undiluted	99%	Fisher	1	2	2	22	2A		II		R36		Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	S	undiluted	99%	Fisher	2	1.7	2.3	76	2A	2A	II	II	R36	R36	Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	S	undiluted	99%	Fisher	3	2	2	25.08	2A		II		R36		Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	S	undiluted	99%	Fisher	4	3	2	23	2A		II		R36		Balls et al. (1995)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Reference

Substance/Product Name	CASRN	Form Tested	Water Solubility[1]	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
2-Ethyl-1-hexanol	104-76-7	liquid	S	undiluted	99%	Fisher	1	2	2.2	43	2A		II		R36		Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	liquid	S	undiluted	99%	Fisher	2	1	2.3	62	2A	2A	II	II	R36	R36	Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	liquid	S	undiluted	99%	Fisher	3	3	1.5	13.31	2A		II		R36		Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	liquid	S	undiluted	99%	Fisher	4	1	2	52.4	2A		II		R36		Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	S*	undiluted	97%	Fluka	1	0.4	0.3	-2.8	NI		IV		NI		Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	S*	undiluted	97%	Fluka	2	1	0	7	2B	2B	III	III	NI	NI	Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	S*	undiluted	97%	Fluka	3	0.67	1	11.52	2B		III		NI		Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	S*	undiluted	97%	Fluka	4	1	0.5	4.5	NI		IV		NI		Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	I*	undiluted	99%	Aldrich	1	1.2	0.4	7.2	2B		III		NI		Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	I*	undiluted	99%	Aldrich	2	2	2	31	2A	2B	II	III	R36	NI	Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	I*	undiluted	99%	Aldrich	3	0	0	1.44	NI		IV		NI		Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	I*	undiluted	99%	Aldrich	4	1	0.5	6.7	2B		III		NI		Balls et al. (1995)
Fomesafen	72128-02-0	solid	S	neat	97.5%	EPA	1	0.9	1.2	5.3	2B		III		NI		Balls et al. (1995)
Fomesafen	72128-02-0	solid	S	neat	97.5%	EPA	2	0	0.2	11	NI	2B	IV	III	NI	NI	Balls et al. (1995)
Fomesafen	72128-02-0	solid	S	neat	97.5%	EPA	3	1	0.5	2.82	NI		IV		NI		Balls et al. (1995)
Fomesafen	72128-02-0	solid	S	neat	97.5%	EPA	4	1	1	4.3	2B		III		NI		Balls et al. (1995)
Glycerol	56-81-5	liquid	S	undiluted	>99.5%	Mallinkrodt	1	1.2	1	5	2B		III		NI		Balls et al. (1995)
Glycerol	56-81-5	liquid	S	undiluted	>99.5%	Mallinkrodt	2	0	0	11	NI	2B	IV	III	NI	NI	Balls et al. (1995)
Glycerol	56-81-5	liquid	S	undiluted	>99.5%	Mallinkrodt	3	1.17	1	8.3	2B		III		NI		Balls et al. (1995)
Glycerol	56-81-5	liquid	S	undiluted	>99.5%	Mallinkrodt	4	2	0.5	29.4	2A		II		R36		Balls et al. (1995)
n-Hexanol	111-27-3	liquid	I*	undiluted	98%	E-Kodak	1	2.8	1.6	17.4	2A		II		R36		Balls et al. (1995)
n-Hexanol	111-27-3	liquid	I*	undiluted	98%	E-Kodak	2	0.2	1.7	82	2A	1	II	I	R36	R41	Balls et al. (1995)
n-Hexanol	111-27-3	liquid	I*	undiluted	98%	E-Kodak	3	3	2.83	28.89	1		I		R41		Balls et al. (1995)
n-Hexanol	111-27-3	liquid	I*	undiluted	98%	E-Kodak	4	3	3	58.9	1		I		R41		Balls et al. (1995)
Imidazole	288-32-4	solid	S	neat	99%	Aldrich	1	3	4	40.3	1		I		R41		Balls et al. (1995)
Imidazole	288-32-4	solid	S	neat	99%	Aldrich	2	3	3	224	1	1	I	I	R41	R41	Balls et al. (1995)
Imidazole	288-32-4	solid	S	neat	99%	Aldrich	3	3	2.5	36.96	1		I		R41		Balls et al. (1995)
Imidazole	288-32-4	solid	S	neat	99%	Aldrich	4	3	3	97.8	1		I		R41		Balls et al. (1995)
Isobutanol	78-83-1	liquid	I*	undiluted	99.9%	Fisher	1	2.8	2.5	46.4	1		I		R41		Balls et al. (1995)
Isobutanol	78-83-1	liquid	I*	undiluted	99.9%	Fisher	2	3	2.7	93	1	1	I	I	R41	R41	Balls et al. (1995)
Isobutanol	78-83-1	liquid	I*	undiluted	99.9%	Fisher	3	3	2.5	37.06	1		I		R41		Balls et al. (1995)
Isobutanol	78-83-1	liquid	I*	undiluted	99.9%	Fisher	4	3	2	69.2	1		I		R41		Balls et al. (1995)
Isopropanol	67-63-0	liquid	S	undiluted	99.9%	Fisher	1	2	1.6	23.3	2A		II		R36		Balls et al. (1995)
Isopropanol	67-63-0	liquid	S	undiluted	99.9%	Fisher	2	0.7	2.7	72	1	1	I	I	R41	R41	Balls et al. (1995)
Isopropanol	67-63-0	liquid	S	undiluted	99.9%	Fisher	3	3	2.5	37.84	1		I		R41		Balls et al. (1995)
Isopropanol	67-63-0	liquid	S	undiluted	99.9%	Fisher	4	2.3	0.5	8.9	2B		III		NI		Balls et al. (1995)
Maneb	12427-38-2	solid	S	neat	90%	EPA	1	0	0.5	2.8	NI		IV		NI		Balls et al. (1995)
Maneb	12427-38-2	solid	S	neat	90%	EPA	2	1	2	33	2A	NI	II	IV	R36	NI	Balls et al. (1995)
Maneb	12427-38-2	solid	S	neat	90%	EPA	3	0	0.5	8.03	NI		IV		NI		Balls et al. (1995)
Maneb	12427-38-2	solid	S	neat	90%	EPA	4	1	1	6.7	2B		III		NI		Balls et al. (1995)
Methyl acetate	79-20-9	liquid	S	undiluted	98%	Fisher	1	1.4	2.4	20.3	2A		II		R36		Balls et al. (1995)
Methyl acetate	79-20-9	liquid	S	undiluted	98%	Fisher	2	1	2.7	93	1	1	I	I	R41	R41	Balls et al. (1995)
Methyl acetate	79-20-9	liquid	S	undiluted	98%	Fisher	3	2	2	22.5	2A		II		R36		Balls et al. (1995)
Methyl acetate	79-20-9	liquid	S	undiluted	98%	Fisher	4	3	3	17.5	1		I		R41		Balls et al. (1995)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Reference

Substance/Product Name	CASRN	Form Tested	Water Solubility[1]	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
Methyl cyanoacetate	105-34-0	liquid	S*	undiluted	99%	Aldrich	1	0.4	0.3	4.5	NI	NI	IV	IV	NI	NI	Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	S*	undiluted	99%	Aldrich	2	0.5	0.7	44	2A		II		R36		Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	S*	undiluted	99%	Aldrich	3	0.17	0.5	4.93	NI		IV		NI		Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	S*	undiluted	99%	Aldrich	4	1	1	10.7	2B		III		NI		Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	I*	undiluted	>99%	Fluka	1	0.4	0.5	2.3	NI	NI	IV	IV	NI	NI	Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	I*	undiluted	>99%	Fluka	2	1	0	22	2B		III		NI		Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	I*	undiluted	>99%	Fluka	3	0	0.5	5.83	NI		IV		NI		Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	I*	undiluted	>99%	Fluka	4	1	0.5	0	NI		IV		NI		Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	S	undiluted	99%	Fisher	1	2	2.2	23.1	2A	1	II	I	R36	R41	Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	S	undiluted	99%	Fisher	2	2.7	3	99	1		I		R41		Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	S	undiluted	99%	Fisher	3	3	2.33	34.88	1		I		R41		Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	S	undiluted	99%	Fisher	4	3	2	12.6	2A		II		R36		Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	I*	undiluted	98%	Fisher	1	2.6	2	26.5	2A	2A	II	II	R36	R36	Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	I*	undiluted	98%	Fisher	2	3	3	64	1		I		R41		Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	I*	undiluted	98%	Fisher	3	2	2.17	21.69	2A		II		R36		Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	I*	undiluted	98%	Fisher	4	2	2	12.3	2A		II		R36		Balls et al. (1995)
1-Napthaleneacetic acid	86-87-3	solid	I*	neat	96%	EPA	1	1	0.9	5.6	2B	2B	III	III	NI	R36	Balls et al. (1995)
1-Napthaleneacetic acid	86-87-3	solid	I*	neat	96%	EPA	2	1	1	24	2B		III		R36		Balls et al. (1995)
1-Napthaleneacetic acid	86-87-3	solid	I*	neat	96%	EPA	3	2	1	8.86	2B		III		R36		Balls et al. (1995)
1-Napthaleneacetic acid	86-87-3	solid	I*	neat	96%	EPA	4	1	1	46.7	2A		II		R36		Balls et al. (1995)
1-Napthaleneacetic acid, sodium salt	61-31-4	solid	S*	neat	95%	EPA	1	3	3	46.6	1	1	I	I	R41	R41	Balls et al. (1995)
1-Napthaleneacetic acid, sodium salt	61-31-4	solid	S*	neat	95%	EPA	2	3	2.7	122	1		I		R41		Balls et al. (1995)
1-Napthaleneacetic acid, sodium salt	61-31-4	solid	S*	neat	95%	EPA	3	3	2.5	44.19	1		I		R41		Balls et al. (1995)
1-Napthaleneacetic acid, sodium salt	61-31-4	solid	S*	neat	95%	EPA	4	3	3	64.1	1		I		R41		Balls et al. (1995)
n-Octanol	111-87-5	liquid	I*	undiluted	>99%	Aldrich	1	2	2.4	36.5	2A	2A	II	II	R36	R36	Balls et al. (1995)
n-Octanol	111-87-5	liquid	I*	undiluted	>99%	Aldrich	2	1.3	2	108	2A		II		R36		Balls et al. (1995)
n-Octanol	111-87-5	liquid	I*	undiluted	>99%	Aldrich	3	1.17	1.5	10.18	2B		III		NI		Balls et al. (1995)
n-Octanol	111-87-5	liquid	I*	undiluted	>99%	Aldrich	4	2	1	25.7	2A		II		R36		Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	I	undiluted	99%	Aldrich	1	3	2.2	35.3	1	1	I	I	R41	R41	Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	I	undiluted	99%	Aldrich	2	3	2	79	1		I		R41		Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	I	undiluted	99%	Aldrich	3	3	2	33.44	1		I		R41		Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	I	undiluted	99%	Aldrich	4	3	2	38.5	1		I		R41		Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	Sf	undiluted	n.p.	Aldrich	1	1.4	1	9.8	2B	2B	III	III	NI	R36	Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	Sf	undiluted	n.p.	Aldrich	2	0.2	0	26	NI		IV		NI		Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	Sf	undiluted	n.p.	Aldrich	3	2	1	5.88	2B		III		R36		Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	Sf	undiluted	n.p.	Aldrich	4	1	0.5	14.8	2B		III		R36		Balls et al. (1995)
Potassium cyanate	590-28-3	solid	S	neat	97%	Degussa	1	1	0.7	8	2B	2B	III	III	NI	R36	Balls et al. (1995)
Potassium cyanate	590-28-3	solid	S	neat	97%	Degussa	2	0.2	0	25	2B		III		NI		Balls et al. (1995)
Potassium cyanate	590-28-3	solid	S	neat	97%	Degussa	3	1.67	1	10.45	2B		III		R36		Balls et al. (1995)
Potassium cyanate	590-28-3	solid	S	neat	97%	Degussa	4	1.3	1.7	25.3	2A		II		R36		Balls et al. (1995)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Reference

Substance/Product Name	CASRN	Form Tested	Water Solubility[1]	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
Promethazine HCl	58-33-3	solid	S*	neat	98%	Aldrich	1	2.6	1.6	33	1	1	I	I	R41	R41	Balls et al. (1995)
Promethazine HCl	58-33-3	solid	S*	neat	98%	Aldrich	2	3	3	143	1		I		R41		Balls et al. (1995)
Promethazine HCl	58-33-3	solid	S*	neat	98%	Aldrich	3	3	2	23.02	2A		II		R36		Balls et al. (1995)
Promethazine HCl	58-33-3	solid	S*	neat	98%	Aldrich	4	2	3	28.6	1/2A		I/II		R36/R41		Balls et al. (1995)
Pyridine	110-86-1	liquid	S	undiluted	>99.9%	Aldrich	1	3	2	32.7	1	1	I	I	R41	R41	Balls et al. (1995)
Pyridine	110-86-1	liquid	S	undiluted	>99.9%	Aldrich	2	3	3	95	1		I		R41		Balls et al. (1995)
Pyridine	110-86-1	liquid	S	undiluted	>99.9%	Aldrich	3	3	2.5	37.47	1		I		R41		Balls et al. (1995)
Pyridine	110-86-1	liquid	S	undiluted	>99.9%	Aldrich	4	3	3	78.6	1		I		R41		Balls et al. (1995)
Quinacrine	69-05-6	solid	S*	neat	n.p.	Sigma	1	1.2	0.6	4.1	2B	2B	III	III	NI	NI	Balls et al. (1995)
Quinacrine	69-05-6	solid	S*	neat	n.p.	Sigma	2	0.2	0.2	12	NI		IV		NI		Balls et al. (1995)
Quinacrine	69-05-6	solid	S*	neat	n.p.	Sigma	3	2	2	11.49	2A		II		R36		Balls et al. (1995)
Quinacrine	69-05-6	solid	S*	neat	n.p.	Sigma	4	1	0.5	6.8	2B		III		NI		Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	S	1%	RG	Fisher	1	1	0.6	14.1	2B	2A	III	II	NI/R36	R36	Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	S	1%	RG	Fisher	2	0.7	2.3	55	2A		II		R36		Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	S	1%	RG	Fisher	3	2.33	2.5	30.31	2A		II		R36		Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	S	1%	RG	Fisher	4	2	2	33.3	2A		II		R36		Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	S	10%	RG[2]	Fisher	1	3	4	32	1	1	I	I	R41	R41	Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	S	10%	RG	Fisher	2	3	3.3	194	1		I		R41		Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	S	10%	RG	Fisher	3	3	3.17	68.86	1		I		R41		Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	S	10%	RG	Fisher	4	3	4	151.7	1		I		R41		Balls et al. (1995)
Sodium lauryl sulfate (15%)	151-21-3	liquid	Sf	15%	98%	Sigma	1	0.6	0.4	7	2B	2B	III	III	NI	R36	Balls et al. (1995)
Sodium lauryl sulfate (15%)	151-21-3	liquid	Sf	15%	98%	Sigma	2	1	0.2	33	2A		II		R36		Balls et al. (1995)
Sodium lauryl sulfate (15%)	151-21-3	liquid	Sf	15%	98%	Sigma	3	1.67	1	9.56	2B		III		R36		Balls et al. (1995)
Sodium lauryl sulfate (15%)	151-21-3	liquid	Sf	15%	98%	Sigma	4	1	1	12.2	2B		III		NI		Balls et al. (1995)
Sodium lauryl sulfate (3%)	151-21-3	liquid	Sf	3%	98%	Sigma	1	1	0.2	3.9	NI	2B	IV	III	NI	NI	Balls et al. (1995)
Sodium lauryl sulfate (3%)	151-21-3	liquid	Sf	3%	98%	Sigma	2	0	0	39	2B		III		R36		Balls et al. (1995)
Sodium lauryl sulfate (3%)	151-21-3	liquid	Sf	3%	98%	Sigma	3	1	0	2.75	NI		IV		NI		Balls et al. (1995)
Sodium lauryl sulfate (3%)	151-21-3	liquid	Sf	3%	98%	Sigma	4	1	1	15.9	2B		III		NI/R36		Balls et al. (1995)
Sodium oxalate	62-76-0	solid	S	neat	>99%	Aldrich	1	0.7	0.7	6.3	2B	2B	III	III	NI	NI	Balls et al. (1995)
Sodium oxalate	62-76-0	solid	S	neat	>99%	Aldrich	2	0.2	0	24	2B		III		NI		Balls et al. (1995)
Sodium oxalate	62-76-0	solid	S	neat	>99%	Aldrich	3	0.5	0.5	2.62	NI		IV		NI		Balls et al. (1995)
Sodium oxalate	62-76-0	solid	S	neat	>99%	Aldrich	4	1	0	2.4	NI		IV		NI		Balls et al. (1995)
Sodium perborate, $4H_2O$	10486-00-7	solid	S	neat	98.6%	Dupont	1	0.6	0.5	3.1	NI	2B	IV	III	NI	NI	Balls et al. (1995)
Sodium perborate, $4H_2O$	10486-00-7	solid	S	neat	98.6%	Dupont	2	0.2	0.7	23	2B		III		NI		Balls et al. (1995)
Sodium perborate, $4H_2O$	10486-00-7	solid	S	neat	98.6%	Dupont	3	1.33	1	7.54	2B		III		NI		Balls et al. (1995)
Sodium perborate, $4H_2O$	10486-00-7	solid	S	neat	98.6%	Dupont	4	1	0.5	14.6	2B		III		NI		Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	I*	neat	97%	Aldrich	1	1.3	1	7.5	2B	2B	III	III	NI	NI	Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	I*	neat	97%	Aldrich	2	1	2	31	2A		II		R36		Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	I*	neat	97%	Aldrich	3	1.5	1.5	7.3	2B		III		NI		Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	I*	neat	97%	Aldrich	4	1	1	8.9	2B		III		NI		Balls et al. (1995)
Toluene	108-88-3	liquid	I*	undiluted	99%	Fisher	2	1.4	1	5.2	2B	2A	III	II	NI	R36	Balls et al. (1995)
Toluene	108-88-3	liquid	I*	undiluted	99%	Fisher	3	2	1.3	29	2A		II		R36		Balls et al. (1995)
Toluene	108-88-3	liquid	I*	undiluted	99%	Fisher	4	1.33	2	13.87	2A/2B		II/III		R36		Balls et al. (1995)
Toluene	108-88-3	liquid	I*	undiluted	99%	Fisher	1	1	2	58.2	2A		II		R36		Balls et al. (1995)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Reference

Substance/Product Name	CASRN	Form Tested	Water Solubility[1]	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
Trichloroacetic acid (3%)	76-03-9	liquid	S	3%	RG	Fisher	2	2.4	1.2	13.2	2A/2B		II/III		R36		Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	S	3%	RG	Fisher	3	2.3	2	38	2A	2A	II	II	R36	R36	Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	S	3%	RG	Fisher	4	1.5	2.5	27.88	2A		II		R36		Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	S	3%	RG	Fisher	1	1.7	2	26.4	2A		II		R36		Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	S	30%	RG	Fisher	2	3	4	32	1		I		R41		Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	S	30%	RG	Fisher	3	3	4	153	1	1	I	I	R41	R41	Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	S	30%	RG	Fisher	4	*	4	*	*		*		*		Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	S	30%	RG	Fisher	1	3	4	*	1		I		R41		Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	Sf	10%	98%	Sigma	2	1.4	0.1	9.9	2B		III		NI		Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	Sf	10%	98%	Sigma	3	1	0.8	29	2A/2B	2A/2B	II/III	II/III	R36	R36	Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	Sf	10%	98%	Sigma	4	2.67	1.17	20.2	2A		II		R36		Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	Sf	10%	98%	Sigma	1	1.7	1	11.2	2B		III		R36		Balls et al. (1995)
Triton X-100 (5%)	9002-93-1	liquid	Sf	5%	98%	Sigma	2	1	0.6	9.8	2B		III		NI		Balls et al. (1995)
Triton X-100 (5%)	9002-93-1	liquid	Sf	5%	98%	Sigma	3	1.3	0	38	2A	2A	II	II	R36	R36	Balls et al. (1995)
Triton X-100 (5%)	9002-93-1	liquid	Sf	5%	98%	Sigma	4	2	0	3.97	2B		III		NI		Balls et al. (1995)
Triton X-100 (5%)	9002-93-1	liquid	Sf	5%	98%	Sigma	1	1	2	39.6	2A		II		R36		Balls et al. (1995)
Tween 20	9005-64-5	liquid	Sf	undiluted	98%	Sigma	2	1	1	3.6	2B		III		NI		Balls et al. (1995)
Tween 20	9005-64-5	liquid	Sf	undiluted	98%	Sigma	3	0.2	0	31	2B	2B	III	III	NI/R36	NI	Balls et al. (1995)
Tween 20	9005-64-5	liquid	Sf	undiluted	98%	Sigma	4	2.5	1	5.63	2B		III		R36		Balls et al. (1995)
Tween 20	9005-64-5	liquid	Sf	undiluted	98%	Sigma	1	1	0.5	6.7	2B		III		NI		Balls et al. (1995)
TNO-01 (Formulation-1)[7]	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-02 (Formulation-2)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	2.7	2	24	2A	2A	II	II	R36	R36	Prinsen (1996)
TNO-03 (Pesticide-1)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.6	0.3	3	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-04 (Detergent-1)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.5	1.5	9	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-05 (Silicone powder-1)	n.p.	solid	I	undiluted	n.p.	n.p.	-	0	0	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-06 (Lubricant)	n.p.	gel	n.p.	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-07 (Ink-1)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.8	0	2	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-08 (Ink-2)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.1	0	3	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-09 (Paint)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.3	0.5	5	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-10 (Silicone powder-2)	n.p.	solid	I	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-11 (Sodium p-styrene sulfonate)	2695-37-6	solid	n.p.	undiluted	n.p.	n.p.	-	2	1.3	19	2A	2A	II	II	R36	R36	Prinsen (1996)
TNO-12 (Formulation-3)	n.p.	paste	n.p.	undiluted	n.p.	n.p.	-	2.5	2	35	2A	2A	II	II	R36	R36	Prinsen (1996)
TNO-13 (Pesticide-2)	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0.7	0	1	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-14 (Polydisaccharide)	n.p.	liquid	n.p.	14.5%	n.p.	n.p.	-	0.3	0	2	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-15 (Polydisaccharide)	n.p.	liquid	n.p.	50%	n.p.	n.p.	-	0	0	2	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-16 (Liquid nylon product)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-17 (Solvent-1)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.3	0	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-18 (Solvent-2)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-19 (Solvent-3)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-20 (Solvent-4)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.5	0.3	3	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-21 (Solvent-5)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.3	0.3	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-22 (Solvent-6)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.2	0.3	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-23 (Solvent-7)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.2	0	2	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-24 (Solvent-8)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.2	0	3	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-25 (Solvent-9)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	IV	IV	NI	NI	Prinsen (1996)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Reference

Substance/Product Name	CASRN	Form Tested	Water Solubility[1]	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
TNO-26 (Ink-3)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.1	0	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-27 (Thermal paper coating-1)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1	0.6	9	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-28 (Toilet cleaner-1)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.4	0.8	12	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-29 (Toilet cleaner-2)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.3	1	11	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-30 (Pesticide-3)	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	1.5	1	7	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-31 (Sulfur)	7704-34-9	solid	I	undiluted	n.p.	n.p.	-	0.2	0	1	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-32 (Ink-4)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1	0.5	7	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-33 (Thermal paper coating-2)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	2	0.5	5	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-34 (Detergent-2)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1	1	25	1	1	I	I	R41	R41	Prinsen (1996)
TNO-35 (Propyl-lactate)	616-09-1	liquid	S	undiluted	n.p.	n.p.	-	3	3	45	1	1	I	I	R41	R41	Prinsen (1996)
TNO-36 (Ethylhexyl lactate)	6283-86-9	liquid	n.p.	undiluted	n.p.	n.p.	-	2	2	18	2A	2A	II	II	R36	R36	Prinsen (1996)
TNO-37 (Pesticide-4)	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	1.5	1	15	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-38 (Solvent-10)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	3	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-39 (Detergent-3)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.5	0.5	4	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-40 (Glycolbromoacetate form.)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	2.6	1.9	41	1	1	I	I	R41	R41	Prinsen (1996)
TNO-41 (Amidosulfonic acid)	5329-14-6	solid	n.p.	undiluted	n.p.	n.p.	-	2.7	4	46	1	1	I	I	R41	R41	Prinsen (1996)
TNO-42 (Glycolbromoacetate)	3785-34-0	liquid	n.p.	85%	n.p.	n.p.	-	3	3	36	1	1	I	I	R41	R41	Prinsen (1996)
TNO-43 (Monobromoacetic acid)	79-08-3	solid	S	undiluted	n.p.	n.p.	-	3	4	80	1	1	I	I	R41	R41	Prinsen (1996)
TNO-44 (Didecyldimethylammoniumchloride (23% in propyl glycol))	7173-51-5	liquid	n.p.	23%	n.p.	n.p.	-	3	3.5	39	1	1	I	I	R41	R41	Prinsen (1996)
Cetylpyridinium bromide (6%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	2.0	2.0	22	1		I		R41		Prinsen (2000)
Cetylpyridinium bromide (6%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	1.8	1.7	21	1		I		R41		Prinsen (2000)
Cetylpyridinium bromide (6%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	2.0	2.0	21	1	1	I	I	R41	R41	Prinsen (2000)
Cetylpyridinium bromide (6%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	1.7	1.7	18	1		I		R41		Prinsen (2000)
Cyclohexylamino-functional PMS	——	liquid	n.p.	undiluted	n.p.	n.p.	1	1.8	2.5	14	2A		II		R36		Prinsen (2000)
Cyclohexylamino-functional PMS	——	liquid	n.p.	undiluted	n.p.	n.p.	1	1.7	2.0	13	2A		II		R36		Prinsen (2000)
Cyclohexylamino-functional PMS	——	liquid	n.p.	undiluted	n.p.	n.p.	1	2.0	2.3	17	2A	2A	II	II	R36	R36	Prinsen (2000)
Cyclohexylamino-functional PMS	——	liquid	n.p.	undiluted	n.p.	n.p.	1	2.0	2.3	14	2A		II		R36		Prinsen (2000)
Cyclohexylamino-functional PMS	——	liquid	n.p.	undiluted	n.p.	n.p.	1	2.0	2.0	13	2A		II		R36		Prinsen (2000)
Decamethylcyclopentasiloxane	——	liquid	n.p.	undiluted	n.p.	n.p.	1	0.3	0.3	1	NI		NI		NI		Prinsen (2000)
Decamethylcyclopentasiloxane	——	liquid	n.p.	undiluted	n.p.	n.p.	1	0.3	0.3	1	NI		NI		NI		Prinsen (2000)
Decamethylcyclopentasiloxane	——	liquid	n.p.	undiluted	n.p.	n.p.	1	0.0	0.5	2	NI	NI	NI	NI	NI	NI	Prinsen (2000)
Decamethylcyclopentasiloxane	——	liquid	n.p.	undiluted	n.p.	n.p.	1	0.0	0.0	0	NI		NI		NI		Prinsen (2000)
Decamethylcyclopentasiloxane	——	liquid	n.p.	undiluted	n.p.	n.p.	1	0.0	0.0	2	NI		NI		NI		Prinsen (2000)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Reference

Substance/Product Name	CASRN	Form Tested	Water Solubility[1]	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
Triton X-500 (5%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	1.0	0.7	14	2B		III		NI		Prinsen (2000)
Triton X-500 (5%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	1.0	0.7	14	2B		III		NI		Prinsen (2000)
Triton X-500 (5%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	1.0	0.7	13	2B	2B	III	III	NI	NI	Prinsen (2000)
Triton X-500 (5%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	1.0	0.8	8	2B		III		NI		Prinsen (2000)
Triton X-500 (5%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	1.0	0.7	11	2B		III		NI		Prinsen (2000)
TNO-45	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1	0.5	5	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-46	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-47	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-48	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	3	1	25	2A	2A	II	II	R36	R36	Prinsen (2005)
TNO-49	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	3	4	-	1	1	I	I	R41	R41	Prinsen (2005)
TNO-50	n.p.		n.p.	undiluted	n.p.	n.p.	-	3	3	41.1	1	1	I	I	R41	R41	Prinsen (2005)
TNO-51	n.p.		n.p.	undiluted	n.p.	n.p.	-	3	3	33.9	1	1	I	I	R41	R41	Prinsen (2005)
TNO-52	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.7	1	5	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-53	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.5	0.2	3	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-54	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	1	1	9	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-55	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.7	1.3	10	2B	2B	III	III	R36	R36	Prinsen (2005)
TNO-56	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	2	1.3	10	2B	2B	III	III	R36	R36	Prinsen (2005)
TNO-57	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.5	1.3	12	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-58	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0	0	-1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-59	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0.2	0	-2	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-60	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.2	0.5	1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-61	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-62	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	2	1	12	2B	2B	III	III	R36	R36	Prinsen (2005)
TNO-63	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0.3	0.5	3	NI	NI	IV	IV	NI	NI	Prinsen (2005)
TNO-64	n.p.	emulsion	n.p.	undiluted	n.p.	n.p.	-	1	1	5	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-65	n.p.	emulsion	n.p.	undiluted	n.p.	n.p.	-	0.7	0.5	4	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-66	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0	0	0	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-67	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1	1	6	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-68	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1/2	1	8	2A	2A	II	II	R36	R36	Prinsen (2005)
TNO-69	n.p.	liquid	n.p.	50%	n.p.	n.p.	-	1	0	0	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-70	n.p.	emulsion	n.p.	undiluted	n.p.	n.p.	-	2	1	20	2A	2A	II	II	R36	R36	Prinsen (2005)
TNO-71	n.p.	emulsion	n.p.	undiluted	n.p.	n.p.	-	1	0.5	13	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-72	n.p.	emulsion	n.p.	undiluted	n.p.	n.p.	-	1.5	0.5	5	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-73	n.p.	emulsion	n.p.	undiluted	n.p.	n.p.	-	2.7	2	18	1	1	I	I	R41	R41	Prinsen (2005)
TNO-74	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0.5	0	0	NI	NI	IV	IV	NI	NI	Prinsen (2005)
TNO-75	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	2	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-76	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0	0	2	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-77	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1	0.5	7	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-78	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.3	1	15	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-79	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	1	1	10	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-80	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0.3	0	-1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-81	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-82	n.p.	n.p.	n.p.	undiluted	n.p.	n.p.	-	0	0	-2	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-83	n.p.	n.p.	n.p.	undiluted	n.p.	n.p.	-	0.8	0.7	10	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-84	n.p.	n.p.	n.p.	undiluted	n.p.	n.p.	-	0.7	0.7	2	2B	2B	III	III	NI	NI	Prinsen (2005)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Reference

Substance/Product Name	CASRN	Form Tested	Water Solubility[1]	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
TNO-85	n.p.	n.p.	n.p.	undiluted	n.p.	n.p.	-	2	1.3	14	2B	2B	III	III	R36	R36	Prinsen (2005)
TNO-86	n.p.	n.p.	n.p.	undiluted	n.p.	n.p.	-	1	1	7	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-87	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.7	1	1	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-88	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.3	0.7	3	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-89	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0.2	0.7	1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-90	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	2	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-91	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0.2	1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-92	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0.8	1.7	16	2B	2B	III	III	R36	R36	Prinsen (2005)
TNO-93	n.p.	emulsion	n.p.	undiluted	n.p.	n.p.	-	3	2	17	2A	2A	II	II	R36	R36	Prinsen (2005)
TNO-94	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1	0.5	2	NI	NI	NI	NI	NI	NI	Prinsen (2005)
1-Butanol	71-36-3	liquid	S	undiluted	99%	Aldrich	-	2.9	2	54	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
2-Butoxyethyl acetate	112-07-2	liquid	S	undiluted	99%	Aldrich	-	1	1	5	2B	2B	III	III	NI	NI	Prinsen and Koëter (1993)
2-Methoxyethanol	109-86-4	liquid	S	undiluted	99.9%	Aldrich	-	2	2	18	2A	2A	II	II	R36	R36	Prinsen and Koëter (1993)
Acetaldehyde	75-07-0	liquid	S	undiluted	99%	Aldrich	-	2	1.4	24	2A	2A	II	II	R36	R36	Prinsen and Koëter (1993)
Acetic acid	64-19-7	liquid	S	10%	99%	Aldrich	-	3	2.6	31	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Benzalkonium chloride (100%)	8001-54-5	liquid	S	undiluted	n.p.	Aldrich	-	3	3	40	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Brij 35	9002-92-0	liquid	S	undiluted	n.p.	Aldrich	-	0.9	0	5	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Chloroform	67-66-3	liquid	I*	undiluted	99.8%	Aldrich	-	2.5	1	21	2A	2A	II	II	R36	R36	Prinsen and Koëter (1993)
Dibutyltin dichloride	683-18-1	solid	S	undiluted	97%	Aldrich	-	3	2.5	34	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Dimethyl sulfoxide	67-68-5	liquid	S	undiluted	99.9%	Aldrich	-	1	0.5	4	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Glycerol	56-81-5	liquid	S	undiluted	99%	Aldrich	-	0.5	0.4	4	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Mercury (II) chloride	7487-94-7	solid	I	undiluted	99.5%	Aldrich	-	2	3.1	55	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
n-Hexane	110-54-3	liquid	I	undiluted	99%	Aldrich	-	0.5	0	1	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Silver (I) nitrate	7761-88-8	solid	S	3%	99.5%	Aldrich	-	1	1	12	2B	2B	III	III	NI	NI	Prinsen and Koëter (1993)
Sodium dodecyl sulfate	151-21-3	solid	S	undiluted	70%	Aldrich	-	0.8	1	22	2B	2B	III	III	R41	R41	Prinsen and Koëter (1993)
Sodium fluorescein	518-47-8	liquid	S	20%	70%[8]	Aldrich	-	0.1	0	0	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Sodium hydroxide	1310-73-2	liquid	S	1%	97%	Aldrich	-	3	3	60	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Toluene	108-88-3	liquid	I*	undiluted	99.9%	Aldrich	-	1.1	1.4	4	2B	2B	III	III	NI	NI	Prinsen and Koëter (1993)
Triacetin	102-76-1	liquid	I*	undiluted	99%	Aldrich	-	0.5	0.4	4	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Tributyltin chloride	1461-22-9	liquid	n.p.	undiluted	96%	Aldrich	-	3	2.5	48	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Triethanolamine	102-71-6	liquid	S	undiluted	99%	Aldrich	-	0.9	0.7	4	2B	2B	III	III	NI	NI	Prinsen and Koëter (1993)

Abbreviations: S = soluble; Sf = surfactant; I = insoluble; *solubility uncertain; RG = reagent grade; n.p. = not provided and not obtained

[1]GHS=Globally Harmonized System (UN 2007)

[2]Eye Irritant Category 1 = irreversible effects on the eye/serious damage to the eye; Category 2A = reversible effects on the eye/irritating to the eyes; Category 2B = reversible effects on the eye/mildly irritating to the eyes; Nonirritant = not an eye irritant.

[3]EPA=U.S. Environmental Protection Agency (EPA 2003a)

[4]Toxicity Category I for the Primary Eye Irritation Study = Corrosive, or corneal involvement or irritation not reversible within 21 days; Category II = Corneal involvement or irritation clearing in 8-21 days; Category III = Corneal involvement or irritation clearing in 1-7 days; Category IV: minimal effects clearing in less than 24 hr.

[5]EU=European Union (EU 2001).

[6]Risk phrase R41 = risk of serious damage to the eyes; R36 = irritating to the eyes; nonirritant = not an eye irritant.

[7]Numbering for substances from this reference assigned based on order of appearance in Table 3 of Prinsen (1996)

[8]Dye content

Annex II-2

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Substance Name

This page intentionally left blank

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Substance Name

Substance/Product Name	CASRN	Form Tested	Water Solubility	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
Acetaldehyde	75-07-0	liquid	S	undiluted	99%	Aldrich	-	2	1.4	24	2A	2A	II	II	R36	R36	Prinsen and Koëter (1993)
Acetic acid	64-19-7	liquid	S	10%	99%	Aldrich	-	3	2.6	31	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Acetone	67-64-1	liquid	S	undiluted	99%	Fisher	1	1.4	0.4	9.6	2B	2A	III	II	NI	R36	Balls et al. (1995)
Acetone	67-64-1	liquid	S	undiluted	99%	Fisher	2	1	1.7	49	2A		II		R36		Balls et al. (1995)
Acetone	67-64-1	liquid	S	undiluted	99%	Fisher	3	1.83	1.17	7.64	2B		III		R36		Balls et al. (1995)
Acetone	67-64-1	liquid	S	undiluted	99%	Fisher	4	3	1	13.8	2A		II		R36		Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	S	undiluted	>99.9%	Aldrich	1	1.2	0.9	6.7	2B	2B	III	III	NI	NI	Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	S	undiluted	>99.9%	Aldrich	2	2	1.3	42	2A		II		R36		Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	S	undiluted	>99.9%	Aldrich	3	1.33	1.5	12.33	2B		III		NI		Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	S	undiluted	>99.9%	Aldrich	4	2	0.5	6	2B		III		NI		Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	S	neat	100%	Degussa	1	1	0.7	3.2	2B	2A	III	II	NI	R36	Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	S	neat	100%	Degussa	2	2	2	56	2A		II		R36		Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	S	neat	100%	Degussa	3	1.83	1.67	14.67	2A		II		R36		Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	S	neat	100%	Degussa	4	2	1	10	2B		III		R36		Balls et al. (1995)
Benzalkonium chloride (1%)	8001-54-5	liquid	S	1%	98%	Sigma	1	1.8	0.6	18	2A/2B	2A	II/III	II	R36	R36	Balls et al. (1995)
Benzalkonium chloride (1%)	8001-54-5	liquid	S	1%	98%	Sigma	2	1.3	2.3	47	2A		II		R36		Balls et al. (1995)
Benzalkonium chloride (1%)	8001-54-5	liquid	S	1%	98%	Sigma	3	2.67	1.5	12.66	2A		II		R36		Balls et al. (1995)
Benzalkonium chloride (1%)	8001-54-5	liquid	S	1%	98%	Sigma	4	2	3	8.8	2A		II		R36		Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	S	10%	98%	Sigma	1	3	3	37.7	1	1	I	I	R41	R41	Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	S	10%	98%	Sigma	2	3	2.3	95	1		I		R41		Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	S	10%	98%	Sigma	3	3	2.33	40.72	1		I		R41		Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	S	10%	98%	Sigma	4	3	2	41.1	1		I		R41		Balls et al. (1995)
Benzalkonium chloride (100%)	8001-54-5	liquid	S	undiluted	n.p.	Aldrich	-	3	3	40	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Benzalkonium chloride (5%)	8001-54-5	liquid	S	5%	98%	Sigma	1	3	2.6	36.3	1	1	I	I	R41	R41	Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	S	5%	98%	Sigma	2	1	2	42	2A		II		R36		Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	S	5%	98%	Sigma	3	3	2	33.77	1		I		R41		Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	S	5%	98%	Sigma	4	3	3	68.9	1		I		R41		Balls et al. (1995)
Brij 35	9002-92-0	liquid	S	undiluted	n.p.	Aldrich	-	0.9	0	5	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
1-Butanol	71-36-3	liquid	S	undiluted	99%	Aldrich	-	2.9	2	54	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
2-Butoxyethyl acetate	112-07-2	liquid	S	undiluted	99%	Aldrich	-	1	1	5	2B	2B	III	III	NI	NI	Prinsen and Koëter (1993)
n-Butyl acetate	123-86-4	liquid	I*	undiluted	99%	Fisher	1	1.8	1.8	13.9	2A	2A	II	II	R36	R36	Balls et al. (1995)
n-Butyl acetate	123-86-4	liquid	I*	undiluted	99%	Fisher	2	0.5	2.7	42	1		I		R41		Balls et al. (1995)
n-Butyl acetate	123-86-4	liquid	I*	undiluted	99%	Fisher	3	1	2	14.67	2A/2B		II/III		R36		Balls et al. (1995)
n-Butyl acetate	123-86-4	liquid	I*	undiluted	99%	Fisher	4	1	2	32.2	2A		II		R36		Balls et al. (1995)
Gammabutyrolactone	96-48-0	liquid	S	undiluted	>99%	Aldrich	1	2.6	1.4	15.8	2A	2A	II	II	R36	R36	Balls et al. (1995)
Gammabutyrolactone	96-48-0	liquid	S	undiluted	>99%	Aldrich	2	1.3	2	47	2A		II		R36		Balls et al. (1995)
Gammabutyrolactone	96-48-0	liquid	S	undiluted	>99%	Aldrich	3	1.67	1.5	13.1	2A/2B		II/III		R36		Balls et al. (1995)
Gammabutyrolactone	96-48-0	liquid	S	undiluted	>99%	Aldrich	4	1	2	13	2A/2B		II/III		R36		Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	S	neat	90%	EPA	1	0	0.4	1.7	NI	2B	IV	III	NI	NI	Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	S	neat	90%	EPA	2	0.2	1	27	2B		III		NI/R36		Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	S	neat	90%	EPA	3	0	1.33	19.17	2B		III		NI		Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	S	neat	90%	EPA	4	1	1	20	2B		III		R36		Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	I*	neat	95%	Sigma	1	1	0.5	5.4	NI	NI	IV	IV	NI	NI	Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	I*	neat	95%	Sigma	2	1.3	3	89	1		I		R41		Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	I*	neat	95%	Sigma	3	0.67	0.5	-1.4	NI		IV		NI		Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	I*	neat	95%	Sigma	4	2	1	12.7	2A/2B		II/III		R36		Balls et al. (1995)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Substance Name

Substance/Product Name	CASRN	Form Tested	Water Solubility	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
Cetylpyridinium bromide (6%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	2.0	2.0	22	1		I		R41		Prinsen (2000)
Cetylpyridinium bromide (6%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	1.8	1.7	21	1	1	I	I	R41	R41	Prinsen (2000)
Cetylpyridinium bromide (6%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	2.0	2.0	21	1		I		R41		Prinsen (2000)
Cetylpyridinium bromide (6%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	1.7	1.7	18	1		I		R41		Prinsen (2000)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	Sf	0.1%	98%	Sigma	1	1	0	2.2	NI		IV		NI		Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	Sf	0.1%	98%	Sigma	2	0.7	0	21	2B	2B	III	III	NI	NI	Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	Sf	0.1%	98%	Sigma	3	0.67	1	10.29	2B		III		NI		Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	Sf	0.1%	98%	Sigma	4	1	1	14.6	2B		III		NI/R36		Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	Sf	10%	98%	Sigma	1	2.6	1	25.8	2A		II		R36		Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	Sf	10%	98%	Sigma	2	1.7	2	41	2A	2A	II	II	R36	R36	Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	Sf	10%	98%	Sigma	3	2	1.67	27.2	2A		II		R36		Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	Sf	10%	98%	Sigma	4	3	3	17.8	1		I		R41		Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	Sf	6%	98%	Sigma	1	2	1.2	27.2	2A		II		R36		Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	Sf	6%	98%	Sigma	2	2	0.5	49	2A	2A	II	II	R36	R36	Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	Sf	6%	98%	Sigma	3	3	1.83	24.55	2A		II		R36		Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	Sf	6%	98%	Sigma	4	2.7	1.7	13.5	2A		II		R36		Balls et al. (1995)
Chlorhexidine	55-56-1	solid	I*	neat	n.p.	Degussa	1	3	4	32	1		I		R41		Balls et al. (1995)
Chlorhexidine	55-56-1	solid	I*	neat	n.p.	Degussa	2	3	4	150	1	1	I	I	R41	R41	Balls et al. (1995)
Chlorhexidine	55-56-1	solid	I*	neat	n.p.	Degussa	3	3	3	53.13	1		I		R41		Balls et al. (1995)
Chlorhexidine	55-56-1	solid	I*	neat	n.p.	Degussa	4	3	4	-	1		I		R41		Balls et al. (1995)
Chloroform	67-66-3	liquid	I*	undiluted	99.8%	Aldrich	-	2.5	1	21	2A	2A	II	II	R36	R36	Prinsen and Kočter (1993)
Cyclohexanol	108-93-0	liquid	S	undiluted	97%	Fisher	1	2.2	2.2	24.7	2A		II		R36		Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	S	undiluted	97%	Fisher	2	3	2	103	1	1	I	I	R41	R41	Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	S	undiluted	97%	Fisher	3	3	2.5	35.74	1		I		R41		Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	S	undiluted	97%	Fisher	4	3	2.5	45.3	1		I		R41		Balls et al. (1995)
Cyclohexylamino-functional PMS	——	liquid	n.p.	undiluted	n.p.	n.p.	1	1.8	2.5	14	2A		II		R36		Prinsen (2000)
Cyclohexylamino-functional PMS	——	liquid	n.p.	undiluted	n.p.	n.p.	1	1.7	2.0	13	2A		II		R36		Prinsen (2000)
Cyclohexylamino-functional PMS	——	liquid	n.p.	undiluted	n.p.	n.p.	1	2.0	2.3	17	2A	2A	II	II	R36	R36	Prinsen (2000)
Cyclohexylamino-functional PMS	——	liquid	n.p.	undiluted	n.p.	n.p.	1	2.0	2.3	14	2A		II		R36		Prinsen (2000)
Cyclohexylamino-functional PMS	——	liquid	n.p.	undiluted	n.p.	n.p.	1	2.0	2.0	13	2A		II		R36		Prinsen (2000)
Decamethylcyclopentasiloxane	——	liquid	n.p.	undiluted	n.p.	n.p.	1	0.3	0.3	1	NI		NI		NI		Prinsen (2000)
Decamethylcyclopentasiloxane	——	liquid	n.p.	undiluted	n.p.	n.p.	1	0.3	0.3	1	NI		NI		NI		Prinsen (2000)
Decamethylcyclopentasiloxane	——	liquid	n.p.	undiluted	n.p.	n.p.	1	0.0	0.5	2	NI	NI	NI	NI	NI	NI	Prinsen (2000)
Decamethylcyclopentasiloxane	——	liquid	n.p.	undiluted	n.p.	n.p.	1	0.0	0.0	0	NI		NI		NI		Prinsen (2000)
Decamethylcyclopentasiloxane	——	liquid	n.p.	undiluted	n.p.	n.p.	1	0.0	0.0	2	NI		NI		NI		Prinsen (2000)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	I	neat	98%	Aldrich	1	2.8	3	12.8	1		I		R41		Balls et al. (1995)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	I	neat	98%	Aldrich	2	1	2.7	75	1	1	I	I	R41	R41	Balls et al. (1995)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	I	neat	98%	Aldrich	3	2	1.5	6.36	2B		III		R36		Balls et al. (1995)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	I	neat	98%	Aldrich	4	1	2	6.7	2B		III		R36		Balls et al. (1995)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Substance Name

Substance/Product Name	CASRN	Form Tested	Water Solubility	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
Dibenzyl phosphate	1623-08-1	solid	I*	neat	99%	Aldrich	1	2.6	2	12.2	2A	2A/2B	II	II/III	R36	R36	Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	I*	neat	99%	Aldrich	2	1	0	22	2B		III		NI		Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	I*	neat	99%	Aldrich	3	2	1.5	17.07	2A/2B		II/III		R36		Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	I*	neat	99%	Aldrich	4	2	2	40.9	1		I		R36		Balls et al. (1995)
Dibutyltin dichloride	683-18-1	solid	S	undiluted	97%	Aldrich	-	3	2.5	34	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	I*	undiluted	99%	Aldrich	1	2.3	0.8	12.7	2A/2B	2A	II/III	II	R36	R36	Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	I*	undiluted	99%	Aldrich	2	2	1.3	26	2A		II		R36		Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	I*	undiluted	99%	Aldrich	3	1.83	1.67	17.15	2A		II		R36		Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	I*	undiluted	99%	Aldrich	4	1.8	0.8	16.8	2A/2B		II/III		R36		Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	I	undiluted	96%	Aldrich	1	3	2.4	43.8	1	1	I	I	R41	R41	Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	I	undiluted	96%	Aldrich	2	3	2.7	74	1		I		R41		Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	I	undiluted	96%	Aldrich	3	3	2.5	35.9	1		I		R41		Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	I	undiluted	96%	Aldrich	4	3	3	62.7	1		I		R41		Balls et al. (1995)
2,5-Dimethylohexanediol	110-03-2	solid	I	neat	99.5%	BASF	1	2	1	11.9	2B	2B	III	III	R36	R36	Balls et al. (1995)
2,5-Dimethylohexanediol	110-03-2	solid	I	neat	99.5%	BASF	2	3	3	64	1		I		R41		Balls et al. (1995)
2,5-Dimethylohexanediol	110-03-2	solid	I	neat	99.5%	BASF	3	1.33	1.67	11.57	2B		III		R36		Balls et al. (1995)
2,5-Dimethylohexanediol	110-03-2	solid	I	neat	99.5%	BASF	4	2	1	6.7	2B		III		R36		Balls et al. (1995)
Dimethyl sulfoxide	67-68-5	liquid	S	undiluted	99.9%	Aldrich	-	1	0.5	4	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Ethanol	64-17-5	liquid	S	undiluted	n.p.	Local vendor	1	2.8	2.8	30.7	1	1	I	I	R41	R41	Balls et al. (1995)
Ethanol	64-17-5	liquid	S	undiluted	n.p.	Local vendor	2	2	3	74	1		I		R41		Balls et al. (1995)
Ethanol	64-17-5	liquid	S	undiluted	n.p.	Local vendor	3	2.5	2.33	35.88	2A		II		R36		Balls et al. (1995)
Ethanol	64-17-5	liquid	S	undiluted	n.p.	Local vendor	4	2	2.3	34.6	2A		II		R36		Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	S	undiluted	99%	Fisher	1	2	2	22	2A	2A	II	II	R36	R36	Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	S	undiluted	99%	Fisher	2	1.7	2.3	76	2A		II		R36		Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	S	undiluted	99%	Fisher	3	2	2	25.08	2A		II		R36		Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	S	undiluted	99%	Fisher	4	3	2	23	2A		II		R36		Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	liquid	S	undiluted	99%	Fisher	1	2	2.2	43	2A	2A	II	II	R36	R36	Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	liquid	S	undiluted	99%	Fisher	2	1	2.3	62	2A		II		R36		Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	liquid	S	undiluted	99%	Fisher	3	3	1.5	13.31	2A		II		R36		Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	liquid	S	undiluted	99%	Fisher	4	1	2	52.4	2A		II		R36		Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	S*	undiluted	97%	Fluka	1	0.4	0.3	-2.8	NI	2B	IV	III	NI	NI	Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	S*	undiluted	97%	Fluka	2	1	0	7	2B		III		NI		Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	S*	undiluted	97%	Fluka	3	0.67	1	11.52	2B		III		NI		Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	S*	undiluted	97%	Fluka	4	1	0.5	4.5	NI		IV		NI		Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	I*	undiluted	99%	Aldrich	1	1.2	0.4	7.2	2B	2B	III	III	NI	NI	Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	I*	undiluted	99%	Aldrich	2	2	2	31	2A		II		R36		Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	I*	undiluted	99%	Aldrich	3	0	0	1.44	NI		IV		NI		Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	I*	undiluted	99%	Aldrich	4	1	0.5	6.7	2B		III		NI		Balls et al. (1995)
Fomesafen	72128-02-0	solid	S	neat	97.5%	EPA	1	0.9	1.2	5.3	2B	2B	III	III	NI	NI	Balls et al. (1995)
Fomesafen	72128-02-0	solid	S	neat	97.5%	EPA	2	0	0.2	11	NI		IV		NI		Balls et al. (1995)
Fomesafen	72128-02-0	solid	S	neat	97.5%	EPA	3	1	0.5	2.82	NI		IV		NI		Balls et al. (1995)
Fomesafen	72128-02-0	solid	S	neat	97.5%	EPA	4	1	1	4.3	2B		III		NI		Balls et al. (1995)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Substance Name

Substance/Product Name	CASRN	Form Tested	Water Solubility	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
Glycerol	56-81-5	liquid	S	undiluted	>99.5%	Mallinkrodt	1	1.2	1	5	2B		III		NI		Balls et al. (1995)
Glycerol	56-81-5	liquid	S	undiluted	>99.5%	Mallinkrodt	2	0	0	11	NI	2B	IV	III	NI	NI	Balls et al. (1995)
Glycerol	56-81-5	liquid	S	undiluted	>99.5%	Mallinkrodt	3	1.17	1	8.3	2B		III		NI		Balls et al. (1995)
Glycerol	56-81-5	liquid	S	undiluted	>99.5%	Mallinkrodt	4	2	0.5	29.4	2A		II		R36		Balls et al. (1995)
Glycerol	56-81-5	liquid	S	undiluted	99%	Aldrich	-	0.5	0.4	4	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
n-Hexane	110-54-3	liquid	I	undiluted	99%	Aldrich	-	0.5	0	1	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
n-Hexanol	111-27-3	liquid	I*	undiluted	98%	E-Kodak	1	2.8	1.6	17.4	2A		II		R36		Balls et al. (1995)
n-Hexanol	111-27-3	liquid	I*	undiluted	98%	E-Kodak	2	0.2	1.7	82	2A	1	II	I	R36	R41	Balls et al. (1995)
n-Hexanol	111-27-3	liquid	I*	undiluted	98%	E-Kodak	3	3	2.83	28.89	1		I		R41		Balls et al. (1995)
n-Hexanol	111-27-3	liquid	I*	undiluted	98%	E-Kodak	4	3	3	58.9	1		I		R41		Balls et al. (1995)
Imidazole	288-32-4	solid	S	neat	99%	Aldrich	1	3	4	40.3	1		I		R41		Balls et al. (1995)
Imidazole	288-32-4	solid	S	neat	99%	Aldrich	2	3	3	224	1	1	I	I	R41	R41	Balls et al. (1995)
Imidazole	288-32-4	solid	S	neat	99%	Aldrich	3	3	2.5	36.96	1		I		R41		Balls et al. (1995)
Imidazole	288-32-4	solid	S	neat	99%	Aldrich	4	3	3	97.8	1		I		R41		Balls et al. (1995)
Isobutanol	78-83-1	liquid	I*	undiluted	99.9%	Fisher	1	2.8	2.5	46.4	1		I		R41		Balls et al. (1995)
Isobutanol	78-83-1	liquid	I*	undiluted	99.9%	Fisher	2	3	2.7	93	1	1	I	I	R41	R41	Balls et al. (1995)
Isobutanol	78-83-1	liquid	I*	undiluted	99.9%	Fisher	3	3	2.5	37.06	1		I		R41		Balls et al. (1995)
Isobutanol	78-83-1	liquid	I*	undiluted	99.9%	Fisher	4	3	2	69.2	1		I		R41		Balls et al. (1995)
Isopropanol	67-63-0	liquid	S	undiluted	99.9%	Fisher	1	2	1.6	23.3	2A		II		R36		Balls et al. (1995)
Isopropanol	67-63-0	liquid	S	undiluted	99.9%	Fisher	2	0.7	2.7	72	1	1	I	I	R41	R41	Balls et al. (1995)
Isopropanol	67-63-0	liquid	S	undiluted	99.9%	Fisher	3	3	2.5	37.84	1		I		R41		Balls et al. (1995)
Isopropanol	67-63-0	liquid	S	undiluted	99.9%	Fisher	4	2.3	0.5	8.9	2B		III		NI		Balls et al. (1995)
Maneb	12427-38-2	solid	S	neat	90%	EPA	1	0	0.5	2.8	NI		IV		NI		Balls et al. (1995)
Maneb	12427-38-2	solid	S	neat	90%	EPA	2	1	2	33	2A	NI	II	IV	R36	NI	Balls et al. (1995)
Maneb	12427-38-2	solid	S	neat	90%	EPA	3	0	0.5	8.03	NI		IV		NI		Balls et al. (1995)
Maneb	12427-38-2	solid	S	neat	90%	EPA	4	1	1	6.7	2B		III		NI		Balls et al. (1995)
Mercury (II) chloride	7487-94-7	solid	I	undiluted	99.5%	Aldrich	-	2	3.1	55	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
2-Methoxyethanol	109-86-4	liquid	S	undiluted	99.9%	Aldrich	-	2	2	18	2A	2A	II	II	R36	R36	Prinsen and Koëter (1993)
Methyl acetate	79-20-9	liquid	S	undiluted	98%	Fisher	1	1.4	2.4	20.3	2A		II		R36		Balls et al. (1995)
Methyl acetate	79-20-9	liquid	S	undiluted	98%	Fisher	2	1	2.7	93	1	1	I	I	R41	R41	Balls et al. (1995)
Methyl acetate	79-20-9	liquid	S	undiluted	98%	Fisher	3	2	2	22.5	2A		II		R36		Balls et al. (1995)
Methyl acetate	79-20-9	liquid	S	undiluted	98%	Fisher	4	3	3	17.5	1		I		R41		Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	S*	undiluted	99%	Aldrich	1	0.4	0.3	4.5	NI		IV		NI		Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	S*	undiluted	99%	Aldrich	2	0.5	0.7	44	2A	NI	II	IV	R36	NI	Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	S*	undiluted	99%	Aldrich	3	0.17	0.5	4.93	NI		IV		NI		Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	S*	undiluted	99%	Aldrich	4	1	1	10.7	2B		III		NI		Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	I*	undiluted	>99%	Fluka	1	0.4	0.5	2.3	NI		IV		NI		Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	I*	undiluted	>99%	Fluka	2	1	0	22	2B	NI	III	IV	NI	NI	Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	I*	undiluted	>99%	Fluka	3	0	0.5	5.83	NI		IV		NI		Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	I*	undiluted	>99%	Fluka	4	1	0.5	0	NI		IV		NI		Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	S	undiluted	99%	Fisher	1	2	2.2	23.1	2A		II		R36		Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	S	undiluted	99%	Fisher	2	2.7	3	99	1	1	I	I	R41	R41	Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	S	undiluted	99%	Fisher	3	3	2.33	34.88	1		I		R41		Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	S	undiluted	99%	Fisher	4	3	2	12.6	2A		II		R36		Balls et al. (1995)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Substance Name

Substance/Product Name	CASRN	Form Tested	Water Solubility	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
Methyl isobutyl ketone	108-10-1	liquid	I*	undiluted	98%	Fisher	1	2.6	2	26.5	2A	2A	II	II	R36	R36	Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	I*	undiluted	98%	Fisher	2	3	3	64	1		I		R41		Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	I*	undiluted	98%	Fisher	3	2	2.17	21.69	2A		II		R36		Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	I*	undiluted	98%	Fisher	4	2	2	12.3	2A		II		R36		Balls et al. (1995)
1-Napthaleneacetic acid	86-87-3	solid	I*	neat	96%	EPA	1	1	0.9	5.6	2B	2B	III	III	NI	R36	Balls et al. (1995)
1-Napthaleneacetic acid	86-87-3	solid	I*	neat	96%	EPA	2	1	1	24	2B		III		R36		Balls et al. (1995)
1-Napthaleneacetic acid	86-87-3	solid	I*	neat	96%	EPA	3	2	1	8.86	2B		III		R36		Balls et al. (1995)
1-Napthaleneacetic acid	86-87-3	solid	I*	neat	96%	EPA	4	1	1	46.7	2A		II		R36		Balls et al. (1995)
1-Napthaleneacetic acid, sodium salt	61-31-4	solid	S*	neat	95%	EPA	1	3	3	46.6	1	1	I	I	R41	R41	Balls et al. (1995)
1-Napthaleneacetic acid, sodium salt	61-31-4	solid	S*	neat	95%	EPA	2	3	2.7	122	1		I		R41		Balls et al. (1995)
1-Napthaleneacetic acid, sodium salt	61-31-4	solid	S*	neat	95%	EPA	3	3	2.5	44.19	1		I		R41		Balls et al. (1995)
1-Napthaleneacetic acid, sodium salt	61-31-4	solid	S*	neat	95%	EPA	4	3	3	64.1	1		I		R41		Balls et al. (1995)
n-Octanol	111-87-5	liquid	I*	undiluted	>99%	Aldrich	1	2	2.4	36.5	2A	2A	II	II	R36	R36	Balls et al. (1995)
n-Octanol	111-87-5	liquid	I*	undiluted	>99%	Aldrich	2	1.3	2	108	2A		II		R36		Balls et al. (1995)
n-Octanol	111-87-5	liquid	I*	undiluted	>99%	Aldrich	3	1.17	1.5	10.18	2B		III		NI		Balls et al. (1995)
n-Octanol	111-87-5	liquid	I*	undiluted	>99%	Aldrich	4	2	1	25.7	2A		II		R36		Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	I	undiluted	99%	Aldrich	1	3	2.2	35.3	1	1	I	I	R41	R41	Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	I	undiluted	99%	Aldrich	2	3	2	79	1		I		R41		Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	I	undiluted	99%	Aldrich	3	3	2	33.44	1		I		R41		Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	I	undiluted	99%	Aldrich	4	3	2	38.5	1		I		R41		Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	Sf	undiluted	n.p.	Aldrich	1	1.4	1	9.8	2B	2B	III	III	NI	R36	Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	Sf	undiluted	n.p.	Aldrich	2	0.2	0	26	NI		IV		NI		Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	Sf	undiluted	n.p.	Aldrich	3	2	1	5.88	2B		III		R36		Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	Sf	undiluted	n.p.	Aldrich	4	1	0.5	14.8	2B		III		R36		Balls et al. (1995)
Potassium cyanate	590-28-3	solid	S	neat	97%	Degussa	1	1	0.7	8	2B	2B	III	III	NI	R36	Balls et al. (1995)
Potassium cyanate	590-28-3	solid	S	neat	97%	Degussa	2	0.2	0	25	2B		III		NI		Balls et al. (1995)
Potassium cyanate	590-28-3	solid	S	neat	97%	Degussa	3	1.67	1	10.45	2B		III		R36		Balls et al. (1995)
Potassium cyanate	590-28-3	solid	S	neat	97%	Degussa	4	1.3	1.7	25.3	2A		II		R36		Balls et al. (1995)
Promethazine HCl	58-33-3	solid	S*	neat	98%	Aldrich	1	2.6	1.6	33	1	1	I	I	R41	R41	Balls et al. (1995)
Promethazine HCl	58-33-3	solid	S*	neat	98%	Aldrich	2	3	3	143	1		I		R41		Balls et al. (1995)
Promethazine HCl	58-33-3	solid	S*	neat	98%	Aldrich	3	3	2	23.02	2A		II		R36		Balls et al. (1995)
Promethazine HCl	58-33-3	solid	S*	neat	98%	Aldrich	4	2	3	28.6	1/2A		I/II		R36/R41		Balls et al. (1995)
Pyridine	110-86-1	liquid	S	undiluted	>99.9%	Aldrich	1	3	2	32.7	1	1	I	I	R41	R41	Balls et al. (1995)
Pyridine	110-86-1	liquid	S	undiluted	>99.9%	Aldrich	2	3	3	95	1		I		R41		Balls et al. (1995)
Pyridine	110-86-1	liquid	S	undiluted	>99.9%	Aldrich	3	3	2.5	37.47	1		I		R41		Balls et al. (1995)
Pyridine	110-86-1	liquid	S	undiluted	>99.9%	Aldrich	4	3	3	78.6	1		I		R41		Balls et al. (1995)
Quinacrine	69-05-6	solid	S*	neat	n.p.	Sigma	1	1.2	0.6	4.1	2B	2B	III	III	NI	NI	Balls et al. (1995)
Quinacrine	69-05-6	solid	S*	neat	n.p.	Sigma	2	0.2	0.2	12	NI		IV		NI		Balls et al. (1995)
Quinacrine	69-05-6	solid	S*	neat	n.p.	Sigma	3	2	2	11.49	2A		II		R36		Balls et al. (1995)
Quinacrine	69-05-6	solid	S*	neat	n.p.	Sigma	4	1	0.5	6.8	2B		III		NI		Balls et al. (1995)
Silver (I) nitrate	7761-88-8	solid	S	3%	99.5%	Aldrich	-	1	1	12	2B	2B	III	III	NI	NI	Prinsen and Koëter (1993)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Substance Name

Substance/Product Name	CASRN	Form Tested	Water Solubility	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
Sodium dodecyl sulfate	151-21-3	solid	S	undiluted	70%	Aldrich	-	0.8	1	22	2B	2B	III	III	R41	R41	Prinsen and Koëter (1993)
Sodium fluorescein	518-47-8	liquid	S	20%	70%[7]	Aldrich	-	0.1	0	0	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Sodium hydroxide	1310-73-2	liquid	S	1%	97%	Aldrich	-	3	3	60	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Sodium hydroxide (1%)	1310-73-2	liquid	S	1%	RG	Fisher	1	1	0.6	14.1	2B	2A	III	II	NI/R36	R36	Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	S	1%	RG	Fisher	2	0.7	2.3	55	2A		II		R36		Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	S	1%	RG	Fisher	3	2.33	2.5	30.31	2A		II		R36		Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	S	1%	RG	Fisher	4	2	2	33.3	2A		II		R36		Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	S	10%	RG	Fisher	1	3	4	32	1	1	I	I	R41	R41	Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	S	10%	RG	Fisher	2	3	3.3	194	1		I		R41		Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	S	10%	RG	Fisher	3	3	3.17	68.86	1		I		R41		Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	S	10%	RG	Fisher	4	3	4	151.7	1		I		R41		Balls et al. (1995)
Sodium lauryl sulfate (15%)	151-21-3	liquid	Sf	15%	98%	Sigma	1	0.6	0.4	7	2B	2B	III	III	NI	R36	Balls et al. (1995)
Sodium lauryl sulfate (15%)	151-21-3	liquid	Sf	15%	98%	Sigma	2	1	0.2	33	2A		II		R36		Balls et al. (1995)
Sodium lauryl sulfate (15%)	151-21-3	liquid	Sf	15%	98%	Sigma	3	1.67	1	9.56	2B		III		R36		Balls et al. (1995)
Sodium lauryl sulfate (15%)	151-21-3	liquid	Sf	15%	98%	Sigma	4	1	1	12.2	2B		III		NI		Balls et al. (1995)
Sodium lauryl sulfate (3%)	151-21-3	liquid	Sf	3%	98%	Sigma	1	1	0.2	3.9	NI	2B	IV	III	NI	NI	Balls et al. (1995)
Sodium lauryl sulfate (3%)	151-21-3	liquid	Sf	3%	98%	Sigma	2	0	0	39	2B		III		R36		Balls et al. (1995)
Sodium lauryl sulfate (3%)	151-21-3	liquid	Sf	3%	98%	Sigma	3	1	0	2.75	NI		IV		NI		Balls et al. (1995)
Sodium lauryl sulfate (3%)	151-21-3	liquid	Sf	3%	98%	Sigma	4	1	1	15.9	2B		III		NI/R36		Balls et al. (1995)
Sodium oxalate	62-76-0	solid	S	neat	>99%	Aldrich	1	0.7	0.7	6.3	2B	2B	III	III	NI	NI	Balls et al. (1995)
Sodium oxalate	62-76-0	solid	S	neat	>99%	Aldrich	2	0.2	0	24	2B		III		NI		Balls et al. (1995)
Sodium oxalate	62-76-0	solid	S	neat	>99%	Aldrich	3	0.5	0.5	2.62	NI		IV		NI		Balls et al. (1995)
Sodium oxalate	62-76-0	solid	S	neat	>99%	Aldrich	4	1	0	2.4	NI		IV		NI		Balls et al. (1995)
Sodium perborate, $4H_2O$	10486-00-7	solid	S	neat	98.6%	Dupont	1	0.6	0.5	3.1	NI	2B	IV	III	NI	NI	Balls et al. (1995)
Sodium perborate, $4H_2O$	10486-00-7	solid	S	neat	98.6%	Dupont	2	0.2	0.7	23	2B		III		NI		Balls et al. (1995)
Sodium perborate, $4H_2O$	10486-00-7	solid	S	neat	98.6%	Dupont	3	1.33	1	7.54	2B		III		NI		Balls et al. (1995)
Sodium perborate, $4H_2O$	10486-00-7	solid	S	neat	98.6%	Dupont	4	1	0.5	14.6	2B		III		NI		Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	I*	neat	97%	Aldrich	1	1.3	1	7.5	2B	2B	III	III	NI	NI	Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	I*	neat	97%	Aldrich	2	1	2	31	2A		II		R36		Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	I*	neat	97%	Aldrich	3	1.5	1.5	7.3	2B		III		NI		Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	I*	neat	97%	Aldrich	4	1	1	8.9	2B		III		NI		Balls et al. (1995)
TNO-01 (Formulation-1)[8]	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-02 (Formulation-2)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	2.7	2	24	2A	2A	II	II	R36	R36	Prinsen (1996)
TNO-03 (Pesticide-1)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.6	0.3	3	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-04 (Detergent-1)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.5	1.5	9	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-05 (Silicone powder-1)	n.p.	solid	I	undiluted	n.p.	n.p.	-	0	0	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-06 (Lubricant)	n.p.	gel	n.p.	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-07 (Ink-1)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.8	0	2	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-08 (Ink-2)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.1	0	3	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-09 (Paint)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.3	0.5	5	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-10 (Silicone powder-2)	n.p.	solid	I	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-11 (Sodium p-styrene sulfonate)	2695-37-6	solid	n.p.	undiluted	n.p.	n.p.	-	2	1.3	19	2A	2A	II	II	R36	R36	Prinsen (1996)
TNO-12 (Formulation-3)	n.p.	paste	n.p.	undiluted	n.p.	n.p.	-	2.5	2	35	2A	2A	II	II	R36	R36	Prinsen (1996)
TNO-13 (Pesticide-2)	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0.7	0	1	NI	NI	IV	IV	NI	NI	Prinsen (1996)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Substance Name

Substance/Product Name	CASRN	Form Tested	Water Solubility	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
TNO-14 (Polydisaccharide)	n.p.	liquid	n.p.	14.5%	n.p.	n.p.	-	0.3	0	2	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-15 (Polydisaccharide)	n.p.	liquid	n.p.	50%	n.p.	n.p.	-	0	0	2	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-16 (Liquid nylon product)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-17 (Solvent-1)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.3	0	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-18 (Solvent-2)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-19 (Solvent-3)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-20 (Solvent-4)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.5	0.3	3	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-21 (Solvent-5)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.3	0.3	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-22 (Solvent-6)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.2	0.3	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-23 (Solvent-7)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.2	0	2	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-24 (Solvent-8)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.2	0	3	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-25 (Solvent-9)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-26 (Ink-3)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.1	0	0	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-27 (Thermal paper coating-1)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1	0.6	9	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-28 (Toilet cleaner-1)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.4	0.8	12	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-29 (Toilet cleaner-2)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.3	1	11	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-30 (Pesticide-3)	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	1.5	1	7	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-31 (Sulfur)	7704-34-9	solid	I	undiluted	n.p.	n.p.	-	0.2	0	1	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-32 (Ink-4)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1	0.5	7	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-33 (Thermal paper coating-2)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	2	0.5	5	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-34 (Detergent-2)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1	1	25	1	1	I	I	R41	R41	Prinsen (1996)
TNO-35 (Propyl-lactate)	616-09-1	liquid	S	undiluted	n.p.	n.p.	-	3	3	45	1	1	I	I	R41	R41	Prinsen (1996)
TNO-36 (Ethylhexyl lactate)	6283-86-9	liquid	n.p.	undiluted	n.p.	n.p.	-	2	2	18	2A	2A	II	II	R36	R36	Prinsen (1996)
TNO-37 (Pesticide-4)	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	1.5	1	15	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-38 (Solvent-10)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	3	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-39 (Detergent-3)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.5	0.5	4	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-40 (Glycolbromoacetate form.)	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	2.6	1.9	41	1	1	I	I	R41	R41	Prinsen (1996)
TNO-41 (Amidosulfonic acid)	5329-14-6	solid	n.p.	undiluted	n.p.	n.p.	-	2.7	4	46	1	1	I	I	R41	R41	Prinsen (1996)
TNO-42 (Glycolbromoacetate)	3785-34-0	liquid	n.p.	85%	n.p.	n.p.	-	3	3	36	1	1	I	I	R41	R41	Prinsen (1996)
TNO-43 (Monobromoacetic acid)	79-08-3	solid	S	undiluted	n.p.	n.p.	-	3	4	80	1	1	I	I	R41	R41	Prinsen (1996)
TNO-44 (Didecyldimethylammoniumchloride (23% in propyl glycol))	7173-51-5	liquid	n.p.	23%	n.p.	n.p.	-	3	3.5	39	1	1	I	I	R41	R41	Prinsen (1996)
TNO-45	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1	0.5	5	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-46	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-47	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-48	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	3	1	25	2A	2A	II	II	R36	R36	Prinsen (2005)
TNO-49	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	3	4	-	1	1	I	I	R41	R41	Prinsen (2005)
TNO-50	n.p.		n.p.	undiluted	n.p.	n.p.	-	3	3	41.1	1	1	I	I	R41	R41	Prinsen (2005)
TNO-51	n.p.		n.p.	undiluted	n.p.	n.p.	-	3	3	33.9	1	1	I	I	R41	R41	Prinsen (2005)
TNO-52	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.7	1	5	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-53	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.5	0.2	3	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-54	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	1	1	9	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-55	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.7	1.3	10	2B	2B	III	III	R36	R36	Prinsen (2005)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Substance Name

Substance/Product Name	CASRN	Form Tested	Water Solubility	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
TNO-56	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	2	1.3	10	2B	2B	III	III	R36	R36	Prinsen (2005)
TNO-57	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.5	1.3	12	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-58	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0	0	-1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-59	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0.2	0	-2	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-60	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.2	0.5	1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-61	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-62	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	2	1	12	2B	2B	III	III	R36	R36	Prinsen (2005)
TNO-63	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0.3	0.5	3	NI	NI	IV	IV	NI	NI	Prinsen (2005)
TNO-64	n.p.	emulsion	n.p.	undiluted	n.p.	n.p.	-	1	1	5	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-65	n.p.	emulsion	n.p.	undiluted	n.p.	n.p.	-	0.7	0.5	4	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-66	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0	0	0	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-67	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1	1	6	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-68	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1/2	1	8	2A	2A	II	II	R36	R36	Prinsen (2005)
TNO-69	n.p.	liquid	n.p.	50%	n.p.	n.p.	-	1	0	0	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-70	n.p.	emulsion	n.p.	undiluted	n.p.	n.p.	-	2	1	20	2A	2A	II	II	R36	R36	Prinsen (2005)
TNO-71	n.p.	emulsion	n.p.	undiluted	n.p.	n.p.	-	1	0.5	13	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-72	n.p.	emulsion	n.p.	undiluted	n.p.	n.p.	-	1.5	0.5	5	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-73	n.p.	emulsion	n.p.	undiluted	n.p.	n.p.	-	2.7	2	18	1	1	I	I	R41	R41	Prinsen (2005)
TNO-74	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0.5	0	0	NI	NI	IV	IV	NI	NI	Prinsen (2005)
TNO-75	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	2	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-76	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0	0	2	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-77	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1	0.5	7	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-78	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1.3	1	15	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-79	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	1	1	10	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-80	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0.3	0	-1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-81	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-82	n.p.	n.p.	n.p.	undiluted	n.p.	n.p.	-	0	0	-2	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-83	n.p.	n.p.	n.p.	undiluted	n.p.	n.p.	-	0.8	0.7	10	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-84	n.p.	n.p.	n.p.	undiluted	n.p.	n.p.	-	0.7	0.7	2	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-85	n.p.	n.p.	n.p.	undiluted	n.p.	n.p.	-	2	1.3	14	2B	2B	III	III	R36	R36	Prinsen (2005)
TNO-86	n.p.	n.p.	n.p.	undiluted	n.p.	n.p.	-	1	1	7	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-87	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.7	1	1	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-88	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0.3	0.7	3	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-89	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0.2	0.7	1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-90	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0	2	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-91	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	0	0.2	1	NI	NI	NI	NI	NI	NI	Prinsen (2005)
TNO-92	n.p.	solid	n.p.	undiluted	n.p.	n.p.	-	0.8	1.7	16	2B	2B	III	III	R36	R36	Prinsen (2005)
TNO-93	n.p.	emulsion	n.p.	undiluted	n.p.	n.p.	-	3	2	17	2A	2A	II	II	R36	R36	Prinsen (2005)
TNO-94	n.p.	liquid	n.p.	undiluted	n.p.	n.p.	-	1	0.5	2	NI	NI	NI	NI	NI	NI	Prinsen (2005)
Toluene	108-88-3	liquid	I*	undiluted	99%	Fisher	2	1.4	1	5.2	2B	2A	III	II	NI	R36	Balls et al. (1995)
Toluene	108-88-3	liquid	I*	undiluted	99%	Fisher	3	2	1.3	29	2A		II		R36		Balls et al. (1995)
Toluene	108-88-3	liquid	I*	undiluted	99%	Fisher	4	1.33	2	13.87	2A/2B		II/III		R36		Balls et al. (1995)
Toluene	108-88-3	liquid	I*	undiluted	99%	Fisher	1	1	2	58.2	2A		II		R36		Balls et al. (1995)
Toluene	108-88-3	liquid	I*	undiluted	99.9%	Aldrich	-	1.1	1.4	4	2B	2B	III	III	NI	NI	Prinsen and Koëter (1993)
Triacetin	102-76-1	liquid	I*	undiluted	99%	Aldrich	-	0.5	0.4	4	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Tributyltin chloride	1461-22-9	liquid	n.p.	undiluted	96%	Aldrich	-	3	2.5	48	1	1	I	I	R41	R41	Prinsen and Koëter (1993)

In Vitro Data for Substances Tested in the ICE Test Method: Sorted by Substance Name

Substance/Product Name	CASRN	Form Tested	Water Solubility	Concentration Tested	Purity	Source	Lab No.	Fluorescein Retention	Corneal Opacity	Corneal Swelling	*In Vitro* Classif. (GHS)[1,2]	Overall *In Vitro* Classif. (GHS)	*In Vitro* Classif. (EPA)[3,4]	Overall *In Vitro* Classif. (EPA)	*In Vitro* Classif. (EU)[5,6]	Overall *In Vitro* Classif. (EU)	Reference
Trichloroacetic acid (3%)	76-03-9	liquid	S	3%	RG	Fisher	2	2.4	1.2	13.2	2A/2B		II/III		R36		Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	S	3%	RG	Fisher	3	2.3	2	38	2A	2A	II	II	R36	R36	Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	S	3%	RG	Fisher	4	1.5	2.5	27.88	2A		II		R36		Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	S	3%	RG	Fisher	1	1.7	2	26.4	2A		II		R36		Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	S	30%	RG	Fisher	2	3	4	32	1		I		R41		Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	S	30%	RG	Fisher	3	3	4	153	1	1	I	I	R41	R41	Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	S	30%	RG	Fisher	4	*	4	*	*		*		*		Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	S	30%	RG	Fisher	1	3	4	*	1		I		R41		Balls et al. (1995)
Triethanolamine	102-71-6	liquid	S	undiluted	99%	Aldrich	-	0.9	0.7	4	2B	2B	III	III	NI	NI	Prinsen and Koëter (1993)
Triton X-500 (5%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	1.0	0.7	14	2B		III		NI		Prinsen (2000)
Triton X-500 (5%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	1.0	0.7	14	2B		III		NI		Prinsen (2000)
Triton X-500 (5%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	1.0	0.7	13	2B	2B	III	III	NI	NI	Prinsen (2000)
Triton X-500 (5%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	1.0	0.8	8	2B		III		NI		Prinsen (2000)
Triton X-500 (5%)	——	liquid	Sf	undiluted	n.p.	n.p.	1	1.0	0.7	11	2B		III		NI		Prinsen (2000)
Triton X-100 (10%)	9002-93-1	liquid	Sf	10%	98%	Sigma	2	1.4	0.1	9.9	2B		III		NI		Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	Sf	10%	98%	Sigma	3	1	0.8	29	2A/2B		II/III		R36		Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	Sf	10%	98%	Sigma	4	2.67	1.17	20.2	2A	2A/2B	II	II/III	R36	R36	Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	Sf	10%	98%	Sigma	1	1.7	1	11.2	2B		III		R36		Balls et al. (1995)
Triton X-100 (5%)	9002-93-1	liquid	Sf	5%	98%	Sigma	2	1	0.6	9.8	2B		III		NI		Balls et al. (1995)
Triton X-100 (5%)	9002-93-1	liquid	Sf	5%	98%	Sigma	3	1.3	0	38	2A		II		R36		Balls et al. (1995)
Triton X-100 (5%)	9002-93-1	liquid	Sf	5%	98%	Sigma	4	2	0	3.97	2B	2A	III	II	NI	R36	Balls et al. (1995)
Triton X-100 (5%)	9002-93-1	liquid	Sf	5%	98%	Sigma	1	1	2	39.6	2A		II		R36		Balls et al. (1995)
Tween 20	9005-64-5	liquid	Sf	undiluted	98%	Sigma	2	1	1	3.6	2B		III		NI		Balls et al. (1995)
Tween 20	9005-64-5	liquid	Sf	undiluted	98%	Sigma	3	0.2	0	31	2B		III		NI/R36		Balls et al. (1995)
Tween 20	9005-64-5	liquid	Sf	undiluted	98%	Sigma	4	2.5	1	5.63	2B	2B	III	III	R36	NI	Balls et al. (1995)
Tween 20	9005-64-5	liquid	Sf	undiluted	98%	Sigma	1	1	0.5	6.7	2B		III		NI		Balls et al. (1995)

Abbreviations: Classif. = classification; S = soluble; Sf = surfactant; I = insoluble; *solubility uncertain; RG = reagent grade; n.p. = not provided and not obtained

[1] GHS=Globally Harmonized System (UN 2007)

[2] Eye Irritant Category 1 = irreversible effects on the eye/serious damage to the eye; Category 2A = reversible effects on the eye/irritating to the eyes; Category 2B = reversible effects on the eye/mildly irritating to the eyes; Nonirritant = not an eye irritant.

[3] EPA=U.S. Environmental Protection Agency (EPA 2003a).

[4] Toxicity Category I for the Primary Eye Irritation Study = Corrosive, or corneal involvement or irritation not reversible within 21 days; Category II = Corneal involvement or irritation clearing in 8-21 days; Category III = Corneal involvement or irritation clearing in 1-7 days; Category IV: minimal effects clearing in less than 24 hr.

[5] EU=European Union (EU 2001).

[6] Risk phrase R41 = risk of serious damage to the eyes; R36 = irritating to the eyes; nonirritant = not an eye irritant.

[7] Dye content

[8] Numbering for substances from this reference assigned based on order of appearance in Table 3 of Prinsen (1996)

Annex III

In Vivo and *In Vitro* Data Comparison of Ocular Irritancy Classification

This page intentionally left blank

Annex III-1

In Vivo and *In Vitro* Data Comparison of Ocular Irritancy Classification: Sorted by Reference

This page intentionally left blank

In Vivo and *In Vitro* Data Comparison of Ocular Irritancy Classification: Sorted by Reference

Substance/Product Name	CASRN[1]	Concentration Tested	Overall *In Vivo* Classification (GHS)	*In Vitro* Classification (GHS)	*In Vivo* Classification (GHS)[2,3]	Overall *In Vivo* Classification (EPA)	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)[4,5]	Overall *In Vivo* Classification (EU)	*In Vitro* Classification (EU)	*In Vivo* Classification (EU)[6,7]	FHSA-20%[8]	FHSA-67%[9]	Reference
1-Napthaleneacetic acid	86-87-3	neat	1	2B	1	I	III	I	R41	R36	SCNM	Irritant	Irritant	Balls et al. (1995)
1-Napthaleneacetic acid, sodium salt	61-31-4	neat	1	1	1	I	I	I	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	undiluted	2B	2A	2A	II	II	II	R36	R36	R36	Irritant	Irritant	Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	undiluted	1	1	SCNM[10]	I	I	I	R41	R41	SCNM	Irritant	Irritant	Balls et al. (1995)
2,5-Dimethylohexanediol	110-03-2	neat	1	2B	1	I	III	I	R41	R36	R41	Irritant	Irritant	Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	undiluted	2A	2A	2A	II	II	II	R36	R36	SCNM	Irritant	Irritant	Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	neat	2A	NI	2A	II	IV	II	R36	NI	R36	Irritant	Irritant	Balls et al. (1995)
Acetone	67-64-1	undiluted	2A	2A	2A	II	II	II	R36	R36	R36	Irritant	Irritant	Balls et al. (1995)
Ammonium nitrate	6484-52-2	undiluted	2B	2B	2B	III	III	III	R36	NI	R36	Irritant	Irritant	Balls et al. (1995)
Benzalkonium chloride (1%)	8001-54-5	1%	1	2A	1	I	II	I	R41	R36	R41	Irritant	Irritant	Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	10%	1	1	1	I	I	I	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	5%	1	1	1	I	I	I	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
Captan 90 concentrate	133-06-2	neat	1	2B	1	I	III	I	R41	NI	R41	Irritant	Irritant	Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	0.1%	NI	2B	NI	III	III	III	NI	NI	NI	Irritant	Inconclusive	Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	10%	1	2A	1	I	II	I	R41	R36	R41	Irritant	Irritant	Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	6%	1	2A	1	SCNM	II	SCNM	R41	R36	R41	Irritant	Irritant	Balls et al. (1995)
Chlorhexidine	55-56-1	neat	1	1	1	I	I	SCNM	R41	R41	SCNM	Irritant	Irritant	Balls et al. (1995)
Cyclohexanol	108-93-0	undiluted	1	1	1	I	I	I	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
Dibenzoyl-L-tartaric acid	2743-38-6	neat	1	1	1	SCNM	I	SCNM	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	neat	2A	2A/2B	2A	II	II/III	II	R36	R36	R36	Irritant	Irritant	Balls et al. (1995)
Ethanol	64-17-5	undiluted	2B	1	2A	III	I	III	NI	R41	NI	Irritant	Irritant	Balls et al. (1995)
Ethyl acetate	141-78-6	undiluted	NI	2A	NI	III	II	III	NI	R36	NI	Irritant	Irritant	Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	undiluted	NI	2B	NI	III	III	III	NI	NI	NI	Not Labeled	Not Labeled	Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	undiluted	2B	2B	2B	III	III	III	NI	NI	NI	Irritant	Irritant	Balls et al. (1995)
Fomesafen	72128-02-0	neat	NI	2B	NI	III	III	III	NI	NI	NI	Irritant	Irritant	Balls et al. (1995)
Gammabutyrolactone	96-48-0	undiluted	2B	2A	2A	II	II	II	R36	R36	R36	Irritant	Irritant	Balls et al. (1995)
Glycerol	56-81-5	undiluted	NI	2B	NI	IV	III	IV	NI	NI	NI	Not Labeled	Not Labeled	Balls et al. (1995)
Imidazole	288-32-4	neat	1	1	1	I	I	I	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
Isobutanol	78-83-1	undiluted	2B	1	2A	II	I	II	R36	R41	R36	Irritant	Irritant	Balls et al. (1995)
Isopropanol	67-63-0	undiluted	2B	1	2A	III	I	III	R36	R41	SCNM	Irritant	Irritant	Balls et al. (1995)
L-Aspartic acid	70-47-3	neat	SCNM	2A	SCNM	SCNM	II	SCNM	R36	R36	SCNM	Irritant	Irritant	Balls et al. (1995)
Maneb	12427-38-2	neat	2B	NI	SCNM	III	IV	III	R36	NI	SCNM	Irritant	Irritant	Balls et al. (1995)
Methyl acetate	79-20-9	undiluted	2B	1	2A	II	I	II	R36	R41	R36	Irritant	Irritant	Balls et al. (1995)
Methyl cyanoacetate	105-34-0	undiluted	2A	NI	2A	II	IV	II	R36	NI	R36	Irritant	Irritant	Balls et al. (1995)
Methyl ethyl ketone	78-93-3	undiluted	2B	1	2A	III	I	III	R36	R41	R36	Irritant	Irritant	Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	undiluted	NI	2A	NI	III	II	III	NI	R36	NI	Irritant	Inconclusive	Balls et al. (1995)
Methylcyclopentane	96-37-7	undiluted	NI	NI	NI	III	IV	III	NI	NI	NI	Not Labeled	Not Labeled	Balls et al. (1995)
n-Butyl acetate	123-86-4	undiluted	NI	2A	NI	III	II	III	NI	R36	NI	Irritant	Inconclusive	Balls et al. (1995)
n-Hexanol	111-27-3	undiluted	2A	1	2A	II	I	II	R36	R41	R36	Irritant	Irritant	Balls et al. (1995)
n-Octanol	111-87-5	undiluted	2B	2A	2B	II	II	II	R36	R36	R36	Irritant	Irritant	Balls et al. (1995)
Parafluoraniline	371-40-4	undiluted	SCNM	1	SCNM	SCNM	I	SCNM	R36	R41	SCNM	Irritant	Irritant	Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	undiluted	NI	2B	NI	IV	III	IV	NI	R36	NI	Not Labeled	Not Labeled	Balls et al. (1995)
Potassium cyanate	590-28-3	neat	SCNM	2B	SCNM	SCNM	III	SCNM	R36	R36	SCNM	Irritant	Irritant	Balls et al. (1995)
Promethazine HCl	58-33-3	neat	1	1	1	I	I	I	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
Pyridine	110-86-1	undiluted	1	1	1	I	I	I	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
Quinacrine	69-05-6	neat	1	2B	1	I	III	I	R41	NI	R41	Irritant	Irritant	Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	1%	2B	2A	2B	III	II	III	R36	R36	R36	Irritant	Irritant	Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	10%	1	1	1	I	I	I	R41	R41	R41	scnm	scnm	Balls et al. (1995)
Sodium lauryl sulfate (15%)	151-21-3	15%	1	2B	1	I	III	I	R36	R36	R36	Irritant	Irritant	Balls et al. (1995)
Sodium lauryl sulfate (3%)	151-21-3	3%	NI	2B	NI	III	III	III	NI	NI	NI	Irritant	Irritant	Balls et al. (1995)
Sodium oxalate	62-76-0	neat	1	2B	1	I	III	I	R41	NI	R41	Irritant	Irritant	Balls et al. (1995)

In Vivo and *In Vitro* Data Comparison of Ocular Irritancy Classification: Sorted by Reference

Substance/Product Name	CASRN[1]	Concentration Tested	Overall *In Vivo* Classification (GHS)	*In Vitro* Classification (GHS)	*In Vivo* Classification (GHS)[2,3]	Overall *In Vivo* Classification (EPA)	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)[4,5]	Overall *In Vivo* Classification (EU)	*In Vitro* Classification (EU)	*In Vivo* Classification (EU)[6,7]	FHSA-20%[8]	FHSA-67%[9]	Reference
Sodium perborate, $4H_2O$	10486-00-7	neat	1	2B	1	I	III	I	R41	NI	R41	Irritant	Irritant	Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	neat	NI	2B	NI	III	III	III	NI	NI	NI	Irritant	Irritant	Balls et al. (1995)
Toluene	108-88-3	undiluted	NI	2A	NI	III	II	III	NI	R36	NI	Irritant	Irritant	Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	3%	NI	2A	NI	III	II	III	NI	R36	NI	Irritant	Inconclusive	Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	30%	1	1	1	I	I	I	R41	R41	R41	scnm	scnm	Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	10%	2A	2A/2B	1	II	II/III	II	R36	R36	R41	Irritant	Irritant	Balls et al. (1995)
Triton X-100 (5%)	9002-93-1	5%	2B	2A	2A	III	II	III	R36	R36	NI	Irritant	Irritant	Balls et al. (1995)
Tween 20	9005-64-5	undiluted	NI	2B	NI	III	III	III	NI	NI	NI	Irritant	Inconclusive	Balls et al. (1995)
TNO-01 (Formulation-1)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-02 (Formulation-2)	n.p.	undiluted		2A	2A		II	II		R36	R36	Irritant	Irritant	Prinsen (1996)
TNO-03 (Pesticide-1)	n.p.	undiluted		NI	NI		IV	III		NI	NI	Irritant	Irritant	Prinsen (1996)
TNO-04 (Detergent-1)	n.p.	undiluted		2B	2A		III	III		NI	NI	Irritant	Irritant	Prinsen (1996)
TNO-05 (Silicone powder-1)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-06 (Lubricant)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-07 (Ink-1)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-08 (Ink-2)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-09 (Paint)	n.p.	undiluted		NI	NI		IV	II		NI	NI	Irritant	Irritant	Prinsen (1996)
TNO-10 (Silicone powder-2)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-11 (Sodium p-styrene sulfonate)	2695-37-6	undiluted		2A	SCNM		II	SCNM		R36	SCNM	Irritant	Irritant	Prinsen (1996)
TNO-12 (Formulation-3)	n.p.	undiluted		2A	NI		II	SCNM		R36	R36	Irritant	Irritant	Prinsen (1996)
TNO-13 (Pesticide-2)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-14 (Polydisaccharide)	n.p.	14.5%		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-15 (Polydisaccharide)	n.p.	50%		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-16 (Liquid nylon product)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-17 (Solvent-1)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-18 (Solvent-2)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-19 (Solvent-3)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-20 (Solvent-4)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-21 (Solvent-5)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-22 (Solvent-6)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-23 (Solvent-7)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-24 (Solvent-8)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-25 (Solvent-9)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-26 (Ink-3)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-27 (Thermal paper coating-1)	n.p.	undiluted		2B	2B		III	III		NI	NI	Irritant	Irritant	Prinsen (1996)
TNO-28 (Toilet cleaner-1)	n.p.	undiluted		2B	1		III	I		NI	R41	Irritant	Irritant	Prinsen (1996)
TNO-29 (Toilet cleaner-2)	n.p.	undiluted		2B	2A		III	III		NI	R36	Irritant	Irritant	Prinsen (1996)
TNO-30 (Pesticide-3)	n.p.	undiluted		2B	NI		III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-31 (Sulfur)	7704-34-9	undiluted		NI	NI		IV	III		NI	NI	Irritant	Inconclusive	Prinsen (1996)
TNO-32 (Ink-4)	n.p.	undiluted		2B	NI		III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-33 (Thermal paper coating-2)	n.p.	undiluted		2B	NI		III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-34 (Detergent-2)	n.p.	undiluted		1	SCNM		I	SCNM		R41	SCNM	Irritant	Irritant	Prinsen (1996)
TNO-35 (Propyl-lactate)	616-09-1	undiluted		1	1		I	I		R41	R41	scnm	scnm	Prinsen (1996)
TNO-36 (Ethylhexyl lactate)	6283-86-9	undiluted		2A	SCNM		II	II		R36	SCNM	Irritant	Irritant	Prinsen (1996)
TNO-37 (Pesticide-4)	n.p.	undiluted		2B	2B		III	III		NI	NI	Irritant	Irritant	Prinsen (1996)
TNO-38 (Solvent-10)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-39 (Detergent-3)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-40 (Glycolbromoacetate form.)	n.p.	undiluted		1	-		I	-		R41	R41 (SC)			Prinsen (1996)
TNO-41 (Amidosulfonic acid)	5329-14-6	undiluted		1	-		I	-		R41	R41 (SC)			Prinsen (1996)
TNO-42 (Glycolbromoacetate)	3785-34-0	85%		1	-		I	-		R41	R41 (SC)			Prinsen (1996)
TNO-43 (Monobromoacetic acid)	79-08-3	undiluted		1	-		I	-		R41	R41 (SC)			Prinsen (1996)

In Vivo and *In Vitro* Data Comparison of Ocular Irritancy Classification: Sorted by Reference

Substance/Product Name	CASRN[1]	Concentration Tested	Overall *In Vivo* Classification (GHS)	*In Vitro* Classification (GHS)	*In Vivo* Classification (GHS)[2,3]	Overall *In Vivo* Classification (EPA)	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)[4,5]	Overall *In Vivo* Classification (EU)	*In Vitro* Classification (EU)	*In Vivo* Classification (EU)[6,7]	FHSA-20%[8]	FHSA-67%[9]	Reference
TNO-44 (Didecyldimethylammoniumchloride (23% in propyl glycol))	7173-51-5	23%		1	-		I	-		R41	R41 (SC)			Prinsen (1996)
Cetylpyridinium bromide (6%)	——	undiluted	R41	1	1		I	SCNM	I	R41	R41	Irritant	Irritant	Prinsen (2000)
cyclohexylamino-functional PMS	——	undiluted	R36	2A	-		II	-	II	R36	R36			Prinsen (2000)
decamethylcyclopentasiloxane	——	undiluted	NI	NI	-		NI	-	NI	NI	NI			Prinsen (2000)
Triton X-500 (5%)	——	undiluted	NI	2B	-		III	-	III	NI	R36			Prinsen (2000)
TNO-45	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-46	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-47	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-48	n.p.	undiluted		2A	-	2A	II	-		R36	R41 (SC)			Prinsen (2005)
TNO-49	n.p.	undiluted		1	-	1	I	-		R41	R41 (SC)			Prinsen (2005)
TNO-50	n.p.	undiluted		1	-	1	I	-		R41	R41 (SC)			Prinsen (2005)
TNO-51	n.p.	undiluted		1	-	1	I	-		R41	R41 (SC)			Prinsen (2005)
TNO-52	n.p.	undiluted		2B	2A	2B	III	III		NI	R36	Irritant	Irritant	Prinsen (2005)
TNO-53	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-54	n.p.	undiluted		2B	2B	2B	III	III		NI	NI	Irritant	Irritant	Prinsen (2005)
TNO-55	n.p.	undiluted		2B	2A	2B	III	III		R36	R36	Irritant	Irritant	Prinsen (2005)
TNO-56	n.p.	undiluted		2B	2B	2B	III	III		R36	NI	Irritant	Irritant	Prinsen (2005)
TNO-57	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-58	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-59	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-60	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-61	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-62	n.p.	undiluted		2B	NI	2B	III	III		R36	NI	Irritant	Irritant	Prinsen (2005)
TNO-63	n.p.	undiluted		NI	NI	NI	IV	III		NI	NI	Irritant	Irritant	Prinsen (2005)
TNO-64	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-65	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-66	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-67	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-68	n.p.	undiluted		2A	2A	2A	II	II		R36	R36	Irritant	Irritant	Prinsen (2005)
TNO-69	n.p.	50%		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-70	n.p.	undiluted		2A	2A	2A	II	III		R36	R36	Irritant	Irritant	Prinsen (2005)
TNO-71	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-72	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-73	n.p.	undiluted		1	2A	1 (LE)	I	II		R41	R36	Irritant	Irritant	Prinsen (2005)
TNO-74	n.p.	undiluted		NI	NI	NI	IV	III		NI	NI	Irritant	Inconclusive	Prinsen (2005)
TNO-75	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-76	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-77	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-78	n.p.	undiluted		2B	2B	2B	III	III		NI	NI	Irritant	Irritant	Prinsen (2005)
TNO-79	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-80	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-81	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-82	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-83	n.p.	undiluted		2B	2B	2B	III	III		NI	R36	Irritant	Irritant	Prinsen (2005)
TNO-84	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-85	n.p.	undiluted		2B	1	2B	III	I		R36	R41	Irritant	Irritant	Prinsen (2005)
TNO-86	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)

In Vivo and *In Vitro* Data Comparison of Ocular Irritancy Classification: Sorted by Reference

Substance/Product Name	CASRN[1]	Concentration Tested	Overall *In Vivo* Classification (GHS)	*In Vitro* Classification (GHS)	*In Vivo* Classification (GHS)[2,3]	Overall *In Vivo* Classification (EPA)	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)[4,5]	Overall *In Vivo* Classification (EU)	*In Vitro* Classification (EU)	*In Vivo* Classification (EU)[6,7]	FHSA-20%[8]	FHSA-67%[9]	Reference
TNO-87	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-88	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-89	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-90	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-91	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-92	n.p.	undiluted		2B	1	2B	III	I		R36	R41	scnm	scnm	Prinsen (2005)
TNO-93	n.p.	undiluted		2A	1	2A	II	I		R36	R41	scnm	scnm	Prinsen (2005)
TNO-94	n.p.	undiluted		NI	1	NI	NI	I		NI	R41	Irritant	Irritant	Prinsen (2005)
1-Butanol	71-36-3	undiluted		1	2A		I	II		R41	R41	Irritant	Irritant	Prinsen and Koëter (1993)
2-Butoxyethyl acetate	112-07-2	undiluted		2B	-		III	-		NI	NI			Prinsen and Koëter (1993)
2-Methoxyethanol	109-86-4	undiluted		2A	-		II	-		R36	R36	Irritant	Irritant	Prinsen and Koëter (1993)
Acetaldehyde	75-07-0	undiluted		2A	-		II	-		R36	R36	Irritant	Irritant	Prinsen and Koëter (1993)
Acetic acid	64-19-7	10%		1	1		I	I		R41	R41	Irritant	Irritant	Prinsen and Koëter (1993)
Benzalkonium chloride (100%)	8001-54-5	undiluted		1	1		I	I		R41	R41	Irritant	Irritant	Prinsen and Koëter (1993)
Brij 35	9002-92-0	undiluted		NI	-		IV	-		NI	NI	Not Labeled	Not Labeled	Prinsen and Koëter (1993)
Chloroform	67-66-3	undiluted		2A	-		II	-		R36	R36			Prinsen and Koëter (1993)
Dibutyltin dichloride	683-18-1	undiluted		1	-		I	-		R41	R41			Prinsen and Koëter (1993)
Dimethyl sulfoxide	67-68-5	undiluted		NI	2B		IV	III		NI	NI	Irritant	Inconclusive	Prinsen and Koëter (1993)
Glycerol	56-81-5	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen and Koëter (1993)
Mercury (II) chloride	7487-94-7	undiluted		1	-		I	-		R41	R41			Prinsen and Koëter (1993)
n-Hexane	110-54-3	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen and Koëter (1993)
Silver (I) nitrate	7761-88-8	3%		2B	-		III	-		NI	NI			Prinsen and Koëter (1993)
Sodium dodecyl sulfate	151-21-3	undiluted		2B	-		III	-		R41	R41			Prinsen and Koëter (1993)
Sodium fluorescein	518-47-8	20%		NI	-		IV	-		NI	NI			Prinsen and Koëter (1993)
Sodium hydroxide	1310-73-2	1%		1	1		I	I		R41	R41			Prinsen and Koëter (1993)
Toluene	108-88-3	undiluted		2B	2B		III	III		NI	NI			Prinsen and Koëter (1993)
Triacetin	102-76-1	undiluted		NI	NI		IV	IV		NI	NI			Prinsen and Koëter (1993)
Tributyltin chloride	1461-22-9	undiluted		1	-		I	-		R41	R41			Prinsen and Koëter (1993)
Triethanolamine	102-71-6	undiluted		2B	NI		III	III		NI	NI	Not Labeled	Not Labeled	Prinsen and Koëter (1993)

[1] CASRN=Chemical Abstract Services Registry Number

[2] GHS=Globally Harmonized System (UN 2007)

[3] Eye Irritant Category 1 = irreversible effects on the eye/serious damage to the eye; Category 2A = reversible effects on the eye/irritating to the eyes; Category 2B = reversible effects on the eye/mildly irritating to the eyes; Nonirritant = not an eye irritant.

[6] NICEATM-defined subgroups assigned based on the lesions that drove classification of a GHS Category 1 substance. 1: based on lesions that are persistent; 2: based on lesions that are severe (not including corneal opacity score equal to 4); 3: based on lesions that are both severe and persistent; and 4: corneal opacity score equal to 4 at any time; NC: not classified because none of the above criteria were met

[4] EPA=U.S. Environmental Protection Agency (EPA 2003a).

[5] Toxicity Category I for the Primary Eye Irritation Study = Corrosive, or corneal involvement or irritation not reversible within 21 days; Category II = Corneal involvement or irritation clearing in 8-21 days; Category III = Corneal involvement or irritation clearing in 1-7 days; Category IV: minimal effects clearing in less than 24 hr

[6] EU=European Union (EU 2001).

[7] Risk phrase R41 = risk of serious damage to the eyes; R36 = irritating to the eyes; NI = not an eye irritant.

[8] FHSA=Federal Hazardous Substance Act (2005). FHSA-20% is based on the proportion of positive animals needed to identify a substance as an irritant using the FHSA sequential testing strategy, where 20% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥1 positive animal in a 3 to 5 animal test or >2 positive animals in a 6 animal test

[9] FHSA=Federal Hazardous Substances Act (2005). FHSA-67% is based on the proportion of positive animals needed to identify a substance as an irritant using the "first test" of the FHSA sequential testing strategy, where 67% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were >2/3, 3/4, 4/5, or 4/6 positive animals. If 1/3, 1/4, 2/4, 1/5, 2/5, 3/5, 2/6, or 3/6 animals were positive, further testing would be required.

[10] SCNM = study criteria not met

Annex III-2

In Vivo and *In Vitro* Data Comparison of Ocular Irritancy Classification: Sorted by Substance Name

This page intentionally left blank

In Vivo and *In Vitro* Data Comparison of Ocular Irritancy Classification: Sorted by Substance Name

Substance/Product Name	CASRN[1]	Concentration Tested	Overall In Vivo Classification (GHS)	*In Vitro* Classification (GHS)	*In Vivo* Classification (GHS)[2,3]	Overall In Vivo Classification (EPA)	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)[4,5]	Overall In Vivo Classification (EU)	*In Vitro* Classification (EU)	*In Vivo* Classification (EU)[6,7]	FHSA-20%[8]	FHSA-67%[9]	Reference
1-Butanol	71-36-3	undiluted		1	2A		I	II		R41	R41	Irritant	Irritant	Prinsen and Koëter (1993)
1-Napthaleneacetic acid	86-87-3	neat	1	2B	1	I	III	I	R41	R36	SCNM	Irritant	Irritant	Balls et al. (1995)
1-Napthaleneacetic acid, sodium salt	61-31-4	neat	1	1	1	I	I	I	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	undiluted	1	1	SCNM	I	I	I	R41	R41	SCNM	Irritant	Irritant	Balls et al. (1995)
2,5-Dimethylohexanediol	110-03-2	neat	1	2B	1	I	III	I	R41	R36	R41	Irritant	Irritant	Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	undiluted	2A	2A	2A	II	II	II	R36	R36	SCNM	Irritant	Irritant	Balls et al. (1995)
2-Butoxyethyl acetate	112-07-2	undiluted		2B	-		III	-		NI	NI			Prinsen and Koëter (1993)
2-Ethyl-1-hexanol	104-76-7	undiluted	2B	2A	2A	II	II	II	R36	R36	R36	Irritant	Irritant	Balls et al. (1995)
2-Methoxyethanol	109-86-4	undiluted		2A	-		II	-		R36	R36	Irritant	Irritant	Prinsen and Koëter (1993)
4-Carboxybenzaldehyde	619-66-9	neat	2A	NI	2A	II	IV	II	R36	NI	R36	Irritant	Irritant	Balls et al. (1995)
Acetaldehyde	75-07-0	undiluted		2A	-		II	-		R36	R36	Irritant	Irritant	Prinsen and Koëter (1993)
Acetic acid	64-19-7	10%		1	1		I	I		R41	R41	Irritant	Irritant	Prinsen and Koëter (1993)
Acetone	67-64-1	undiluted	2A	2A	2A	II	II	II	R36	R36	R36	Irritant	Irritant	Balls et al. (1995)
Ammonium nitrate	6484-52-2	undiluted	2B	2B	2B	III	III	III	R36	NI	R36	Irritant	Irritant	Balls et al. (1995)
Benzalkonium chloride (1%)	8001-54-5	1%	1	2A	1	I	II	I	R41	R36	R41	Irritant	Irritant	Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	10%	1	1	1	I	I	I	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
Benzalkonium chloride (100%)	8001-54-5	undiluted		1	1		I	I		R41	R41	Irritant	Irritant	Prinsen and Koëter (1993)
Benzalkonium chloride (5%)	8001-54-5	5%	1	1	1	I	I	I	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
Brij 35	9002-92-0	undiluted		NI	-		IV	-		NI	NI	Not Labeled	Not Labeled	Prinsen and Koëter (1993)
Captan 90 concentrate	133-06-2	neat	1	2B	1	I	III	I	R41	NI	R41	Irritant	Irritant	Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	0.1%	NI	2B	NI	III	III	III	NI	NI	NI	Irritant	Inconclusive	Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	10%	1	2A	1	I	II	I	R41	R36	R41	Irritant	Irritant	Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	6%	1	2A	1	SCNM	II	SCNM	R41	R36	R41	Irritant	Irritant	Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	undiluted	R41	1	1		I	SCNM	I	R41	R41			Prinsen (2000)
Chlorhexidine	55-56-1	neat	1	1	1	I	I	SCNM	R41	R41	SCNM	Irritant	Irritant	Balls et al. (1995)
Chloroform	67-66-3	undiluted		2A	-		II	-		R36	R36			Prinsen and Koëter (1993)
Cyclohexanol	108-93-0	undiluted	1	1	1	I	I	I	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
Cyclohexylamino-functional PMS	——	undiluted	R36	2A	-		II	-	II	R36	R36			Prinsen (2000)
Decamethylcyclopentasiloxane	——	undiluted	NI	NI	-		NI	-	NI	NI	NI			Prinsen (2000)
Dibenzoyl-L-tartaric acid	2743-38-6	neat	1	1	1	SCNM	I	SCNM	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	neat	2A	2A/2B	2A	II	II/III	II	R36	R36	R36	Irritant	Irritant	Balls et al. (1995)
Dibutyltin dichloride	683-18-1	undiluted		1	-		I	-		R41	R41			Prinsen and Koëter (1993)
Dimethyl sulfoxide	67-68-5	undiluted		NI	2B		IV	III		NI	NI	Irritant	Inconclusive	Prinsen and Koëter (1993)
Ethanol	64-17-5	undiluted	2B	1	2A	III	I	III	NI	R41	NI	Irritant	Irritant	Balls et al. (1995)
Ethyl acetate	141-78-6	undiluted	NI	2A	NI	III	II	III	NI	R36	NI	Irritant	Irritant	Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	undiluted	NI	2B	NI	III	III	III	NI	NI	NI	Not Labeled	Not Labeled	Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	undiluted	2B	2B	2B	III	III	III	NI	NI	NI	Irritant	Irritant	Balls et al. (1995)
Fomesafen	72128-02-0	neat	NI	2B	NI	III	III	III	NI	NI	NI	Irritant	Irritant	Balls et al. (1995)
gamma-Butyrolactone	96-48-0	undiluted	2B	2A	2A	II	II	II	R36	R36	R36	Irritant	Irritant	Balls et al. (1995)
Glycerol	56-81-5	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen and Koëter (1993)
Imidazole	288-32-4	neat	1	1	1	I	I	I	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
Isobutanol	78-83-1	undiluted	2B	1	2A	II	I	II	R36	R41	R36	Irritant	Irritant	Balls et al. (1995)
Isopropanol	67-63-0	undiluted	2B	1	2A	III	I	III	R36	R41	SCNM	Irritant	Irritant	Balls et al. (1995)
L-Aspartic acid	70-47-3	neat	SCNM	2A	SCNM	SCNM	II	SCNM	R36	R36	SCNM	Irritant	Irritant	Balls et al. (1995)
Maneb	12427-38-2	neat	2B	NI	SCNM	III	IV	III	R36	NI	SCNM	Irritant	Irritant	Balls et al. (1995)
Mercury (II) chloride	7487-94-7	undiluted		1	-		I	-		R41	R41			Prinsen and Koëter (1993)
Methyl acetate	79-20-9	undiluted	2B	1	2A	II	I	II	R36	R41	R36	Irritant	Irritant	Balls et al. (1995)
Methyl cyanoacetate	105-34-0	undiluted	2A	NI	2A	II	IV	II	R36	NI	R36	Irritant	Irritant	Balls et al. (1995)
Methyl ethyl ketone	78-93-3	undiluted	2B	1	2A	III	I	III	R36	R41	R36	Irritant	Irritant	Balls et al. (1995)

In Vivo and *In Vitro* Data Comparison of Ocular Irritancy Classification: Sorted by Substance Name

Substance/Product Name	CASRN[1]	Concentration Tested	Overall In Vivo Classification (GHS)	*In Vitro* Classification (GHS)	*In Vivo* Classification (GHS)[2,3]	Overall In Vivo Classification (EPA)	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)[4,5]	Overall In Vivo Classification (EU)	*In Vitro* Classification (EU)	*In Vivo* Classification (EU)[6,7]	FHSA-20%[8]	FHSA-67%[9]	Reference
Methyl isobutyl ketone	108-10-1	undiluted	NI	2A	NI	III	II	III	NI	R36	NI	Irritant	Inconclusive	Balls et al. (1995)
Methylcyclopentane	96-37-7	undiluted	NI	NI	NI	III	IV	III	NI	NI	NI	Not Labeled	Not Labeled	Balls et al. (1995)
n-Butyl acetate	123-86-4	undiluted	NI	2A	NI	III	II	III	NI	R36	NI	Irritant	Inconclusive	Balls et al. (1995)
n-Hexane	110-54-3	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen and Koëter (1993)
n-Hexanol	111-27-3	undiluted	2A	1	2A	II	I	II	R36	R41	R36	Irritant	Irritant	Balls et al. (1995)
n-Octanol	111-87-5	undiluted	2B	2A	2B	II	II	II	R36	R36	R36	Irritant	Irritant	Balls et al. (1995)
Parafluoraniline	371-40-4	undiluted	SCNM	1	SCNM	SCNM	I	SCNM	R36	R41	SCNM	Irritant	Irritant	Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	undiluted	NI	2B	NI	IV	III	IV	NI	R36	NI	Not Labeled	Not Labeled	Balls et al. (1995)
Potassium cyanate	590-28-3	neat	SCNM	2B	SCNM	SCNM	III	SCNM	R36	R36	SCNM	Irritant	Irritant	Balls et al. (1995)
Promethazine HCl	58-33-3	neat	1	1	1	I	I	I	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
Pyridine	110-86-1	undiluted	1	1	1	I	I	I	R41	R41	R41	Irritant	Irritant	Balls et al. (1995)
Quinacrine	69-05-6	neat	1	2B	1	I	III	I	R41	NI	R41	Irritant	Irritant	Balls et al. (1995)
Silver (I) nitrate	7761-88-8	3%		2B	-		III	-		NI	NI			Prinsen and Koëter (1993)
Sodium dodecyl sulfate	151-21-3	undiluted		2B	-		III	-		R41	R41			Prinsen and Koëter (1993)
Sodium fluorescein	518-47-8	20%		NI	-		IV	-		NI	NI			Prinsen and Koëter (1993)
Sodium hydroxide	1310-73-2	1%		1	1		I	I		R41	R41			Prinsen and Koëter (1993)
Sodium hydroxide (1%)	1310-73-2	1%	2B	2A	2B	III	II	III	R36	R36	R36	Irritant	Irritant	Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	10%	1	1	1	I	I	I	R41	R41	R41	scnm	scnm	Balls et al. (1995)
Sodium lauryl sulfate (15%)	151-21-3	15%	1	2B	1	I	III	I	R36	R36	R36	Irritant	Irritant	Balls et al. (1995)
Sodium lauryl sulfate (3%)	151-21-3	3%	NI	2B	NI	III	III	III	NI	NI	NI	Irritant	Irritant	Balls et al. (1995)
Sodium oxalate	62-76-0	neat	1	2B	1	I	III	I	R41	NI	R41	Irritant	Irritant	Balls et al. (1995)
Sodium perborate, $4H_2O$	10486-00-7	neat	1	2B	1	I	III	I	R41	NI	R41	Irritant	Irritant	Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	neat	NI	2B	NI	III	III	III	NI	NI	NI	Irritant	Irritant	Balls et al. (1995)
TNO-01 (Formulation-1)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-02 (Formulation-2)	n.p.	undiluted		2A	2A		II	II		R36	R36	Irritant	Irritant	Prinsen (1996)
TNO-03 (Pesticide-1)	n.p.	undiluted		NI	NI		IV	III		NI	NI	Irritant	Irritant	Prinsen (1996)
TNO-04 (Detergent-1[3)]	n.p.	undiluted		2B	2A		III	III		NI	NI	Irritant	Irritant	Prinsen (1996)
TNO-05 (Silicone powder-1)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-06 (Lubricant)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-07 (Ink-1)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-08 (Ink-2)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-09 (Paint)	n.p.	undiluted		NI	NI		IV	II		NI	NI	Irritant	Irritant	Prinsen (1996)
TNO-10 (Silicone powder-2)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-11 (Sodium p-styrene sulfonate)	2695-37-6	undiluted		2A	SCNM		II	SCNM		R36	SCNM	Irritant	Irritant	Prinsen (1996)
TNO-12 (Formulation-3)	n.p.	undiluted		2A	NI		II	SCNM		R36	R36	Irritant	Irritant	Prinsen (1996)
TNO-13 (Pesticide-2)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-14 (Polydisaccharide)	n.p.	14.5%		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-15 (Polydisaccharide)	n.p.	50%		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-16 (Liquid nylon product)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-17 (Solvent-1)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-18 (Solvent-2)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-19 (Solvent-3)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-20 (Solvent-4)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-21 (Solvent-5)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-22 (Solvent-6)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-23 (Solvent-7)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-24 (Solvent-8)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-25 (Solvent-9)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-26 (Ink-3)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)

In Vivo and *In Vitro* Data Comparison of Ocular Irritancy Classification: Sorted by Substance Name

Substance/Product Name	CASRN[1]	Concentration Tested	Overall In Vivo Classification (GHS)	*In Vitro* Classification (GHS)	*In Vivo* Classification (GHS)[2,3]	Overall In Vivo Classification (EPA)	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)[4,5]	Overall In Vivo Classification (EU)	*In Vitro* Classification (EU)	*In Vivo* Classification (EU)[6,7]	FHSA-20%[8]	FHSA-67%[9]	Reference
TNO-27 (Thermal paper coating-1)	n.p.	undiluted		2B	2B		III	III		NI	NI	Irritant	Irritant	Prinsen (1996)
TNO-28 (Toilet cleaner-1)	n.p.	undiluted		2B	1		III	I		NI	R41	Irritant	Irritant	Prinsen (1996)
TNO-29 (Toilet cleaner-2)	n.p.	undiluted		2B	2A		III	III		NI	R36	Irritant	Irritant	Prinsen (1996)
TNO-30 (Pesticide-3)	n.p.	undiluted		2B	NI		III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-31 (Sulfur)	7704-34-9	undiluted		NI	NI		IV	III		NI	NI	Irritant	Inconclusive	Prinsen (1996)
TNO-32 (Ink-4)	n.p.	undiluted		2B	NI		III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-33 (Thermal paper coating-2)	n.p.	undiluted		2B	NI		III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-34 (Detergent-2)	n.p.	undiluted		1	SCNM		I	SCNM		R41	SCNM	Irritant	Irritant	Prinsen (1996)
TNO-35 (Propyl-lactate)	616-09-1	undiluted		1	1		I	I		R41	R41	scnm	scnm	Prinsen (1996)
TNO-36 (Ethylhexyl lactate)	6283-86-9	undiluted		2A	SCNM		II	II		R36	SCNM	Irritant	Irritant	Prinsen (1996)
TNO-37 (Pesticide-4)	n.p.	undiluted		2B	2B		III	III		NI	NI	Irritant	Irritant	Prinsen (1996)
TNO-38 (Solvent-10)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-39 (Detergent-3)	n.p.	undiluted		NI	NI		IV	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (1996)
TNO-40 (Glycolbromoacetate form.)	n.p.	undiluted		1	-		I	-		R41	R41 (SC)			Prinsen (1996)
TNO-41 (Amidosulfonic acid)	5329-14-6	undiluted		1	-		I	-		R41	R41 (SC)			Prinsen (1996)
TNO-42 (Glycolbromoacetate)	3785-34-0	85%		1	-		I	-		R41	R41 (SC)			Prinsen (1996)
TNO-43 (Monobromoacetic acid)	79-08-3	undiluted		1	-		I	-		R41	R41 (SC)			Prinsen (1996)
TNO-44 (Didecyldimethylammoniumchloride (23% in propyl glycol))	7173-51-5	23%		1	-		I	-		R41	R41 (SC)			Prinsen (1996)
TNO-45	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-46	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-47	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-48	n.p.	undiluted		2A	-	2A	II	-		R36	R41 (SC)			Prinsen (2005)
TNO-49	n.p.	undiluted		1	-	1	I	-		R41	R41 (SC)			Prinsen (2005)
TNO-50	n.p.	undiluted		1	-	1	I	-		R41	R41 (SC)			Prinsen (2005)
TNO-51	n.p.	undiluted		1	-	1	I	-		R41	R41 (SC)			Prinsen (2005)
TNO-52	n.p.	undiluted		2B	2A	2B	III	III		NI	R36	Irritant	Irritant	Prinsen (2005)
TNO-53	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-54	n.p.	undiluted		2B	2B	2B	III	III		NI	NI	Irritant	Irritant	Prinsen (2005)
TNO-55	n.p.	undiluted		2B	2A	2B	III	III		R36	R36	Irritant	Irritant	Prinsen (2005)
TNO-56	n.p.	undiluted		2B	2B	2B	III	III		R36	NI	Irritant	Irritant	Prinsen (2005)
TNO-57	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-58	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-59	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-60	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-61	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-62	n.p.	undiluted		2B	NI	2B	III	III		R36	NI	Irritant	Irritant	Prinsen (2005)
TNO-63	n.p.	undiluted		NI	NI	NI	IV	III		NI	NI	Irritant	Irritant	Prinsen (2005)
TNO-64	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-65	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-66	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-67	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-68	n.p.	undiluted		2A	2A	2A	II	II		R36	R36	Irritant	Irritant	Prinsen (2005)
TNO-69	n.p.	50%		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-70	n.p.	undiluted		2A	2A	2A	II	III		R36	R36	Irritant	Irritant	Prinsen (2005)
TNO-71	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-72	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-73	n.p.	undiluted		1	2A	1 (LE)	I	II		R41	R36	Irritant	Irritant	Prinsen (2005)
TNO-74	n.p.	undiluted		NI	NI	NI	IV	III		NI	NI	Irritant	Inconclusive	Prinsen (2005)
TNO-75	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-76	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)

In Vivo and *In Vitro* Data Comparison of Ocular Irritancy Classification: Sorted by Substance Name

Substance/Product Name	CASRN[1]	Concentration Tested	Overall In Vivo Classification (GHS)	*In Vitro* Classification (GHS)	*In Vivo* Classification (GHS)[2,3]	Overall In Vivo Classification (EPA)	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)[4,5]	Overall In Vivo Classification (EU)	*In Vitro* Classification (EU)	*In Vivo* Classification (EU)[6,7]	FHSA-20%[8]	FHSA-67%[9]	Reference
TNO-77	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-78	n.p.	undiluted		2B	2B	2B	III	III		NI	NI	Irritant	Irritant	Prinsen (2005)
TNO-79	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-80	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-81	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-82	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-83	n.p.	undiluted		2B	2B	2B	III	III		NI	R36	Irritant	Irritant	Prinsen (2005)
TNO-84	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-85	n.p.	undiluted		2B	1	2B	III	I		R36	R41	Irritant	Irritant	Prinsen (2005)
TNO-86	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-87	n.p.	undiluted		2B	NI	2B	III	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-88	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-89	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-90	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-91	n.p.	undiluted		NI	NI	NI	NI	IV		NI	NI	Not Labeled	Not Labeled	Prinsen (2005)
TNO-92	n.p.	undiluted		2B	1	2B	III	I		R36	R41	scnm	scnm	Prinsen (2005)
TNO-93	n.p.	undiluted		2A	1	2A	II	I		R36	R41	scnm	scnm	Prinsen (2005)
TNO-94	n.p.	undiluted		NI	1	NI	NI	I		NI	R41	Irritant	Irritant	Prinsen (2005)
Toluene	108-88-3	undiluted	NI	2A	NI	III	II	III	NI	R36	NI	Irritant	Irritant	Balls et al. (1995)
Triacetin	102-76-1	undiluted		NI	NI		IV	IV		NI	NI			Prinsen and Koëter (1993)
Tributyltin chloride	1461-22-9	undiluted		1	-		I	-		R41	R41			Prinsen and Koëter (1993)
Trichloroacetic acid (3%)	76-03-9	3%	NI	2A	NI	III	II	III	NI	R36	NI	Irritant	Inconclusive	Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	30%	1	1	1	I	I	I	R41	R41	R41	scnm	scnm	Balls et al. (1995)
Triethanolamine	102-71-6	undiluted		2B	NI		III	III		NI	NI	Not Labeled	Not Labeled	Prinsen and Koëter (1993)
Triton X-100 (10%)	9002-93-1	10%	2A	2A/2B	1	II	II/III	II	R36	R36	R41	Irritant	Irritant	Balls et al. (1995)
Triton X-100 (5%)	9002-93-1	5%	2B	2A	2A	III	II	III	R36	R36	NI	Irritant	Irritant	Balls et al. (1995)
Triton X-500 (5%)	——	undiluted	NI	2B	-		III	-	III	NI	R36			Prinsen (2000)
Tween 20	9005-64-5	undiluted	NI	2B	NI	III	III	III	NI	NI	NI	Irritant	Inconclusive	Balls et al. (1995)

[1] CASRN=Chemical Abstract Services Registry Number

[2] GHS=Globally Harmonized System (UN 2007)

[3] Eye Irritant Category 1 = irreversible effects on the eye/serious damage to the eye; Category 2A = reversible effects on the eye/irritating to the eyes; Category 2B = reversible effects on the eye/mildly irritating to the eyes; NI = not an eye irritant.

[6] NICEATM-defined subgroups assigned based on the lesions that drove classification of a GHS Category 1 substance. 1: based on lesions that are persistent; 2: based on lesions that are severe (not including corneal opacity score equal to 4); 3: based on lesions that are both severe and persistent; and 4: corneal opacity score equal to 4 at any time; NC: not classified because none of the above criteria were met

[4] EPA=U.S. Environmental Protection Agency (EPA 2003a).

[5] Toxicity Category I for the Primary Eye Irritation Study = Corrosive, or corneal involvement or irritation not reversible within 21 days; Category II = Corneal involvement or irritation clearing in 8-21 days; Category III = Corneal involvement or irritation clearing in 1-7 days; Category IV: minimal effects clearing in less than 24 hr

[6] EU=European Union (EU 2001).

[7] Risk phrase R41 = risk of serious damage to the eyes; R36 = irritating to the eyes; nonirritant = not an eye irritant.

[8] FHSA=Federal Hazardous Substance Act (2005). FHSA-20% is based on the proportion of positive animals needed to identify a substance as an irritant using the FHSA sequential testing strategy, where 20% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥1 positive animal in a 3 to 5 animal test or ≥2 positive animals in a 6 animal test.

[9] FHSA=Federal Hazardous Substances Act (2005). FHSA-67% is based on the proportion of positive animals needed to identify a substance as an irritant using the "first test" of the FHSA sequential testing strategy, where 67% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥2/3, 3/4, 4/5, or 4/6 positive animals. If 1/3, 1/4, 2/4, 1/5, 2/5, 3/5, 2/6, or 3/6 animals were positive, further testing would be required.

[10] SCNM = study criteria not met.

Appendix G

Independent Scientific Peer Review Panel Assessment

This page intentionally left blank

Appendix G1

Summary Minutes from the Peer Review Panel Meeting on May 19-21, 2009

This page intentionally left blank

Summary Minutes

Independent Scientific Peer Review Panel Meeting

Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches

Consumer Product Safety Commission Headquarters
Fourth Floor Hearing Room
Bethesda Towers Building
Bethesda, MD

May 19 - 21, 2009

Peer Review Panel Members:

A. Wallace Hayes, Ph.D., DABT, FATS, ERT (Peer Review Panel Chair)	Visiting Scientist (Harvard), Harvard School of Public Health, Andover, MA; Principal Advisor, Spherix Incorporated, Bethesda, MD
Hongshik Ahn, Ph.D.	Professor, Stony Brook University, Stony Brook, NY
Paul Bailey, Ph.D.	Bailey & Associates Consulting, Neshanic Station, NJ
Richard Dubielzig, D.V.M.	Professor, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI
Henry Edelhauser, Ph.D.[1]	Professor of Ophthalmology and Director of Ophthalmic Research, Emory University School of Medicine, Atlanta, GA
Mark Evans, D.V.M., Ph.D., DACVP	Pathology Lead for Ophthalmology Therapeutic Area, Pfizer Global Research and Development at La Jolla Drug Safety Research and Development, San Diego, CA
James Jester, Ph.D.	Professor of Ophthalmology and Biomedical Engineering, Endowed Chair, University of California-Irving, Orange, CA

[1] Unable to attend the Panel meeting, but participated in the review of all materials.

Peer Review Panel Members:

Tadashi Kosaka, D.V.M., Ph.D.	Associate Director, Chief, Laboratory of Immunotoxicology and Acute Toxicology, Toxicology Division, The Institute of Environmental Toxicology, Ibaraki, Japan
Alison McLaughlin, M.Sc., DABT	Health Canada, Environmental Impact Initiative, Office of Science and Risk Management, Health Products and Food Branch, Ottawa, Ontario, Canada
J. Lynn Palmer, Ph.D.	Associate Professor, Department of Palliative Care and Rehabilitation Medicine, University of Texas, MD Anderson Cancer Center, Houston, TX
Robert Peiffer, Jr., D.V.M., Ph.D., DACVO	Senior Investigator, Merck Research Laboratories, Safety Assessment Toxicology, West Point, PA
Denise Rodeheaver, Ph.D., DABT	Assistant Director, Alcon Research Ltd., Department of Toxicology, Fort Worth, TX
Donald Sawyer, D.V.M., Ph.D., DACVA	Professor Emeritus, Retired, College of Veterinary Medicine, Michigan State University, East Lansing, MI
Kirk Tarlo, Ph.D., DABT	Scientific Director, Comparative Biology and Safety Sciences, Amgen, Inc., Thousand Oaks, CA
Daryl Thake, D.V.M., Dipl. ACVP[1]	Midwest ToxPath Sciences, Inc., Chesterfield, MO
Scheffer Tseng, M.D., Ph.D.[1]	Director, Ocular Surface (OS) Center, Medical Director OS Research & Education Foundation, Directory R&D Department, Tissue Tech, Inc., Ocular Surface Center, P.A., Miami, FL
Jan van der Valk, Ph.D.	Senior Scientist, Departments of Animals, Science and Society, Faculty of Veterinary Medicine, Utrecht University, Netherlands Centre Alternatives to Animal Use (NCA), Utrecht, Netherlands
Philippe Vanparys, Ph.D., DABT	Managing Director, CARDAM (VITO), Mol, Belgium
Maria Pilar Vinardell, Ph.D.	Director, Department of Physiology, Professor of Physiology and Pathology, Department Fisologia, Facultat de Farmacia, Universitat de Barcelona, Barcelona, Spain
Sherry Ward, Ph.D., M.B.A.	In Vitro Toxicology Consultant, BioTred Solutions, Science Advisor, International Foundation for Ethical Research, New Market, MD

Peer Review Panel Members:

Daniel Wilson, Ph.D., DABT	Mammalian Toxicology Consultant, Toxicology and Environmental Research Consulting, The Dow Chemical Company, Midland, MI
Fu-Shin Yu, Ph.D.	Director of Research, Department of Ophthalmology & Anatomy, School of Medicine, Wayne State University, Detroit, MI

ICCVAM and ICCVAM Ocular Toxicity Working Group Members:

Meta Bonner, Ph.D.	EPA, OPP, Washington, DC
Robert Bronaugh, Ph.D.	FDA, CFSAN, College Park, MD
Pertti Hakkinen	NLM, Bethesda, MD
Masih Hashim, D.V.M., Ph.D.	EPA, OPP, Washington, DC
Jodie Kulpa-Eddy, D.V.M. (ICCVAM Vice-Chair)	USDA, Riverdale, MD
Donnie Lowther	FDA, CFSAN, College Park, MD
Deborah McCall	EPA, OPP, Washington, DC
Jill Merrill, Ph.D. (OTWG Chair)	FDA, CDER, Silver Spring, MD
John Redden	EPA, OPP, Crystal City, VA
RADM William Stokes, D.V.M., DACLAM (Director, NICEATM)	NIEHS, Research Triangle Park, NC
Marilyn Wind, Ph.D., (ICCVAM Chair)	CPSC, Bethesda, MD

Invited Experts:

Rodger Curren, Ph.D.	Institute for In Vitro Sciences (IIVS), Gaithersburg, MD
Arnhild Schrage, Ph.D.	Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany

European Centre for the Validation of Alternative Methods, ICCVAM OTWG Liaison:

João Barroso, Ph.D.	European Centre for the Validation of Alternative Methods, Ispra, Italy

Public Attendees:

Attendee	**Affiliation**	**Day Attended**		
		1	**2**	**3**
Odelle Alexander	Syngenta Crop Protection, Greensboro, NC	√	√	√
Ian Blackwell	EPA, Antimicrobials Division, Arlington, VA	√	√	-
Krishna Deb	EPA, Antimicrobials Division, Arlington, VA	√	√	-
Noe Galvan	Clorox Services Co., Pleasanton, CA	√	√	√
Earl Goad	EPA, Antimicrobials Division, Arlington, VA	√	√	√
John Harbell	Mary Kay Inc., Addison, TX	√	√	√
Leon Johnson	EPA, Antimicrobials Division, Crystal City, VA	√	-	-
Eli Kumekpor	Invitrogen, Frederick, MD	√	-	√
Pauline McNamee	The Procter & Gamble Co., Egham, Surrey, U.K.	√	√	√
Michelle Piehl	MB Research Laboratories, Spinnerstown, PA	√	-	-
Patrick Quinn	Accord Group, Washington, DC	-	-	√
Hans Raabe	Institute for In Vitro Sciences, Gaithersburg, MD	-	√	√
Mary Richardson	Bausch & Lomb, Rochester, NY	√	√	√
Michael Rohovsky	Johnson & Johnson, New Brunswick, NJ	√	√	√
Kristie Sullivan	Physicians Committee for Responsible Medicine, Oakland, CA	-	-	√
Neil Wilcox	Consultant/FDA, College Park, MD	√	√	-

NICEATM:

RADM William Stokes, D.V.M., DACLAM	Director
Debbie McCarley	Special Assistant to the Director

Support Contract Staff— Integrated Laboratory Systems, Inc.:

David Allen, Ph.D.	Elizabeth Lipscomb, Ph.D.
Jonathan Hamm, Ph.D.	Linda Litchfield
Nelson Johnson	Greg Moyer, M.B.A.
Brett Jones, Ph.D.	James Truax, M.A.

Abbreviations used in participants' affiliations:

CDER = Center for Drug Evaluation and Research

CFSAN = Center for Food Safety and Applied Nutrition

CPSC = U.S. Consumer Product Safety Commission

ECVAM = European Centre for the Validation of Alternative Methods

EPA = U.S. Environmental Protection Agency

FDA = U.S. Food and Drug Administration

ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods

ILS = Integrated Laboratory Systems, Inc.

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

NIEHS = National Institute of Environmental Health Sciences

NLM = National Library of Medicine

OPP = Office of Pesticide Programs

OTWG = Ocular Toxicity Working Group

USDA = U.S. Department of Agriculture

TUESDAY, MAY 19, 2009

Call to Order and Introductions

Dr. Hayes (Peer Review Panel Chair) called the meeting to order at 8:30 a.m. and introduced himself. He then asked all Peer Review Panel (Panel) members to introduce themselves and to state their name and affiliation for the record. He then asked all the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) staff, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) members, the ICCVAM Ocular Toxicity Working Group (OTWG) members, the European Centre for the Validation of Alternative Methods (ECVAM) staff person, and members of the public to introduce themselves. Dr. Hayes stated that there would be opportunities for public comments during the discussions associated with each of the ten test method topics. He asked that those individuals interested in making a comment register at the registration table and provide a written copy of their comments, if available, to NICEATM staff. Dr. Hayes emphasized that the comments would be limited to seven minutes per individual per public comment session, and that, while an individual would be welcome to make comments during each commenting period, repeating the same comments at each comment period would be inappropriate. He further stated that the meeting was being recorded and that Panel members should speak directly into the microphone.

Welcome from the ICCVAM Chair

Dr. Wind, U.S. Consumer Product Safety Commission (CPSC) and Chair of ICCVAM, welcomed everyone to CPSC and to the Panel meeting. Dr. Wind stressed the importance of this Panel's efforts, especially considering the public health importance of ocular safety testing and hazard labeling. Dr. Wind noted that approximately 125,000 home eye injuries occur each year and over 2,000 workers suffer eye injuries each day, many of which are caused by accidental exposure to chemicals or chemical products. Dr. Wind also reviewed the statutes and regulations requiring ocular testing.

Dr. Wind thanked the Panel members for giving their expertise, time, and effort and acknowledged their important role in the ICCVAM test method evaluation process. Dr. Wind also emphasized the importance of public comments that are considered by the Panel in this process and the Panel's role in the development of ICCVAM final test method recommendations.

Welcome from the Director of NICEATM, and Conflict-of-Interest Statements

Dr. Stokes, Director of NICEATM, stated the Panel meeting was being convened as a National Institutes of Health (NIH) Special Emphasis Panel and was being held in accordance with applicable U.S. Federal Advisory Committee Act regulations. As such, Dr. Stokes indicated that he would serve as the Designated Federal Official for this public meeting. He reminded the Panelists that, when they were originally selected, they had signed conflict-of-interest statements in which they identified any potential conflicts of interest. He then read the conflict-of-interest statement and again asked members of the Panel to identify any potential conflicts for the record. Dr. Hayes asked the Panel members to declare any direct or indirect conflicts based on Dr. Stokes' statements and to recuse themselves from voting on any aspect of the meeting where these conflicts were relevant.

Dr. Sawyer declared a potential conflict-of-interest regarding his employment with Minrad Inc., a company that manufactures inhalation anesthetics. Dr. Ward declared a potential conflict-of-interest regarding her consulting relationship with a company that manufactures antimicrobial cleaning products. Dr. Rodeheaver indicated that she worked for Alcon, a manufacturer of the topical anesthetics proparacaine and tetracaine. Dr. Vanparys declared a potential conflict-of-interest regarding his company's involvement in the conduct of the Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) test method.

Overview of the ICCVAM Test Method Evaluation Process

Dr. Stokes opened his presentation by thanking the Panel members for their significant commitment of time and effort preparing for and attending the meeting. He noted that this is an international Panel, made up of 22 different scientists from six different countries (Belgium, Canada, The Netherlands, Japan, Spain, and the United States). He explained that the purpose of the Panel was to conduct an independent scientific peer review of the information provided on several proposed alternative ocular safety test methods, a testing strategy, and proposed refinements to the *in vivo* rabbit eye test method. This assessment is to include an evaluation of the extent that each of the established ICCVAM criteria for validation and regulatory acceptance has been appropriately addressed for each test method or testing strategy. The Panel is then asked to comment on the extent that the available information and test method performance in terms of accuracy and reliability supports the ICCVAM draft recommendations. Dr. Stokes noted that the first ICCVAM Ocular Peer Review Panel met in 2005 to evaluate the validation status of four alternative test methods (Bovine Corneal Opacity and Permeability [BCOP], Isolated Chicken Eye [ICE], Isolated Rabbit Eye [IRE], and the HET-CAM) for their ability to identify ocular corrosives or severe irritants. The Panel recommended two of these test methods (BCOP and ICE) on a case-by-case basis for use in a tiered-testing strategy with test method-specific applicability domain restrictions. ICCVAM and the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) endorsed the Panel's recommended use for these test methods. The Panel also recommended that, while the IRE and HET-CAM test methods were potentially useful in a tiered-testing strategy with appropriate restrictions, additional data were needed to fully assess their usefulness and limitations for regulatory testing. ICCVAM prepared a test method evaluation report (TMER) and provided a transmittal package (i.e., Panel report, SACATM and public comments, TMER and associated materials) to the ICCVAM Federal agencies for their response as required by the ICCVAM Authorization Act of 2000 (ICCVAM 2000). All Federal agencies with ocular testing requirements endorsed the BCOP and ICE test method recommendations. Dr. Stokes noted that five Panel members from the 2005 review are on the current Panel (i.e., Drs. Henry Edelhauser, A. Wallace Hayes, Robert Peiffer, Scheffer Tseng, and Philippe Vanparys).

Dr. Stokes then provided a brief overview of ICCVAM and NICEATM, and identified the 15 Federal agencies that comprise ICCVAM. He summarized the purpose and duties of ICCVAM (as described in the ICCVAM Authorization Act of 2000[2]), noting that ICCVAM, as an interagency committee, does not carry out research and development or validation studies. Instead, ICCVAM, in conjunction with NICEATM, carries out critical scientific evaluations of the results of validation studies for proposed test methods to assess their usefulness and limitations for regulatory testing, and then makes formal recommendations to ICCVAM agencies.

Dr. Stokes then described the ICCVAM test method evaluation process, emphasizing the many opportunities for stakeholder input during numerous public comment periods.

As part of this process, a working group of Federal scientists designated for the relevant toxicity testing area (e.g., the OTWG) and NICEATM prepare a draft background review document (BRD) that provides a comprehensive review of all available data and information. ICCVAM considers all of this available data and information and then develops draft test method recommendations on the proposed usefulness and limitations of the test methods, test method protocol, performance standards, and future studies. The draft BRD and the ICCVAM draft test method recommendations are made available to the Panel and the public for review and comment. The Panel reviews the draft BRD and evaluates the extent to which the established ICCVAM validation and regulatory acceptance criteria have been adequately addressed and the extent that the demonstrated accuracy and reliability support the ICCVAM draft test method recommendations. A Panel report is published and then considered, along with public and SACATM comments, by ICCVAM in developing final recommendations.

[2] http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf

ICCVAM forwards these final recommendations to the ICCVAM member agencies for their consideration and possible incorporation into relevant testing guidelines.

He concluded by summarizing the timeline for 2009 for the ICCVAM evaluation and peer review of the ocular test methods and approaches, including a *Federal Register* notice in March announcing the Panel meeting, the projected publication of the Panel report in July, and transmittal of ICCVAM final recommendations to Federal agencies in November.

ICCVAM Charge to the Panel

Dr. Stokes reviewed the charge to the Panel:

(1) Review the ICCVAM draft BRDs for completeness and identify any errors or omissions (e.g., other relevant publications or available data).

(2) Evaluate the information in the draft BRDs to determine the extent to which each of the applicable ICCVAM criteria for validation and regulatory acceptance of toxicological test methods have been appropriately addressed.

(3) Consider the ICCVAM draft test method recommendations for the following and comment on the extent to which they are supported by the information provided in the BRDs: proposed test method usefulness and limitations, proposed recommended standardized protocols, proposed test method performance standards, and proposed future studies.

Dr. Stokes thanked the OTWG and ICCVAM for their contributions to this project and acknowledged the contributions from the participating liaisons from ECVAM, the Japanese Center for the Validation of Alternative Methods (JaCVAM), and Health Canada. He also acknowledged the NICEATM staff for their support and assistance in organizing the Panel meeting and preparing the review materials.

Overview of the Agenda

Dr. Hayes outlined the process for reviewing each of the topics. First, the test method developer or other expert will describe the test method protocol and procedures, followed by a presentation summarizing the test method validation database and test method performance for each draft BRD or summary review document (SRD) given by a member of the NICEATM staff. An ICCVAM OTWG member will then present the ICCVAM draft test method recommendations. Following presentations, the Evaluation Group Chair responsible for the topic under consideration will present the Evaluation Group's draft recommendations and conclusions followed by Panel discussion. Public comments will then be presented followed by the opportunity for questions to the public commenters and additional Panel discussion. After consideration of the public comments, the Panel will then vote to accept the Panel consensus, with any minority opinions being so noted with a rationale for the minority opinion provided.

Draize Rabbit Eye Test and Current Ocular Regulatory Testing Requirements and Hazard Classification Schemes

Ms. McCall of the U.S. Environmental Protection Agency (EPA) presented the relevant U.S. and international statutes and regulations for ocular safety testing (e.g., EPA, CPSC, Food and Drug Administration [FDA], Occupational Safety and Health Administration [OSHA], European Union [EU], and Organisation for Economic Co-operation and Development [OECD]). She summarized the Draize scoring system for corneal, iridal, and conjunctival lesions in the rabbit, using representative photographs for reference. She also discussed optional but potentially useful assessments of ocular injury (e.g., fluorescein staining, corneal thickness, depth of corneal injury, photographic documentation, and histopathology) that are not routinely included in the Draize eye test. Ms. McCall then provided an overview of the various U.S. and international hazard classification schemes for ocular corrosivity and irritation (i.e., EPA, EU, Globally Harmonized System of Classification and

Labelling of Chemicals [GHS], and Federal Hazardous Substances Act [FHSA]). She noted that, based on the recently adopted European Union Regulation on the Classification, Labelling and Packaging of Substances and Mixtures (i.e., the CLP Regulation), the EU will move to the GHS system after December 1, 2010, for substances and after June 1, 2015, for mixtures. Ms. McCall also identified the required signal words for labeling based on each regulatory classification.

Use of Topical Anesthetics and Systemic Analgesics to Avoid or Minimize Pain and Distress in Ocular Toxicity Testing

On behalf of NICEATM, Dr. Allen reviewed the relevant sections of the draft BRD on the routine use of topical anesthetics and systemic analgesics in *in vivo* ocular irritation testing.

Dr. Merrill then presented the ICCVAM draft recommendations for the routine use of topical anesthetics and systemic analgesics in *in vivo* ocular irritation testing for the Panel to consider.

Panel Evaluation

Dr. Sawyer (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the routine use of topical anesthetics and systemic analgesics in *in vivo* ocular irritation testing and ICCVAM draft test method recommendations. Dr. Sawyer indicated that anesthetic requirements vary enormously among species. For instance, cats require approximately 40% more anesthetic than humans to achieve a similar level of anesthesia. Therefore, any protocol designed to minimize or eliminate pain needs to be individualized to the target species. The Evaluation Group proposed an alternative to the ICCVAM anesthetic/analgesic protocol to be used during all *in vivo* rabbit ocular irritation testing. Dr. Sawyer outlined the Evaluation Group's proposed protocol, which is divided into pretreatment and posttreatment regimens as follows:

Pretreatment Analgesia:
Buprenorphine 0.01 mg/kg subcutaneous (SC) (60 minutes before test substance application [TSA]). Dr. Sawyer noted that buprenorphine is classified as an opioid agonist-antagonist analgesic with a wide margin of safety in rabbits, minimal sedation, and relatively long duration. It has been found to be effective in managing pain in small animals, and is given before application of the test substance because the most effective method of managing pain and distress is to administer the analgesic preemptively to prevent establishment of central sensitization.

One or two drops of 0.5% proparacaine hydrochloride, applied to the eye three times at 5-minute intervals starting 15 minutes pre-TSA. Last application would be five minutes pre-TSA. Anticipated duration of action: 30 - 60 minutes. Dr. Sawyer stated that proparacaine is preferred because application to the eye would be less painful and the suggested application sequence is to assure effective penetration of the epithelial layer.

Eight hours post-TSA:
Buprenorphine 0.01 mg/kg SC and meloxicam 0.5 mg/kg SC. Dr. Sawyer noted that the timing is to reinforce the initial level of analgesia to carry over until the next morning (the duration of analgesia is expected to be at least 12 hours for buprenorphine and at least 24 hours for meloxicam). The combination of an opioid and a nonsteroidal anti-inflammatory drug (NSAID) such as meloxicam is a well-tested approach to balanced analgesia. Used for post-operative or chronic pain in dogs since 1997, meloxicam has been found to have effective application in rabbits.

Day two through day seven post-TSA:
Buprenorphine 0.01 mg/kg SC every 12 hours and meloxicam 0.5 mg/kg SC every 24 hours. Dr. Sawyer noted that buprenorphine and meloxicam should be continued for seven days post-TSA unless signs of ocular injury sufficient to cause pain and discomfort appear. If so, this systemic analgesic protocol would continue until the test is completed.

Rescue Analgesia:
Dr. Sawyer also outlined a procedure where, if a test subject shows signs of physical pain or discomfort during the test interval using the above protocol, a rescue dose of buprenorphine at 0.03 mg/kg SC could be given as needed every eight hours instead of 0.01 mg/kg SC every 12 hours. Meloxicam would continue with the same dose and interval.

Dr. Sawyer pointed out that buprenorphine and meloxicam were synergistic and have an excellent safety profile in clinical practice. A question was raised concerning the interval of dosing throughout the test period and the burden that it would impose on the testing laboratory. The Panel agreed that a ±30-minute interval is appropriate for the administration of the systemic analgesics.

Dr. Dubielzig indicated that the impact of the NSAID on inflammatory aspects of the Draize rabbit eye test is unknown, but the Panel did not consider such affects to be limited and therefore not likely to be a problem. Dr. Jester questioned the need to continue analgesic treatment through day seven when Category III or IV substances would have cleared by day three. He suggested an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approach where treatment is continued through day four. Dr. Peiffer suggested that the temporal aspect be removed and that treatment be continued only if there are signs of discomfort. **The Panel agreed that treatment should be stopped after day four (instead of day 7, as suggested above) if there are no signs of discomfort**. The Panel agreed that pain assessment should be made and recorded daily.

Dr. Jester raised a concern that the use of preservatives in the topical anesthetics may interfere with the irritation response. The Panel agreed that the use of preservative-free proparacaine should be required. Dr. Stokes asked how long after the administration of the systemic analgesics a rescue dose can be administered. Dr. Sawyer indicated that, due to the wide margin of safety, the rescue dose can be given immediately afterward if necessary.

Dr. Jester expressed concern that dilution of the test substance could occur if a significant amount of liquid anesthetic remained in the eye. Dr. Peiffer indicated that, in his experience, the 5-minute interval is reasonable and should not pose a problem for test substance dilution.

In response to the evaluation guidance question specific to testing situations where the use of topical anesthetics would be considered inappropriate, the Panel indicated that drugs to be used for ocular effects, such as eye drops, need to be tested by other means. However, the focus of this evaluation is eye irritation hazard classification; therefore, the proposal would be relevant to all such testing. The Panel did not know of additional systemic analgesics that might have greater efficacy in relieving ophthalmic pain associated with chemically-induced injuries. The Panel also agreed that there were no additional pain-related chemically-induced injuries to the eye that the proposed alternate analgesic proposal would not adequately address.

The Panel expressed general concern about the use of transdermal patches to deliver anesthetics due to the need for shaving prior to patch application and the possibility of skin irritation. In addition, with multiple applications, the availability of irritation-free skin sites may pose a problem. Most importantly, analgesic patches have proven to be unreliable in clinical practice with significant animal-to-animal variation as well as species-to-species variation when comparing effectiveness and duration of effect. The Panel also indicated a greater concern about self-mutilation due to severe pain during eye irritation testing than about the potential for the systemic analgesics to alter the ocular injury response. Dr. Jester indicated that there was insufficient information in the BRD to make this assessment.

The majority of the Panel agreed that the tetracaine information provided in the ICCVAM BRD could be applied to other topical anesthetics such as proparacaine. Dr. Ward indicated that additional studies on cell proliferation, migration, and cytotoxicity could be done with topical anesthetics to provide some assurance that they behave in a manner similar to tetracaine. Although it was previously noted

that anesthetic/analgesic use was for all *in vivo* eye irritation tests, the Panel indicated that administration of post-application analgesics is not a concern if a standard dosing regimen is used throughout and not adjusted for each animal to avoid overdosing side effects.

The Panel also agreed that the clinical signs of post-application pain and distress are adequately described and that no other clinical signs should be added. In the event of an eye infection, the Panel agreed that secondary treatment should be considered, the signs and symptoms of the eye infection should be documented, and the animal should be immediately removed from the study. Finally, the Panel agreed that all relevant data had been adequately considered in the BRD.

The Panel considered its proposal to be more appropriate than the ICCVAM-proposed recommendations in terms of the type and frequency of dosing for topical anesthetics and systemic analgesics. The Panel agreed with the ICCVAM draft recommendations for future studies. Therefore, it recommended refinement of the current *in vivo* test system to evaluate ocular irritation utilizing contemporary/novel technologies to address both concerns. The Panel recommended the following:

- New animal studies should only be considered when absolutely necessary in developing new strategies for testing.
- Products that are overpredicted when anesthetic and analgesic pretreatment is used should be identified.
- Animal responses should be collected in tests currently being conducted to determine whether refinements are warranted in the dosing and timing of anesthetic, analgesic, and antibiotic treatments.
- Rabbit ocular specimens should be submitted for histopathological evaluation to develop an archive of specimens.
- Digital photographs of lesions/observations should be collected.
- Analysis of the variability in rabbit wound-healing responses would help determine whether or not it is due to variability in the ocular defense linking to the neuroanatomic integration.
- Studies should be conducted to determine whether the timing and dosing of systemic analgesics with topical anesthetics might alter the ocular defense enough to change the classification of test substances.
- Cytology samples from the surface of the eye should be collected.
- Studies should be conducted to investigate the appropriateness of using proparacaine instead of tetracaine.
- Studies should be conducted to evaluate the impact of using the NSAID meloxicam with buprenorphine.
- New technologies (e.g., new imaging modalities and quantitative/mechanistic endpoints) should be incorporated into the Draize rabbit eye test, refining/changing it to make it a more humane test that is also more reliable.

Public Comments

No public comments were made.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention,

Dr. Rodeheaver, who cited a potential conflict-of-interest due to her employment by a manufacturer of anesthetic products.

Use of Humane Endpoints in *In Vivo* Ocular Irritation Testing

On behalf of NICEATM, Dr. Allen reviewed the relevant sections of the draft BRD on the use of humane endpoints in *in vivo* ocular irritation testing for the Panel.

Dr. Merrill then presented the ICCVAM draft recommendations for the use of humane endpoints in *in vivo* ocular irritation testing for the Panel to consider.

Panel Evaluation

Dr. Sawyer (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the use of humane endpoints in *in vivo* ocular irritation testing and ICCVAM draft test method recommendations. The Panel agreed that each of the current and proposed humane endpoints detailed in the BRD are sufficiently predictive of irreversible or severe effects (i.e., GHS Category 1, U.S. EPA Category I, EU R41) that they should be used routinely as humane endpoints to terminate a study as soon as they are observed. The Panel also agreed that animals should be observed at least once per day (at least twice daily for the first three days) to ensure that termination decisions are made in a timely manner. The Panel agreed that there was insufficient data in the BRD to determine the adequacy of pannus as a recommended humane endpoint. The Panel also agreed that the use of fluorescein staining was an appropriate technique for evaluating eye injury; however, the technique needs to be better described before a reasonable conclusion regarding its value can be made. Dr. Jester suggested that the use of fluorescein staining had not been adequately discussed in this BRD.

The Panel emphasized that, in some cases, decisions to terminate a study should be based on more than one endpoint. Very severe endpoints (e.g., corneal perforation) would be adequate alone to terminate a study. Other biomarkers considered useful by the Panel as routine humane endpoints included extent of epithelial loss, limbal ischemia, and/or stromal loss, and depth of corneal damage.

In response to the question regarding other earlier biomarkers/criteria indicative that painful lesions can be expected to fully reverse, the Panel indicated eyes with conjunctival scores without corneal/iris scores would be expected to recover. The Panel indicated that the destruction of 50% of the limbus will result in pannus in rabbits and, therefore, the ICCVAM draft recommendation requiring 75% for early termination may be excessive. In addition, the Panel indicated that the humane endpoints described in the BRD were sufficient to ensure that the lesions would not reverse. The Panel did agree that the available data and information supported the ICCVAM draft recommendations on humane endpoints. The Panel recommended that studies be developed to identify better and earlier endpoints, such as those seen with fluorescein staining, and that these endpoints should be incorporated into current testing guidelines.

Public Comments

No public comments were made.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

Adjournment

Dr. Hayes adjourned the Panel for the day at 5:45 p.m., to reconvene at 8:30 a.m. on Wednesday, May 20, 2009.

WEDNESDAY, MAY 20, 2009

Dr. Hayes called the meeting to order at 8:28 a.m. and asked Dr. Stokes to discuss the conflict-of-interest for the day's planned topics. Dr. Stokes read the conflict-of-interest statement and Dr. Hayes asked the Panel to declare any conflicts-of-interest. The conflicts-of-interest declared by Panel members on day one of the meeting were repeated.

Dr. Hayes then asked for introductions from the Panel, NICEATM staff, members of ICCVAM and the OTWG, and those in attendance for the public session.

HET-CAM Test Method

Dr. Schrage reviewed the various HET-CAM test method protocols (i.e., IS[A], IS[B], S-Score, Q-Score, and IT) and BASF experience with the test method. Dr. Schrage stressed the need for harmonization of HET-CAM protocols, endpoints, and scoring methods. BASF has conducted a retrospective review of 145 test substances, including a broad variety of chemicals and formulations, which revealed that overall accuracy, false positive rates, and false negative rates were not acceptable. The specificity and sensitivity were especially affected by solubility in both water and oil. These data were submitted to the journal Alternatives to Laboratory Animals in April 2009. Dr. Schrage said she would be willing to share the HET-CAM data on these 145 substances with NICEATM following publication.

Dr. Vanparys said that he would be willing to provide NICEATM with HET-CAM data using the IS(B) analysis method to determine if conversion to the IS(A) method was feasible. He added that, in his experience, the HET-CAM test method can be sensitive for the identification of substances not labeled as irritants.

On behalf of NICEATM, Dr. Allen reviewed the HET-CAM draft BRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the HET-CAM test method for the Panel to consider.

Panel Evaluation

Dr. Wilson (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the HET-CAM test method and ICCVAM draft test method recommendations. He noted that HET-CAM classified four EPA Category III substances incorrectly as Category IV (i.e., they were false negative in HET-CAM). However, he said that regulators would be more concerned if the false negative substances were EPA Category I or Category II. Some Panelists did not consider these substances likely to be a significant risk. Dr. Stokes suggested adding a statement defining an acceptable rate for false positives and false negatives. Dr. Wilson expressed concern that, while three of the four animals had an EPA Category III classification that cleared in seven days, one animal had a conjunctival redness score of two that cleared to one in seven days but required 14 days to completely resolve (i.e., return to a score of zero). Such lesions would not be considered inconsequential.

The Panel discussed the low number of mild and moderate substances used in the performance analyses, and that additional substances in these categories would be needed before a conclusion on the usefulness of HET-CAM could definitively be reached. The Panel also recognized that the validation database does not include substances currently regulated by EPA and that collection of additional data is needed. Therefore, given the limited data for mild and moderate substances, the Panel did not support the ICCVAM draft test method recommendation for use of the HET-CAM to identify substances not labeled as irritants from all other classes.

Dr. Peiffer said that he was concerned with the recommendation to test increasing concentrations of test substances. He stated that while dose-response curves are preferred for scientific studies, they are

not practical for regulatory testing. Dr. Sawyer agreed that increasing concentrations should not be a requirement. Ms. McLaughlin argued that use of different concentrations allows the investigator to see if increasing the concentration affects the outcome. She stated that poor predictivity might result from use of a concentration that produces an ineffectual or weak response, whereas the comparative effect of a higher concentration would provide useful information. The Panel agreed to remove the concentration requirement from the test method protocol but to include it as a general recommendation for additional research.

Ms. McLaughlin offered a minority opinion with respect to the Panel's recommendation on the use of the HET-CAM test method to identify substances not labeled as irritants from all other classes. Ms. McLaughlin stressed that personal care products are not regulated in the U.S. as they are in Europe and Canada. Ms. McLaughlin stated that the HET-CAM test method could be used as an alternative to the Draize rabbit eye test to evaluate personal care products in situations where they are regulated. Dr. Hayes asked Ms. McLaughlin to write a short paragraph to note the rationale for her opposition to the majority view for inclusion in the Panel report. Ms. McLaughlin drafted the following text:

Based on the demonstrated performance as outlined in the ICCVAM draft recommendations, HET-CAM can be used to screen not labeled as irritants from other irritant categories for the restricted applicability domain (surfactant-based formulations and oil/water emulsions). The rationale for this dissenting view is based on the fact that there were 60 substances in the overall database. The hazard category distribution was: 25 Category I; 2 Category II; 18 Category III; and 15 Category IV. The sensitivity of HET-CAM is 91% (41/45), resulting in a false negative rate of 9% (4/45). Among the four false negatives for the EPA system, 100% (4/4, all oil/water emulsion cosmetic formulations) were EPA Category III substances based on conjunctival redness score of two that required at least three days to resolve. The lesions noted *in vivo* indicated mild ocular irritation and are unlikely to represent a significant hazard. As such, the HET-CAM could be considered useful as a screening test for EPA Category IV substances not labeled as irritants from all other categories for the restricted applicability domain of surfactant-based formulations and oil/water emulsions. The sensitivity for GHS and EU was high enough for each system to warrant HET-CAM test method use (i.e., 100% sensitivity; 31/31 and 26/26, respectively for GHS and EU [from the ICCVAM draft BRD, Tables 6-2 and 6-12]) also with domain restriction. This performance demonstrates that HET-CAM could be used to screen EU or GHS hazard not labeled as irritant classifications from other irritant categories for the restricted applicability domain of surfactant-based formulations and oil/water emulsions. It should be noted that, for regulatory purposes, sensitivity (the proportion of all positive substances that are classified as positive) is most important from a public health perspective and the HET-CAM performed well in this regard.

The Panel discussed the ICCVAM draft recommended protocol for the HET-CAM test method. Dr. Vinardell said that she would like to see a statement added to the protocol to wash out any leftover solids after 30 seconds (as currently recommended in the EU Annex V). Dr. Hayes asked Dr. Vinardell to provide a statement for Dr. Wilson to include in the Panel report.

The Panel discussed the HET-CAM test method performance. One Panelist suggested that a Chi-square analysis should be included to ensure that differences in classification were statistically significant. Dr. Ahn was asked if a power analysis could be used to determine if the number of substances in the mild and moderate classification was adequate to differentiate the irritant classifications. Dr. Ahn said that there should be at least three substances in each classification category to conduct a power analysis.

The Panel discussed the need for Good Laboratory Practice (GLP) studies. Dr. Hayes emphasized that a study is either GLP compliant or it is not. He said that the phrase "spirit of GLP" should not be used in the Panel report. He also said that the term "original data" should be used rather than "raw data."

The Panel agreed that data from studies not conducted under GLP guidelines could be used to increase knowledge about the applicability domain of a test method but that laboratories should provide sufficient detail about the conduct of the study to understand any deviations from GLP guidelines.

The Panel discussed additional sources of HET-CAM data to expand the applicability domain and the number of mild and moderate substances tested. Dr. Allen noted that Dr. Debbasch, a principal contact for data acquisition, had left L'Oreal. Dr. Hayes said that *cosmeceuticals* represented a gray zone between cosmetics and personal-care formulations, and this class of products should be considered. Ms. McLaughlin said that the inclusion of a single ingredient (e.g., a UV-blocking material) could change the regulatory requirements for a formulation from an unregulated personal care product to a regulated material in Canada. She said that the applicability domain and database used in the ICCVAM draft BRD should be adequate to warrant use of the HET-CAM test method for personal care products that are not labeled as irritants. The Panel did not support the use of additional studies to identify the full range of irritation but supported additional studies to identify substances not labeled as irritants from all other classifications.

Public Comments

Dr. Barroso from ECVAM commented that the false negatives using the EPA classification system, which are substances not labeled as irritants using the GHS classification system, result because the EPA classification system categorizes substances based upon the most severe category observed among the test rabbits (i.e., not based on the majority classification among rabbits tested). Dr. Barroso also said that because the types of formulations regulated by EPA are not present in the database that the EPA classification system should not be given too much weight.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted to approve the recommendations as revised during the discussion with one minority opinion, Ms. McLaughlin, and one abstention, Dr. Vanparys, who cited a potential conflict-of-interest with the HET-CAM test method, which he had worked on at Johnson & Johnson.

Isolated Chicken Eye Test Method

On behalf of NICEATM, Dr. Allen presented an overview of the ICE test method protocol and reviewed the ICE draft BRD. One Panelist asked why the test method was limited to three eyes. Dr. Allen explained that the incubation apparatus contained 10 chambers, sufficient for three groups of three eyes and a negative control. However, the ICCVAM ICE test method protocol, upon which the recently submitted OECD Test Guideline is based, includes both positive and negative controls.

Dr. Jester said that the term fluorescein *staining* should be used rather than *retention*. He also asked how the EPA classification categories were determined using the ICE test method. Dr. Allen replied that the four-tiered EPA classification system was considered equivalent to the four-tiered GHS system and used the same ICE test method decision criteria (e.g., EPA Category I – GHS Category 1, EPA Category II = GHS Category 2A, EPA Category III = GHS Category 2B, EPA Category IV = GHS Category Not labeled).

Dr. Yu asked if the evaluation of the eyes was subjective and whether photographs were taken. Dr. Allen said that the evaluation of the eyes for corneal lesions was subjective, except for the measurement of corneal swelling, which is measured quantitatively using a pachymeter. He said that photographs were not typically taken but were recommended by the previous ocular Panel.

Dr. Merrill then presented the ICCVAM draft recommendations for the ICE test method for the Panel to consider.

Panel Evaluation

Dr. Tarlo (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the ICE test method and ICCVAM draft test method recommendations. The Panel agreed that the available data and test method performance supported the ICCVAM draft recommendations that the ICE test method is not recommended to identify substances from all hazard categories as defined by GHS, EPA, and EU classification systems. The Panel further agreed that the ICE test method is not recommended as a screening test to identify substances not labeled as irritants from all other hazard classifications defined by GHS, EPA, and EU, because one of the false negatives included a GHS Category 1 substance. The Panel agreed with the ICCVAM draft recommendation that the ICE test method should not be used as a screening test to identify GHS substances not labeled as irritants. Dr. van der Valk noted that the ICE test method is used by the Netherlands Organisation for Applied Scientific Research (TNO) to obtain good results, but the results obtained by other laboratories using the ICE test method in the validation study were variable. Dr. Vanparys recommended that the source of the variability be noted in the appropriate text.

The Panel agreed that the available data supported the ICCVAM draft recommendations that the proposed standardized protocol appeared acceptable. However, the Panel suggested that the protocol could be improved by adding objective endpoints for corneal opacity and fluorescein staining. The Panel also added that inclusion of a histopathological evaluation might improve ICE test method performance.

The Panel agreed with the ICCVAM draft recommendations for the ICE test method in terms of the proposed future studies that additional optimization studies would be required to validate the test method for the identification of all ocular irritancy hazard categories. The use of histopathology evaluation might add to the accuracy and determination of the test. The Panel also agreed with ICCVAM that the ICE test method performance standards are not warranted at this time.

Public Comments

Dr. Barroso said that variability of the ICE test method was similar to that of the Draize rabbit eye test because of the subjective assessments. He stated that the ICE test method should not be held to a higher standard than the Draize test. He also noted that the concordance among laboratories was reasonable.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

Isolated Rabbit Eye (IRE) Test Method

On behalf of NICEATM, Dr. Allen presented an overview of the IRE test method and reviewed the IRE draft BRD. Dr. Hayes asked whether the rabbits used by GlaxoSmithKline (GSK) were from PelFreeze Biologicals or if fresh eyes were used for each test. Dr. Allen replied that at least some of the rabbits were obtained from other GSK laboratories and had been used as negative controls from other acute safety testing. Dr. Ward noted that PelFreeze ships rabbit eyes from its facility in Rogers, Arkansas, adding that their rabbits are used for multiple purposes. She was not aware of a formal study to determine the acceptability of eyes shipped from the U.S. to Europe. Dr. Peiffer suggested

that shipped eyes should be carefully examined prior to use. Dr. Jester said that his laboratory has compared eyes obtained from an abattoir to fresh eyes and found no significant differences.

Dr. Merrill then presented the ICCVAM draft recommendations for the IRE test method for the Panel to consider.

Panel Evaluation

Dr. Tarlo (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the IRE test method and ICCVAM draft test method recommendations. The Panel agreed with ICCVAM that additional optimization and validation studies using a protocol that includes all four recommended endpoints are needed to further evaluate the relevance and reliability of the IRE test method and to develop more definitive recommendations.

The Panel recommended that the planned validation study with GSK/SafePharm include an evaluation of fresh versus shipped eyes. In general, the Panel felt there should be rigid criteria on the handling and storage of the eyes. Finally, the Panel recommended that criteria on test article administration/washout (e.g., viscous substances) were warranted.

Public Comments

No public comments were made.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

Bovine Corneal Opacity and Permeability Test Method (BCOP)

Dr. Curren, Institute for In Vitro Sciences, provided an overview of the BCOP test method. He noted that Pierre Gautheron and his colleagues initially developed the test method for occupational safety. Dr. Curren said that as many as 30% of bovine eyes are rejected upon inspection because of scratches and other defects, and emphasized the importance of including concurrent positive and negative controls in each study. With respect to histopathology evaluation, he said that it was important to carefully choose a qualified laboratory because of the impact of quality on the evaluation.

Dr. Vanparys pointed out that the 15x OD_{490} value in the *In Vitro* Score calculation was chosen to equate the data to *in vivo* data. One Panel member asked if there was an equilibration period, and Dr. Curren indicated that the bovine corneas were equilibrated for one hour before dosing.

Dr. Bailey asked if there was an example for when histopathology evaluation should be recommended based on effects associated with a particular chemical class. Dr. Curren cited as an example oxidizers, which may not produce opacity or permeability changes, but still produce substantive corneal damage that is observable only by histopathology. A Panel member asked why corneal thickness was not measured to provide a quantitative endpoint. Dr. Curren said that corneal thickness has been evaluated, but is less reliable than the opacity and permeability measurements and therefore is not measured in the current protocol.

Dr. Peiffer asked how the BCOP decision criteria for histopathology evaluation are applied to the EPA categorization scheme. Dr. Curren replied that a substance labeled as EPA Category IV would not penetrate further than the superficial corneal epithelium, whereas a Category III substance would penetrate to the basal layer, a Category II substance into the top third of the stroma, and a Category I substance into the bottom third of the stroma or to the endothelium. Minimal damage to the epithelium heals quickly, moderate damage heals more slowly, and significant damage (e.g., deep stromal or endothelial penetration) may be irreversible.

On behalf of NICEATM, Dr. Hamm reviewed the BCOP draft BRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the BCOP test method for the Panel to consider.

Panel Evaluation

Dr. Tarlo (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the BCOP test method and ICCVAM draft test method recommendations. With respect to the substances used in the validation studies, the Panel requested additional chemical classes be added as data becomes available to provide a more significant statistical inference. The Panel requested that Drs. Ahn and Palmer conduct a power analysis to determine the number of substances needed in each hazard classification to provide statistical significance.

The Panel discussed the performance of the BCOP test method to identify the intended range of classification categories. The Panel indicated that the available data and analyses were adequate for the intended purpose. The Panel indicated that all available and relevant data had been used in the ICCVAM BCOP test method analyses.

The Panel agreed with ICCVAM that the test method performance supported the ICCVAM draft recommendations. Accordingly, the BCOP test method was not recommended to identify substances from all hazard categories as defined by GHS, EPA, and EU classification systems. However, the BCOP test method can be used as a screening test to distinguish substances not labeled as irritants from all other hazard categories when results are to be used for EU or GHS hazard classifications. Because of the significant lesions associated with 50% (4/8) of the EPA Category III substances that tested as false negatives, the BCOP test method cannot be recommended as a screening test to identify EPA Category IV substances.

The Panel agreed with the ICCVAM draft recommendation that the BCOP test method could be used to distinguish substances not labeled as irritants from all other irritant classes, because the false negative rate for the EU and GHS systems was 0% (0/54 or 0/97, respectively). By comparison, the false negative rate was 6% (8/141) for the EPA system. Among the eight false negatives for the EPA system, 100% (8/8) were EPA Category III substances based on Draize rabbit eye test data.

The Panel said that, while the BCOP test method is unable to identify all irritant classifications, further test method development and refinement in future studies was encouraged.

The Panel recommended that performance standards should be developed, because the BCOP test method is now being considered as a screening test for both ocular corrosives/severe irritants and for the identification of substances not labeled as irritants.

Public Comments

Dr. Curren said that, based on his experience with the BCOP test method, performance of the BCOP for the four hazard classification systems was unlikely to improve based on the lack of Draize rabbit eye test reproducibility in the mild and moderate categories. He said that results from Weil and Scala (1971) show that the extremes are reproducible, but the mild and moderate levels of ocular irritation are highly variable. He referenced the antimicrobial cleaning products (AMCP) BRD that includes an analysis of the impact on the ocular hazard category when the results of a six-rabbit Draize test are randomly sampled for a three-rabbit test.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Harbell, Mary Kay Inc., said that his laboratories have used over 30,000 bovine eyes that were kept cold at 4ºC. He added that damaged eyes are quickly removed and excluded from the test. He pointed out that Gautheron et al. (1992) used both fresh eyes and eyes maintained at 4ºC and found no differences in their test method results. Dr. Harbell emphasized the utility of the BCOP in comparison to the other methods being considered given its focus on quantitative measurements.

Dr. Harbell also asked the Panel to consider how histopathology evaluation might contribute to the BCOP test method performance. He said that the experts at the 2005 ICCVAM workshop considered the depth of injury to be an important consideration in the assessment of ocular injury. The purpose of including histopathology evaluation is to evaluate the depth of injury that may not be visible to the naked eye. Dr. Harbell cited the example of oxidizing chemicals that may not affect the opacity or permeability of bovine eyes but do still damage the corneal tissue. Therefore, for these substances, depth-of-injury analysis may be important to differentiate corrosives or severe irritants from moderate irritants. Dr. Harbell said he would like to see histopathology evaluation reconsidered. Dr. Ward asked if he was recommending histopathology evaluation for all classes. Dr. Harbell said that he was but that it would be used primarily for EPA Categories I and II.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Barroso commented on what he referred to as the "top-down" (i.e., screening for corrosives/severe irritants) and "bottom-up" (i.e., screening for substances not labeled as irritants) approaches using the ICE and BCOP test methods. ECVAM is developing a paper to recommend the use of these proposed testing strategies for both ICE and BCOP, where substances could be tested in the BCOP or ICE test methods in order to identify corrosives/severe irritants or substances not labeled as irritants without using an animal test.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion (pending the results of a power analysis by Dr. Ahn) with one abstention, Dr. Vanparys, who cited a potential conflict-of-interest with the BCOP test method, which he had worked on at Johnson & Johnson.

Adjournment

After the discussion, Dr. Hayes adjourned the Panel for the day at 7:25 p.m., to reconvene at 8:30 a.m. on Thursday, May 21, 2009.

THURSDAY, MAY 21, 2009

Dr. Hayes convened the Panel at 8:30 a.m. and asked Dr. Stokes to discuss the conflict-of-interest for the day's planned topics. Dr. Stokes read the conflict-of-interest statement and Dr. Hayes asked the Panel to declare any conflicts-of-interest. The conflicts-of-interest declared by Panel members on day one of the meeting were repeated.

Dr. Hayes then asked for introductions from the Panel, NICEATM staff, members of ICCVAM and the OTWG, and those in attendance for the public session.

The first order of business was to address issues from the preceding day.

BCOP Power Calculation

Dr. Ahn reported on the power calculation requested on Wednesday May 20, 2009, for the BCOP test method. He determined that, for each of the four hazard classification systems, a sample size of 13 substances in each chemical class represented (i.e., 13 x 4 for each chemical class for a four-category hazard classification system) is required to achieve 80% power using a two-group normal approximation test for proportions with a one-sided 0.05 significance level. This is necessary to reject the null hypothesis that the BCOP test is inferior to the Draize rabbit eye test (the accuracy of the BCOP test is more than 0.1 less than that of the Draize test) in favor of the alternative hypothesis that the accuracies in the two groups are equivalent. Dr. Ahn also noted that his analysis included the assumption that the expected accuracy of the BCOP test is 0.6 and the expected accuracy of the Draize rabbit eye test is 0.9.

The Panel voted unanimously to include the recommendation that a sample size of 13 be used for each chemical class in each of the four hazard classifications to achieve statistical significance.

ICE Test Method False Negative Substances

Dr. Vanparys commented on the ability of the ICE test method to identify GHS substances not labeled as irritants. Dr. Vanparys indicated that the false negative substances listed in the ICCVAM BRD were either paints that stick to the cornea or solids, which are known to give inaccurate results with the ICE test method. Dr. Vanparys suggested that the ICE test method is capable of identifying GHS substances not labeled as irritants with the exception of solids and substances that stick to the cornea. The overall Panel recommendations, as stated the previous day, remained unchanged.

Low Volume Eye Test (LVET) Test Method

On behalf of NICEATM, Dr. Allen provided a brief overview of the LVET test method and reviewed the LVET draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the LVET for the Panel to consider.

Panel Evaluation

Dr. Sawyer (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the LVET and ICCVAM draft test method recommendations. The Panel noted that the LVET has been used on a wide range of substances and that it does detect the full range of ocular irritancy, but recognized that the majority of the LVET database was for surfactants and surfactant-containing products. The Panel identified several references that should be added to the SRD and noted the need to review the ECVAM BRD. If any additional historical data were obtained, there might be sufficient data to determine the performance of the LVET on several other chemical classes.

The Panel indicated that pain associated with direct application of the test substance to the cornea should not be an issue in light of the recommendations for topical anesthetic and systemic analgesic use.

When discussing the performance of the LVET compared to the Draize test, the Panel indicated that the evaluation was adequate, noting that the LVET appeared to overpredict the human response to a lesser degree than the Draize rabbit eye test. They also recommended that the full range of irritation categories are represented in the LVET validation database.

In considering whether all available data had been made available, the Panel indicated that all data had not been evaluated. Additional published sources should be considered as well as the ECVAM BRD, on which the Panel was unable to comment during this meeting. The Panel stated that in the absence of all existing data, including a background review document prepared by the European Centre for the Validation of Alternative Methods, it could not make definitive conclusions or recommendations on the validation status of the LVET. Nonetheless, the Panel did consider the limited data that are available for the LVET to support the use of historical LVET data as acceptable *in vivo* reference data on which to base comparisons to *in vitro* study results.

Public Comments

Dr. Harbell commented that eye irritation testing is done to protect the public and that accidental exposure data should be included in the evaluation. Dr. Harbell also commented on Dr. Merrill's presentation that outlined the ICCVAM draft recommendations. He stated that the suggestion in the ICCVAM draft recommendations that severe substances should be tested in humans is terrifying. (Note: This comment was in response to a misinterpretation by the commenter, which was clarified by Dr. Merrill who stated that the ICCVAM draft recommendations do not recommend human testing to be conducted [see below]).

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Curren commented that the LVET is being discussed because it was used as an *in vivo* reference test method for some of the data provided for the antimicrobial cleaning product (AMCP) testing strategy. He stated that only biologic or LVET data exist for many of the AMCPs, and these data were used to determine the prediction model to support registration of these AMCPs. The LVET test method is no longer used, but there is historical data that can and should be used. Dr. Curren stated that the question is whether we are putting people at risk based upon the cut-off points suggested in the AMCP BRD.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. McNamee (Procter & Gamble) reiterated the comments by Dr. Curren regarding the LVET and noted that 30 years of human experience data with a chemical substance are sufficient for licensing in the United Kingdom.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Merrill responded to the comment by Dr. Harbell regarding human testing. Dr. Merrill clarified that the ICCVAM draft recommendation states that if an organization or sponsor desires to more adequately characterize the usefulness and limitations of the LVET, ICCVAM recommends that a comprehensive set of substances be tested and compared with the Draize rabbit eye test results. She stated that there was no recommendation for human testing to be conducted, but that existing accidental human injury data and ethical human study data should always be considered.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention,

Dr. Ward, who cited a potential conflict-of-interest because of her previous consulting work for a company that conducts the LVET.

Cytosensor® Microphysiometer Test Method

Dr. Curren provided an overview of the Cytosensor Microphysiometer (CM) test method protocol.

On behalf of NICEATM, Dr. Lipscomb reviewed the CM test method performance as detailed in the AMCP draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the CM test method for the Panel to consider.

Panel Evaluation

Dr. Bailey (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the CM test method and ICCVAM draft test method recommendations. The Panel indicated that the test method protocol was sufficiently detailed; however, it was unlikely to be widely used because the CM instrument has been discontinued and a new instrument would require revalidation.

The Panel recommended the use of relevant positive controls in any future validation studies and, because surfactants form micelles that can influence response, surfactant concentrations should be included. The Panel recommended that an evaluation of the different classes of surfactants (i.e., nonionic, anionic, cationic, and zwitterionic) be conducted to determine if restrictions should be imposed on use of the CM test method.

The Panel agreed that, based on the database of surfactants and surfactant-based formulations, LVET data could be used to support the validity of the CM test method in the proposed AMCP testing strategy.

The Panel also agreed that the additional data on the surfactants and surfactant-containing formulations in the ECVAM BRD provided sufficient support for the use of the CM test method as a screening test to identify water-soluble surfactant chemicals and certain types of surfactant-containing formulations (e.g., cosmetics and personal care product formulations but not pesticide formulations) as either severe or corrosive irritants or substances not labeled as irritants in a tiered-testing strategy, as part of a weight-of-evidence approach. The Panel also agreed that the intra- and interlaboratory reproducibility of the CM test method had been adequately evaluated, although for a limited range of substances as previously discussed. The Panel again noted that the instrument has been discontinued and is currently not supported by the manufacturer, making its use difficult. However, if the CM instrument were redesigned, the remanufactured instrument would require "catch-up" validation (i.e., not a full validation study).

Based upon the lesions noted for one false negative substance in the EPA classification system, the Panel expressed concern with the ability of the CM test method to identify EPA Category IV substances. The Panel noted that the rabbit data indicated that this substance would be classified as a Category III and, therefore, may cause irritation in a human. The Panel noted that further CM studies are needed, in particular for EPA Categories III and IV substances.

The Panel also expressed concern with the high false positive rate of the CM test method when identifying all four hazard categories.

Public Comments

Dr. Curren noted a correction to his presentation where he did not specifically state that the CM test method is limited to water-soluble substances. He questioned the need for performance standards for the CM test method, given that the Panel did not recommend performance standards for the BCOP

and ICE test methods. Dr. Curren commented that the surfactants referred to as *personal care products* are really detergents.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

EpiOcular Test Method

Dr. Curren provided an overview of the EpiOcular (EO) test method protocol.

On behalf of NICEATM, Dr. Lipscomb reviewed the EO test method performance as detailed in the AMCP draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the EO test method for the Panel to consider.

Panel Evaluation

Dr. Bailey (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the EO test method and ICCVAM draft test method recommendations. The Panel agreed that the EO test method protocol is adequately detailed but emphasized that the manufacturer should provide a "certificate of quality" for each batch of EO. The Panel also agreed that the critical aspects of the protocol had been justified and described in the BRD; however, in order to use the EO test method in a testing strategy to identify mild irritants and substances not labeled as irritants, positive controls that represent these hazard categories should be included in any future validation studies. The Panel noted that the EO test method cannot distinguish Category III from Category IV substances.

The Panel commented that the performance of the EO test method had not been adequately evaluated and compared to the Draize test for the types of substances included in the AMCP database. The Panel noted that the total number of products and their distribution across hazard categories were not sufficient. The Panel commented that the intralaboratory variability was not adequately assessed, although interlaboratory variability was considered to be adequate.

Public Comments

Dr. Curren indicated that he felt that it was appropriate to include EO data that used a different protocol as a measure of test method reproducibility.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention, Dr. Ward, who cited a potential conflict-of-interest because of her previous consulting work for a company that conducts the EO test method.

Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products (AMCPs) Using *In Vitro* Alternative Test Methods

Dr. Curren provided an overview of the AMCP testing strategy.

On behalf of NICEATM, Dr. Lipscomb reviewed the AMCP draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the AMCP testing strategies for the Panel to consider.

Panel Evaluation

Dr. Bailey (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the AMCP testing strategies and ICCVAM draft test method recommendations. The Panel also suggested adding more discussion of the cells used in the CM and EO test methods.

Regarding the BCOP test method, the Panel reflected on its previous discussions of the BCOP test method for the total database. The Panel indicated that use of the BCOP test method in a testing strategy to identify severe irritants (Category I) and moderate irritants (Category II), should include positive controls that represent these hazard categories in any future validation studies. The Panel noted that histopathology evaluation, as it is proposed at this time as an additional endpoint for the BCOP test method, does not justify its use for hazard classification of AMCPs. However, histopathology evaluation may prove to be a useful endpoint and, as such, collection of histopathology data and further efforts to optimize its use are encouraged.

The Panel agreed with the ICCVAM draft recommendations that there is insufficient data to support the testing strategy in terms of the proposed test method usefulness and limitations (i.e., the classification of substances in all four ocular hazard categories). There were also insufficient available data on which to base definitive recommendations on the proposed alternate testing strategy for classifying substances in all four ocular hazard categories. In discussing the validity of retrospective evaluations, the Panel stated that a retrospective evaluation of results could be considered adequate if the studies were performed with GLP compliance, coded samples, and pre-established evaluation criteria. The Panel commented that any definitive recommendations on a testing strategy should be based on prospective testing of a list of reference substances in each of the proposed *in vitro* test methods.

The Panel concurred with the ICCVAM draft recommendations in terms of the proposed test method standardized protocols. The Panel stated that routine fixation of tissue from the BCOP test method for possible histopathology evaluation should be continued. The Panel emphasized that no single *in vitro* test method alone was applicable to all types of test materials, and therefore suggested several future studies that could potentially expand the usefulness of AMCP test strategies.

Finally, the Panel commented that the development of performance standards for the AMCP testing strategy was not currently warranted and that a new approach needed to be defined for comparing testing strategies.

Public Comments

Dr. Barroso commented that ECVAM is working on a guideline for the detection of severe irritants with the BCOP test method. He indicated that they see a small change in classification when the cut-off is changed from 55 to 75. ECVAM considers 55 the best cut-off for their intended purpose.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Curren commented that concern regarding the limited number of AMCPs is misplaced due to the intended narrow applicability domain. He stated that industrial-strength cleaners are mostly severe irritants and that household cleaners are mostly mild irritants. Very few, if any, substances are in the moderate range. Dr. Curren expressed concern with the recommendation by the Panel that substances need to be tested by each test method in the testing strategy. He noted that histopathology evaluation with the BCOP test method was included in the testing strategy to provide additional safety, and clarified that most of the histopathology evaluation was performed by a certified veterinary

pathologist. He also questioned the Panel's suggested use of a transformed ocular cell line rather than a normal epidermal cell line.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention, Dr. Ward, who cited a potential conflict-of-interest because of her previous consulting work for a company that manufactures AMCPs.

Concluding Remarks

Dr. Hayes, on behalf of the Panel, thanked Dr. Stokes and the NICEATM staff for their continued assistance during the review process and Panel meeting. He also thanked Dr. Wind, ICCVAM Chair, and the members of ICCVAM and the OTWG for their contributions to the project. Finally, Dr. Hayes thanked the Panel and the Evaluation Group Chairs.

Drs. Wind and Stokes thanked the Panel again for their hard work, thoughtful and objective deliberations, and advice. Dr. Stokes further thanked public attendees for their participation and the invited test method developers for their excellent test method summaries. Dr. Stokes concluded by saying he looked forward to working further with Panel members to complete the Panel report.

Adjournment

Dr. Hayes adjourned the Panel at 7:40 p.m., concluding the meeting.

Appendix G2

Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches

This document is available at:
https://ntp.niehs.nih.gov/iccvam/docs/ocutox_docs/ocularprprept2009.pdf

The document is also available on request from NICEATM:

NICEATM
National Institute of Environmental Health Sciences
P.O. Box 12233, MD K2-16
Research Triangle Park, NC 27709 USA
E-mail: niceatm@niehs.nih.gov

This page intentionally left blank

Appendix H

Federal Register Notices and Public Comments

This page intentionally left blank

Appendix H1

Federal Register Notices

All Federal Register notices are available at https://www.federalregister.gov/

70 FR 13512 (March 21, 2005)
Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for Nominations for an Independent Expert Panel

72 FR 26396 (May 9, 2007)
Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing

72 FR 31582 (June 7, 2007)
Request for Ocular Irritancy Test Data from Human, Rabbit, and *In Vitro* Studies Using Standardized Testing Methods

73 FR 18535 (April 4, 2008)
Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data

74 FR 14556 (March 31, 2009)
Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents (BRDs); Request for Comments

74 FR 19562 (April 29, 2009)
Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

74 FR 33444 (July 13, 2009)
Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches: Notice of Availability and Request for Public Comments

This page intentionally left blank

Appendix H2

Public Comments Received in Response to *Federal Register* Notices

Public comments are available on request from NICEATM

70 FR 13512 (March 21, 2005)
Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for Nominations for an Independent Expert Panel

- No responses received.

72 FR 26396 (May 9, 2007)
Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing

- Robert Guest (Safepharm Laboratories, Ltd.)

72 FR 31582 (June 7, 2007)
Request for Ocular Irritancy Test Data from Human, Rabbit, and *In Vitro* Studies Using Standardized Testing Methods

- No responses received.

73 FR 18535 (April 4, 2008)
Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data

- No responses received.

74 FR 14556 (March 31, 2009)
Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents (BRDs); Request for Comments

- Dr. Raymond David (BASF Corporation)
- Dr. John Harbell
- MatTek Corporation

- Dr. Wolfgang Pape (R&D Brands)
- Dr. Ruud Woutersen and Mr. Menk Prinsen (TNO)
- Dr. Robert Rapaport (The Procter & Gamble Company)
- Dr. Gerald Renner (Colipa, the European Cosmetics Association)
- Dr. Sherry Ward

74 FR 19562 (April 29, 2009)
Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

- Mr. Troy Seidle, Ms. Sara Amundson, and Dr. Martin Stephens (HSUS), Dr. Kate Willet (PETA), and Dr. Chad Sandusky (PCRM)
- Dr. Catherine Willet (PETA)

74 FR 33444 (July 13, 2009)
Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches: Notice of Availability and Request for Public Comments

- No responses received.

Appendix H3

Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) Comments

SACATM Meeting on June 25-26, 2009

Minutes from past SACATM meetings are available at:
https://ntp.niehs.nih.gov/events/past/index.html?type=SACATM

This page intentionally left blank

Appendix H4

ECVAM Comments on ICCVAM Recommendations for the BCOP Test Method

This page intentionally left blank

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
European Centre for the Validation of Alternative Methods (ECVAM)

Ispra, 27/05/2010
IHCP/I.3/jk-ARES(2010)284892

Marilyn Wind
Deputy Associate Executive Director for Health Sciences
Chair of ICCVAM
P.O. Box 12233, MD EC-17
Research Triangle Park, NC 27709
USA

William S. Stokes
Executive Director, ICCVAM
National Institute of Environmental Health Sciences, NIH, DHHS
79 Alexander Drive
Bldg. 4401 Room 3129
P.O. Box 12233
Research Triangle Park, NC 27709
USA

Dear Marilyn,

Dear Bill,

The development of harmonized test method recommendations is a key element of the ICATM framework and we therefore appreciate to collaborate with you on the development of final recommendations that take the different views among the participating validation bodies into account, with the aim to avoid the situation of a partner VAM issuing a deviating position.

ECVAM agrees with ICCVAM's conclusion that the BCOP should not be recommended for the identification of chemicals not classified as ocular irritants under the EPA (Category IV) and FHSA (Not Labeled) classification systems due to false negative rates of 5-6%.

On the other hand we still strongly disagree with ICCVAM's opinion that the accuracy and reliability of the BCOP test method does not allow its use for identifying chemicals not classified as ocular irritants under the EU DSD (Not Labeled), the UN GHS (No Category), and the EU CLP (No Category) classification systems. The reason for this disagreement is that the BCOP produces reliable results and has shown a rate of 0% false negatives under these classification systems. Obviously this performance is related to the

Joint Research Centre · I-21027 Ispra (VA), Italy · TP 580
In-Vitro Methods Unit/ ECVAM
Institute for Health and Consumer Protection
http://ecvam.jrc.ec.europa.eu
http://ihcp.jrc.ec.europa.eu
Telephone: direct line (+39) 0332 786735, · Telefax: (+39) 0332 786297
E-mail Internet: joachim.kreysa@ec.europa.eu

thresholds applied, which have been established as a result of a long and intensive international process.

Please note that we therefore continue to be in full agreement with the conclusions and recommendations of the ICCVAM Ocular Peer Review Panel (PRP) that convened in Bethesda, USA, on 19-21 May 2009, which concluded that the usefulness of the BCOP for the identification of chemicals not classified as ocular irritants depended on the intended purpose (i.e. the classification system) and that, therefore, the BCOP could be recommended for the identification of chemicals not classified as ocular irritants under the EU DSD and UN GHS classification systems (the CLP was not yet adopted), while such recommendation was not possible when considering use of the BCOP for the EPA classification system.

We therefore believe that the most appropriate approach to evaluate the usefulness and limitations of the four organotypic test methods (BCOP, ICE, IRE and HET-CAM) for the different classification systems (EU DSD, UN GHS/EU CLP, EPA, FHSA) would be through a separate and independent analysis of each test method's predictive capacity for each classification system.

ICCVAM expresses concern of ensuring sufficient protection of public health that could result from classifying substances as "Not Labeled" (EU DSD) or as "No Category" (the UN GHS and the EU-CLP). However, as we all know, all classification systems are simplifications of a rather complex scientific reality and they represent compromises that are (internationally) accepted to sufficiently protect public health. .

We remain, in this context, concerned by a statement in the latest draft version of the ICCVAM Test Method Evaluation Report that in our understanding is – so far – not substantiated by scientific evidence: ICCVAM states that "the nature, severity, and duration of these eye injuries [i.e. those induced in rabbit eyes] **suggest the potential to cause human injury**", when referring to the 70% EPA category III chemicals that are not classified under the UN GHS classification system. In view of the limitations of the Draize test due to species differences and other parameters, we would kindly like to ask ICCVAM to substantiate this claim that these chemicals indeed produce injury to the human eye with further data. Should there be no data available substantiating this claim, ECVAM would not be able to support a recommendation containing the conclusions as they now stand.

Please note, that the EU DSD classification system is in place in Europe since 1967 (Directive 67/548/EEC) without any known case of human eye injury caused by chemicals classified as "not labelled" under this classification system. In this context it is also relevant to recognize that the EU DSD system is even less conservative than the new EU CLP system, which is based on UN GHS (cut-off Draize scores for EU DSD R36 classification are higher than for UN GHS/EU CLP Cat 2 classification). We conclude from this that there is no empirical evidence that the EU-DSD system in reality poses any human health problem with regard to eye irritants.

Nevertheless, we recognize that the reduced eye hazard labeling resulting from the use of GHS instead of current U.S. regulatory classification criteria is of concern to the U.S. and we support that this issue be presented and discussed at international level. This could happen, for example, with experts from the UN Sub-Committee of Experts on the GHS and/or OECD. Importantly, such discussions should occur before judging on the appropriate public protection of one or several internationally agreed classification systems and should not be confounded with recommendations of test methods against the current criteria.

In conclusion, ECVAM suggests that the recommendations on BCOP should be along the lines proposed by the ICCVAM Ocular Peer Review Panel (PRP), cited above, clearly spelling out that the performance of the BCOP differs for the different classification systems.

In addition, ECVAM strongly recommends the development of full Performance Standards (Essential Test Method Components, Reference Chemicals and Target Accuracy Values in function of the target classification system) for BCOP, to allow for the faster evaluation and validation of variations/updates of the method (a new opacitometer is, for example, available from BASF and revised protocols are being developed to address problematic chemical classes, etc.).

Let me underline once more our appreciation of this cooperation and of the opportunity to find together and in true partnership suitable formulations. These should bring forward the concern expressed in the current draft but also make it very clear that for certain current classification systems the BCOP can very well serve as a means to identify substances that are not labelled or not classified with regard to eye irritation. In our view the formulation of the ICCVAM Ocular Peer Review Panel pointed in the right direction.

On the other hand, if ICCVAM cannot recommend the BCOP test for identification of not labelled and no category chemicals under EU DSD and UN GHS/EU CLP, ECVAM will have to issue its own recommendation along this line.

[redacted]

Dr. Joachim Kreysa
In Vitro Methods Unit
Head of Unit
On behalf of ECVAM

Copy: Elke Anklam (IHCP Director)
Sharon Munn (ECVAM Policy Support Action Leader)
Maurice Whelan (Systems Toxicology Head of Unit)

This page intentionally left blank

Appendix I

Relevant U.S. Federal and International Ocular Toxicity Regulations, Labeling, and Test Guidelines

This page intentionally left blank

Appendix I1

Table of Relevant U.S. Federal and International Ocular Testing Regulations for Hazard Classification and Labeling

Note to the Reader:
Regulations may be updated in the future. It is recommended that users review the most current version of all regulations identified.

Electronic versions of United States Code (U.S.C.) can be obtained at:
http://www.gpoaccess.gov/uscode/index.html

Electronic versions of the Code of Federal Regulations (CFR) can be obtained at:
http://www.gpoaccess.gov/cfr/index.html

This page intentionally left blank

Eye Irritation/Corrosion Testing: Relevant U.S. Federal Laws, Regulations, Guidelines, and Recommendations				
Agency, Center, or Office	**Regulated Products**	**Statutory Requirements**	**Regulations (Applications)**	**Guidelines and Recommendations**
CPSC	Consumer Products	Federal Hazardous Substances Act (U.S.C. Title 15, Chapter 47)	16 CFR 1500.3 (Definitions) 16 CFR 1500.42 (Test for Eye Irritants) 16 CFR 1500.121 (Labeling)	Animal Testing Policy (1984)
EPA/OPPTS	Chemicals as defined by the Toxic Substances Control Act Pesticides	Toxic Substances Control Act (U.S.C. Title 15, Chapter 53) Federal Insecticide, Fungicide, and Rodenticide Act (U.S.C. Title 7, Chapter 6)	40 CFR 716 (Safety Data) 40 CFR 717 (Adverse Reactions) 40 CFR 720 (Premanufacture Notification) 40 CFR 156 (Labeling) 40 CFR 158 (Pesticide Data)	OPPTS 870.2400 (1998)[1] Label Review Manual (2003)[2]

continued

[1] See **Appendix F2.**
[2] Available at: http://www.epa.gov/oppfead1/labeling/lrm/.

Eye Irritation/Corrosion Testing: Relevant U.S. Federal Laws, Regulations, Guidelines, and Recommendations (continued)				
Agency, Center, or Office	**Regulated Products**	**Statutory Requirements**	**Regulations (Applications)**	**Guidelines and Recommendations**
FDA/CFSAN FDA/CDER	Cosmetics[3] Pharmaceuticals	Federal Food, Drug, and Cosmetic Act (U.S.C. Title 21, Chapter 9) Public Health Service Act (U.S.C. Title 42, Chapter 6A)	21 CFR 70 (Color additives in food, medical devices, and cosmetics) 21 CFR 312 (IND Application) 21 CFR 314 (IND Approval) 21 CFR 701 (Cosmetic Labeling) 21 CFR 740 (Cosmetic Warning Statement)	No Specific Guidelines or Recommendations on Eye Irritation/Corrosion Testing Are Provided.
OSHA	Chemicals	Occupational Safety and Health Act of 1970 (U.S.C. Title 29, Chapter 15)	29 CFR 1910.1200 (Hazard Communication Standard) 16 CFR 1500.42 (Test for Eye Irritants)	No Specific Guidelines or Recommendations on Eye Irritation/Corrosion Testing Are Provided.

[3] FDA does not have authority for pre-market approval of cosmetics or cosmetic ingredients with the exception of color additives. However, the FDA may enforce action against products or ingredients that are in violation of Federal labeling laws, including provision of adequate safety information.

Relevant Ocular Testing Regulations for Hazard Classification and Labeling: European Union	
Regulated Products	**Regulations and Directives**
Substances and Mixtures	Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 (CLP, Classification Labelling and Packaging), amending and repealing Directives 67/548/EEC (DSD, Dangerous Substances Directive) and 1999/45/EC (DPD, Dangerous Preparations Directive), and amending Regulation (EC) No 1907/2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 (REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals)
Plant Protection Products	Council Directive 91/414/EEC of 15 July 1991 as amended
Cosmetics	Council Directive 76/768/EEC of 27 July 1976 as amended
Biocidal Products	Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 as amended

Relevant Ocular Testing Regulations for Hazard Classification and Labeling: United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS)	
Scope	**Legal Instruments and Recommendations**
Chemicals (Substances and Mixtures)	Globally Harmonized System of Classification and Labelling of Chemicals (UN 2007), Part 3, Chapter 3.2.4 (Serious eye damage/eye irritation)

This page intentionally left blank

Appendix I2

EPA OPPTS Guidance Document 870.2400 (August 1998)

EPA Health Effects Test Guidelines are available at:
https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines

This page intentionally left blank

Appendix I3

EPA Office of Pesticide Programs Label Review Manual (August 2003)

Electronic versions of the EPA LRM can be obtained at:
http://www.epa.gov/oppfead1/labeling/lrm/

This page intentionally left blank

Appendix I4

Organisation for Economic Co-operation and Development (OECD) Test Guideline 405 (Adopted April 2002)

Test Guideline 405 is available at:
https://www.oecd-ilibrary.org/environment/test-no-405-acute-eye-irritation-corrosion_9789264185333-en

This page intentionally left blank

Appendix J

NICEATM Analysis:
Reduced Eye Hazard Labeling Resulting from Using Globally Harmonized System (GHS) Instead of Current U.S. Regulatory Classification Criteria

This page intentionally left blank

Table of Contents

List of Tables

Summary

Recent analyses by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) reveal that up to 36% of substances currently classified and labeled as eye irritation hazards by U.S. hazard classification regulations would not be classified and labeled as eye hazards using United Nations (UN) Globally Harmonized System for the Classification and Labelling of Chemicals (GHS) eye irritation criteria (UN 2007). Current U.S. hazard classification regulations include the Federal Hazardous Substances Act (FHSA) regulations used by the U.S. Consumer Product Safety Commission (CPSC) and the Occupational Safety and Health Administration (OSHA), and U.S. Environmental Protection Agency (EPA) hazard regulations. U.S. agencies are currently considering implementation of GHS criteria, and OSHA has recently proposed to adopt the GHS criteria to replace the current OSHA Hazard Communication Standard (HCS).[1]

ICCVAM discovered the substantial differences in eye hazard labeling between the GHS and current U.S. classification systems while evaluating the validity of several *in vitro* methods proposed for regulatory ocular safety testing. NICEATM subsequently reviewed and analyzed two separate databases of *in vivo* eye irritation studies to assess the extent that using the GHS criteria would result in no hazard labeling for substances currently labeled as eye hazards in the United States.

The first ocular database evaluated for this analysis was constructed for chemicals used to prepare a 1999 OECD *Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries* (DRD; **Annex I**[2]). This document proposed a potential harmonized classification scheme for eye hazards and compared the impact on eye hazard labeling for existing Canadian, EPA, EU, and FHSA classification systems. Careful review of the DRD reveals that using the GHS criteria resulted in no hazard labeling for up to 27% and 33% of substances labeled as eye hazards by current FHSA and EPA classification systems, respectively. This includes 76% of currently labeled EPA Category III irritants (those causing eye injuries persisting for 24 hours to 7 days) that would not require hazard labeling using the GHS criteria. Nonetheless, the scheme was subsequently adopted by GHS.

The second database consisted of a public database of eye irritation studies for 149 chemicals, which revealed similar classification disparities. Using the GHS criteria resulted in no hazard labeling for up to 31% and 36% of substances currently labeled as eye hazards by FHSA and EPA classification systems, respectively.

NICEATM further characterized the nature, duration, and severity of eye injuries produced in these studies for the substances that will no longer be labeled as eye hazards using GHS criteria. Over 50% of these chemicals produced visible eye injuries expected to interfere with normal vision, including corneal opacity, corneal ulceration, and/or iritis (visible damage inside of the eye). Of these substances, 10% produced corneal opacity of a severity grade described as *easily defined translucent areas of the cornea that obscured the details of the iris* (i.e., corneal opacity score of 2/4). While all of the lesions were reversible, they persisted from 24 hours to 7 days.

The high rate of reduced eye hazard labeling resulting from using the GHS criteria compared to U.S. criteria is attributable to two important differences. First, the minimum number/proportion of animals with positive eye injury responses required for classifying a substance as an eye irritation

[1] September 30, 2009, *Federal Register* notice (74 FR 50280): OSHA 29 CFR Parts 1910, 1915, and 1926 Hazard Communication: Proposed Rule.

[2] Available at http://www.olis.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono%2899%294

hazard differs significantly. FHSA regulations classify substances as eye irritation hazards when as few as 22% (4/18) of animals produce positive eye injury responses. EPA regulations classify substances as eye irritants when *any* single test animal exhibits a positive response, regardless of the number of animals tested. In contrast, GHS criteria require *at least* 67% (2/3) of animals tested to produce a positive response for classification as an eye irritant hazard.

Secondly, there is a significant difference in the criteria that must be met for eye injuries to be considered positive responses. U.S. regulations (FHSA) consider it a positive response whenever the minimum severity is reached for any of the four types of ocular injuries at *any* of the three observation time points (24, 48, and 72 hours following test substance administration). In contrast, classification according to the GHS requires calculating the *average* severity across all three time points. This average score must meet or exceed the minimum severity level in order to be considered positive. Taken together, these two major differences account for the significant reduction in eye hazard labeling by GHS compared to current U.S. regulations.

The GHS incorporates the principle that the level of protection offered to workers, consumers, the general public, and the environment should not be reduced as a result of harmonizing the classification and labeling systems (UN 2007). In order to adhere to the GHS principle of not reducing protection that could result from the significant reduction in labeling of eye hazards, GHS classification criteria are needed that can provide hazard labeling at least equivalent to that currently provided by current U.S. hazard regulations. This paper summarizes the eye irritation hazard classification analyses and provides proposals for updating the GHS hazard criteria with an optional hazard category that could continue to provide the same level of hazard labeling and protection as current U.S. hazard regulations.

1.0 Background

Physical trauma or chemical burns due to contact with workplace or household products or chemicals result in about 125,000 household eye injuries each year and approximately 2,000 job-related eye injuries per day that require medical treatment.[3,4] In order to provide warnings to consumers and workers of the potential for chemicals and products to cause eye injuries, regulatory authorities require ocular safety testing to determine if substances may cause eye damage. Such testing characterizes the nature, duration, and severity of eye injuries in an animal model, and whether the injuries are reversible or permanent. Testing results are then used for hazard classification and labeling of eye injury potential according to relevant national and/or international classification systems. These classification systems are intended to warn users of the potential for substances to cause eye injuries, the precautions necessary to avoid injuries, and the immediate first-aid procedures that should be followed in the case of an accidental exposure.

Currently, U.S. Occupational Safety and Health Administration's Hazard Communication Standard (HCS) uses the FHSA classification scheme (16 CFR 1500.42) to classify the ocular irritation hazard potential of regulated substances. The FHSA classification system is based on the proportion of animals that exhibit a minimum severity score for each of three areas of the eye (i.e., corneal ulceration and opacity, conjunctival redness and swelling, iritis) that occur during the first 72 hours after test substance administration, with observations recorded at 24, 48, and 72 hours (**Table 1-1**). **Annex II** provides the grading criteria for each of the types of ocular lesions. By comparison, classification according to the EPA scheme uses the same threshold for positive results in each tissue type but has three severity categories, which are determined based on the maximum score for any of the three tissues in any one animal (**Table 1-2**).

Table 1-1 FHSA Classification System (16 CFR 1500.42)

Positive Response for a Single Rabbit[1] (≥1 of the following at 24, 48, or 72 hours)	***In Vivo* Effect[2]**
Corneal ulceration (other than a fine stippling) Corneal opacity ≥ 1 Iritis ≥ 1 Conjunctival swelling and/or redness ≥ 2	First Test - If ≥4/6 animals are positive, the test is positive. If ≤1 animal is positive, the test is negative.[1] If 2/6 or 3/6 animals are positive, the test is repeated using a different group of six animals. Second Test - If ≥3/6 animals are positive, the test is positive. If 0/6 are positive, the test is negative. If 1/6 or 2/6 animals are positive, the test is repeated using a different group of six animals. Third Test - Should a third test be needed, the test is positive if ≥1/6 animals are positive. If 0/6 are positive, the test is negative. **Note: Classification as an eye irritant hazard can result from as few as 22% of animals showing a positive response (e.g., 2/6 + 1/6 + 1/6 = 4/18).**

Abbreviations: CFR = U.S. Code of Federal Regulations; FHSA = U.S. Federal Hazardous Substances Act.
The following scores are considered positive: CO or IR ≥ 1 or CC or CR ≥ 2. Therefore, CO or IR scores of 0 and CC or CR scores of ≤1 are considered cleared.

[1] In this evaluation, a test was also considered negative for 0/3, 0/4, or 0/5 positive animals in 3-, 4-, or 5-animal tests.

[2] In this evaluation, a test was also considered negative for 0/3, 0/4, or 0/5 positive animals in 3, 4, or 5-animal tests.

[3] Available at: http://www.geteyesmart.org/eyesmart/injuries/home.cfm

[4] Available at: http://www.cdc.gov/niosh/topics/eye/

Table 1-2 EPA Classification System

EPA Category	*In Vivo* Effect
I	Corrosive (irreversible) or corneal involvement or other eye irritation persisting for more than 21 days
II[1]	Corneal involvement or other eye irritation clearing[2] in 8 to 21 days
III[1]	Corneal involvement or other eye irritation clearing[2] in 7 days or less
IV	Minimal effects clearing[3] in less than 24 hours

Abbreviations: CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; EPA = U.S. Environmental Protection Agency; IR = iritis.

At least 3 animals per test (1-animal screen for corrosive/severe irritants permitted).

Maximum score in any animal used for classification.

[1] The EPA currently bases classification decisions on the criteria presented in the EPA Label Review Manual (2003). However, these requirements differ from 40 CFR 156.62 (e.g., EPA Category III is based on no corneal involvement [EPA 2006]).

[2] The following scores are considered positive: CO or IR ≥ 1 or CC or CR ≥ 2. Therefore CO or IR scores of 0 and CC or CR scores of ≤1 are considered cleared.

[3] The following scores are considered positive: CO or IR ≥ 1 or CC ≥ 2. Therefore CO or IR scores of 0 and CC or CR scores of ≤1 are considered cleared.
Most severe response used for classification of substance.

In September 2009, OSHA proposed to modify the HCS to conform to the GHS system.[5] The GHS classification system is based primarily on the severity and timing of reversibility of effects using *mean* values for each endpoint (i.e., corneal opacity, conjunctival redness and swelling, iritis) based on observations assessed at 24, 48, and 72 hours following test substance administration (**Table 1-3**).

Table 1-3 GHS Classification System (UN 2009)

GHS Category	*In Vivo* Effect
I	≥ 1 animal with CO ≥ 4 at any time or ≥ 2 animals with mean[1] CO ≥ 3 or IR ≥ 1.5 or ≥1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 which is not expected to reverse or does not fully reverse[2] within 21 days
2A	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 which fully reverses[2] within 21 days
2B	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 which fully reverses[2] within 7 days

Abbreviations: CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; GHS = UN Globally Harmonized System; IR = iritis; UN = United Nations.

[1] Mean value is calculated from grading at 24, 48, and 72 hours after instillation of the test material.

[2] Fully reversed requires a score of 0.

To understand the potential impact of this change, NICEATM and ICCVAM evaluated 149 Draize rabbit eye tests in the database of the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC; 1998). NICEATM searched for differences in classification

[5] September 30, 2009, *Federal Register* notice (74 FR 50280): OSHA 29 CFR Parts 1910, 1915, and 1926 Hazard Communication: Proposed Rule

of the test substances when comparing the GHS classification system to either the EPA classification system or the FHSA classification system.

NICEATM and ICCVAM also reviewed a 1999 OECD analysis of possible harmonized criteria for eye irritation and corrosion (which were ultimately adopted as the GHS criteria) that assessed the impact of the proposed criteria compared to current Canadian, EPA, EU, and FHSA labeling requirements based on 140 substances and 144 studies (4 repeat tests).

2.0 Overview of NICEATM and ICCVAM Analyses

To evaluate if and to what extent using the proposed HCS/GHS classification system might not identify substances as eye irritation hazards that would be classified as eye irritation hazards by FHSA and EPA criteria, NICEATM evaluated results from Draize rabbit eye test studies from two independent databases:[6] (1) 149 studies obtained from a publicly available database (ECETOC 1998) and (2) 144 studies included in the *Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries* (DRD; **Annex I**[7]).

All of the Draize eye test data used in these analyses are from studies that used no more than six animals. If the current FHSA criteria were applied to these studies, many substances could not be definitively classified for ocular hazard potential based on the results of the initial 6-animal test. To assign a definitive FHSA classification, these substances would require further testing in a second and, in some cases, a third 6-animal test. In order to establish a definitive FHSA classification for all substances, an analysis was first undertaken to determine the most appropriate minimum number of positive animals that could be used to assign an FHSA eye hazard label in such circumstances and that would provide the same level of hazard labeling as current FHSA hazard classification regulations. This analysis (see **Annex III**) indicates that a minimum of one positive response out of three test animals would provide nearly equivalent labeling as current FHSA requirements. Based on this analysis, a threshold of ≥33% positive animals was used to assign a definitive classification for all substances included in the two databases.

3.0 Analysis of the ECETOC Eye Irritation Database

The ECETOC database was assessed to identify examples of substances classified based on Draize rabbit eye test results as GHS Not Classified, but as FHSA Irritants or EPA Category I, II, or III irritants. Conversely, examples were also sought for substances classified as EPA Category IV or FHSA Not Labeled, but as GHS Category 1, 2A, or 2B.

3.1 Comparison of the FHSA and GHS Classification Systems

Where possible, NICEATM assigned FHSA and GHS hazard classifications for each substance in the ECETOC database.[8] Only substances that could be assigned a definitive FHSA and GHS classification were included, which yielded a total of 122 or 134 substances included in the analysis, depending on whether the current FHSA criteria or a threshold of 33% positive animals, respectively, was used. Among these substances, 69/122 (57%) and 81/134 (60%) were identified

---

[6] As noted in **Section 4.0**, the OECD database includes 24 substances that are also in the ECETOC database.

[7] Available at http://www.olis.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono%2899%294

[8] The ECETOC database is composed of 149 studies representing 145 substances. Three substances with duplicate studies, resulting in discordant hazard classifications among one or more of the hazard classification systems, were excluded from these analyses (i.e., 1% benzalkonium chloride is GHS Category 1 or 2A; 5% triton X-100 is GHS Category 2A or 2B; xylene is EPA Category II or IV).

as ocular irritants by the FHSA using the current FHSA criteria and 33% threshold, respectively. NICEATM compared the FHSA ocular hazard classification of these substances with the classification that would be assigned by the GHS system. As indicated in **Table 3-1**, using the GHS criteria would result in no hazard labeling for up to 31% (25/81) of the ECETOC substances that are identified as ocular hazards by FHSA (see also **Annex IV**). Conversely, no substances labeled as ocular hazards by the GHS were not also labeled as hazards by the FHSA (**Table 3-1**).

Table 3-1 ECETOC Database: Substances Classified as Ocular Irritants Using FHSA Compared to Each GHS Ocular Hazard Category

FHSA Classification	No. of ECETOC Substances Classified as FHSA Irritants	GHS Classification			
		1	2A	2B	NC
Irritant (33% threshold)	81	31/81 (38%)	18/81 (22%)	7/81 (9%)	25/81 (31%)
Irritant (16 CFR 1500.42)	69	31/69 (45%)	18/69 (26%)	7/69 (10%)	13/69 (19%)
Not Labeled (either criterion)	53	0/53 (0%)	0/53 (0%)	0/53 (0%)	53/53 (100%)

Abbreviations: CFR = U.S. Code of Federal Regulations; FHSA = U.S. Federal Hazardous Substances Act; GHS = UN Globally Harmonized System; NC = Not Classified.

A closer look at the individual rabbit eye test data for the 25 FHSA eye irritants based on the 33% threshold that would not be labeled using GHS criteria reveals that 48% (12/25) of these substances produced corneal opacity and/or corneal ulceration, including seven that also produced iritis (visible evidence of tissue damage inside the eye; **Table 3-2**). Many of these substances (28% [7/25]) produced corneal opacity that extended beyond 48 hours after test substance administration (**Table 3-2**). **Table 3-2** also provides these data for the subset of 13 substances classified using the current FHSA criteria.

Table 3-2 ECETOC Database: Frequency, Type, and Severity of Ocular Lesions Among Substances Classified as FHSA Irritants, but Not Classified as Ocular Hazards by the Proposed HCS and Current GHS Classification Criteria

In Vivo Finding	No. of Substances	No. of Substances Where More than One Animal Exhibited the *In Vivo* Finding[1]
FHSA Classification Based on ≥33% Positive Animals		
Any CO score ≥ 1	12/25 (48%)	10/12 (83%)
CO Score ≥ 1: duration of 48 hours or more	7/25 (28%)	2/7 (29%)
CR or CC score ≥ 2	22/25 (88%)	17/22 (68%)
CR or CC score ≥ 2: duration of 72 hours or more	5/25 (20%)	2/5 (40%)
Iritis: visible inflammation inside the eye	7/25 (28%)	5/7 (71%)
Iritis: duration of 48 hours or more	3/25 (12%)	-

continued

Table 3-2 ECETOC Database: Frequency, Type, and Severity of Ocular Lesions Among Substances Classified as FHSA Irritants, but Not Classified as Ocular Hazards by the Proposed HCS and Current GHS Classification Criteria (*continued*)

In Vivo Finding	No. of Substances	No. of Substances Where More than One Animal Exhibited the *In Vivo* Finding[1]
FHSA Classification Based on 16 CFR 1500.42		
Any CO score ≥ 1	10/13 (77%)	8/10 (80%)
CO Score ≥ 1: duration of 48 hours or more	7/13 (54%)	2/7 (29%)
CR or CC score ≥ 2	12/13 (92%)	12/12 (100%)
CR or CC score ≥ 2: duration of 72 hours or more	5/13 (38%)	2/5 (40%)
Iritis: visible inflammation inside the eye	6/13 (46%)	5/6 (83%)
Iritis: duration of 48 hours or more	3/13 (23%)	-

Abbreviations: CC = conjunctival chemosis; CFR = U.S. Code of Federal Regulations; CO = corneal opacity; CR = conjunctival redness; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; FHSA = U.S. Federal Hazardous Substances Act; HCS = OSHA Hazard Communication Standard; No. = number; OSHA = U.S. Occupational Safety and Health Administration.

[1] The total number of animals in each test ranged from 3 to 6.

3.2 Comparison of the EPA and GHS Classification Systems

NICEATM also compared the ocular hazard classifications for the ECETOC substances based on EPA and GHS classification systems. Again, NICEATM attempted to assign EPA and GHS hazard classifications for each substance. Only substances that could be assigned definitive EPA and GHS classifications were included in the analysis, a total of 134 substances. Among these substances, 87/134 (65%) are identified as ocular irritants (i.e., EPA Category I, II, or III) by the EPA system. NICEATM compared the EPA ocular hazard classification of these substances with the classification that would be assigned by the GHS system. As indicated in **Table 3-3**, using the GHS criteria would result in no hazard labeling for 36% (31/87) of the ECETOC substances that are identified as ocular hazards by EPA (see also **Annex IV**). This includes 78% of currently labeled EPA Category III irritants (those causing eye injuries persisting for 24 hours to 7 days) that would not require hazard labeling using the GHS (see **Table 3-4**). No substances were labeled as ocular hazards by the GHS that were not also labeled as hazards by the EPA (**Table 3-4**).

Table 3-3 ECETOC Database: Substances Classified in the U.S. as Ocular Hazards Using the EPA Hazard Category Criteria, but Not Classified as Ocular Hazards by GHS Classification Criteria

EPA Category I, II, or III	GHS Hazard Classification	No. of Substances
87	1	31/87 (36%)
	2A	18/87 (21%)
	2B	7/87 (8%)
	NC	31/87 (36%)

Abbreviations: ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; EPA = U.S. Environmental Protection Agency; GHS = UN Globally Harmonized System; NC = Not Classified; No. = number.

Table 3-4 ECETOC Database: Comparison of Substances Classified Using Each EPA and GHS Eye Hazard Category

EPA Classification	No. of Substances	GHS Classification			
		1	2A	2B	NC
EPA I	28	27/27 (100%)	0/27 (0%)	0/27 (0%)	0/27 (0%)
EPA II	21	4/20 (20%)	14/20 (70%)	2/20 (10%)	0/20 (0%)
EPA III	42	0/40 (0%)	4/40 (10%)	5/40 (12%)	31/40 (78%)
EPA IV	47	0/47 (0%)	0/47 (0%)	0/47 (0%)	47/47 (100%)

Abbreviations: ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; EPA = U.S. Environmental Protection Agency; GHS = UN Globally Harmonized System; NC = Not Classified; No. = number.

A closer look at the individual rabbit eye test data for the 31 EPA eye irritants that would not be labeled using GHS criteria reveals that 52% (16/31) of these substances produced corneal opacity and/or corneal ulceration, including eight (26% [8/31]) that extended beyond 48 hours after test substance administration (**Table 3-5**). A total of eight substances produced iritis (visible evidence of tissue damage inside the eye). Seven of the eight also produced corneal opacity.

Table 3-5 ECETOC Database: Responses, Frequency, and Severity of Ocular Lesions Among 31 Substances Classified in the U.S. as Ocular Hazards Using the EPA Hazard Category Criteria, but Not Classified as Ocular Hazards by the Proposed HCS and Current GHS Classification Criteria

In Vivo Finding	No. of Substances	No. of Substances Where More than One Animal Exhibited the *In Vivo* Finding[1]
Any CO Score ≥ 1	16/31 (52%)	10/16 (63%)
CO Score ≥ 1: duration of 48 hours or more	8/31 (26%)	2/8 (25%)
CR or CC score ≥ 2	25/31 (81%)	17/25 (68%)
CR or CC Score ≥ 2: duration of 72 hours or more	5/31 (16%)	2/5 (40%)
Iritis: visible inflammation inside the eye	8/31 (26%)	5/8 (63%)
Iritis: visible inflammation inside the eye; duration of 48 hours or more	3/31 (10%)	1/3 (33%)

Abbreviations: CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; EPA = U.S. Environmental Protection Agency; GHS = UN Globally Harmonized System; HCS = OSHA Hazard Communication Standard; OSHA = U.S. Occupational Safety and Health Administration; No. = number.

[1] The total number of animals in each test ranged from 3 to 6.

4.0 Analysis of the 1999 OECD Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries

During the development of possible harmonized criteria for eye irritation and corrosion hazard categories, the OECD coordinated preparation of a *Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries* (DRD; **Annex I**). This document provides a potential harmonized classification scheme along with a comparison to the impact on eye hazard labeling for several existing national classification systems (i.e., Canada, EPA, EU, and FHSA). The DRD provides clear and concise documentation of the extent that the potential harmonization scheme would significantly reduce the number of chemicals identified as eye irritation hazards compared to current U.S. (EPA and FHSA) requirements. The scheme proposed in the DRD was subsequently incorporated into the GHS (UN 2009). However, it should be noted that the DRD does not provide any conclusions or recommendations, but instead details comparisons of sensitivity offered by the existing classification systems and the proposed scheme. There is no discussion in the document as to why *not* labeling substances currently labeled as eye hazards by EPA and FHSA criteria could be construed as providing the same level of protection. Efforts to locate documentation of further consideration of the severe underlabeling of eye hazards and reduced protection that would result from using the GHS scheme compared to current U.S. requirements were unsuccessful.

The OECD DRD (hereafter, OECD database) includes Draize rabbit eye test data for 140 substances (144 studies – 4 repeat tests) that were obtained from five different sources: (1) ECETOC industrial chemicals (n = 24), (2) EPA pesticide active ingredients (n = 60), (3) EPA pesticide products (n = 18), (4) EPA new industrial chemicals (n = 27), and (5) German

new industrial chemicals (n = 11). NICEATM obtained the individual animal data from all 144 studies and assigned EPA, FHSA, and GHS ocular hazard classifications where possible. However, using the classification rules described in **Tables 1-1** to **1-3**, NICEATM was unable to assign a definitive classification (i.e., either irritant or not classified) for some of the substances (EPA: n = 13; FHSA: n = 14; GHS: n = 19). Accordingly, there are some differences in the numbers of substances classified by NICEATM and those reported in the DRD (**see Annex V**). However, these differences did not result in substantive differences between NICEATM and the DRD database in the proportion of substances classified as irritants.

The OECD database was assessed to identify examples of substances classified based on Draize rabbit eye test results as GHS Not Classified, but as FHSA Irritants or EPA Category I, II, or III irritants. Conversely, examples were also sought for substances classified as EPA Category IV or FHSA Not Labeled, but as GHS Category 1, 2A, or 2B.

4.1 Comparison of the FHSA and GHS Classification Systems

Where possible, NICEATM assigned FHSA and GHS hazard classifications for each substance in the OECD database. Only substances that could be assigned a definitive FHSA and GHS classification were included, which yielded a total of 114 or 125 substances included in the analysis, depending on whether the current FHSA criteria or a threshold of 33% positive animals, respectively, was used. Among these substances, 85/114 (76%) and 95/125 (76%) were identified as ocular irritants by the FHSA using the current FHSA and 33% threshold criteria, respectively. NICEATM compared the FHSA ocular hazard classification of these substances with the classification that would be assigned by the GHS system. As indicated in **Table 4-1**, using the GHS criteria would result in no hazard labeling for up to 27% (26/95) of the OECD substances that are identified as ocular hazards by FHSA (see also **Annex VI**). Conversely, there were no substances labeled as ocular hazards by the GHS that were not also labeled as hazards by the FHSA (**Table 4-1**).

Table 4-1 OECD Database: Substances Classified as Ocular Irritants Using FHSA Compared to Each GHS Ocular Hazard Category

FHSA Classification	No. of OECD Substances Classified as FHSA Irritants	GHS Classification			
		1	2A	2B	NC
FHSA Classification Based on ≥33% Positive Animals					
Irritant	95	38/95 (40%)	22/95 (23%)	9/95 (9%)	26/95 (27%)
Not Labeled	30	0/30 (0%)	0/30 (0%)	0/30 (0%)	30/30 (100%)
FHSA Classification Based on 16 CFR 1500.42					
Irritant	85	38/85 (45%)	22/85 (26%)	9/85 (10%)	16/85 (19%)
Not Labeled	29	0/29 (0%)	0/29 (0%)	0/29 (0%)	29/29 (100%)

Abbreviations: CFR = U.S. Code of Federal Regulations; FHSA = U.S. Federal Hazardous Substances Act; GHS = UN Globally Harmonized System; OECD = Organisation for Economic Co-operation and Development; NC = Not Classified; No. = number.

A closer look at the individual rabbit eye test data for the 26 FHSA eye irritants based on the 33% threshold that would not be labeled using GHS criteria reveals that 46% (12/26) of these

substances produced corneal opacity and/or corneal ulceration, including twelve that also produced iritis (visible evidence of tissue damage inside the eye; **Table 4-2**). Many of these substances (27% [7/26]) produced corneal opacity that extended beyond 48 hours after test substance administration (**Table 4-2**). **Table 4-2** also provides these data for the subset of 16 substances classified using the current FHSA criteria.

Table 4-2 OECD Database: Frequency, Type, and Severity of Ocular Lesions Among Substances Classified as FHSA Irritants, but Not Classified as Ocular Hazards by the Proposed HCS and Current GHS Classification Criteria

***In Vivo* Finding**	**No. of Substances**	**No. of Substances Where More than One Animal Exhibited the *In Vivo* Finding[1]**
FHSA Classification Based on ≥33% Positive Animals		
Any CO score ≥ 1	12/26 (46%)	8/12 (67%)
CO score ≥ 1: duration of 48 hours or more	7/26 (27%)	6/7 (86%)
CR or CC score ≥ 2	22/26 (85%)	20/22 (91%)
CR or CC score ≥ 2: duration of 72 hours or more	4/26 (15%)	4/4 (100%)
Iritis: visible inflammation inside the eye	12/26 (46%)	6/12 (50%)
Iritis: duration of 48 hours or more	2/26 (8%)	1/2 (50%)
FHSA Classification Based on 16 CFR 1500.42		
Any CO score ≥ 1	8/16 (50%)	5/8 (62%)
CO score ≥ 1: duration of 48 hours or more	5/16 (31%)	4/5 (80%)
CR or CC score ≥ 2	16/16 (100%)	15/16 (94%)
CR or CC score ≥ 2: duration of 72 hours or more	3/16 (19%)	2/3 (67%)
Iritis: visible inflammation inside the eye	8/16 (50%)	5/8 (62%)
Iritis: duration of 48 hours or more	2/16 (12%)	2/2 (100%)

Abbreviations; CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; FHSA = U.S. Federal Hazardous Substances Act; GHS = UN Globally Harmonized System; HCS = OSHA Hazard Communication Standard; No. = number; OECD = Organisation for Economic Co-operation and Development; OSHA = U.S. Occupational Safety and Health Administration; UN = United Nations.

[1] The total number of animals in each test ranged from 3 to 6.

4.2 Comparison of the EPA and GHS Classification Systems

NICEATM also compared the ocular hazard classifications for the ECETOC substances based on EPA and GHS classification systems. Again, NICEATM attempted to assign EPA and GHS hazard classifications for each substance, and only substances that could be assigned a definitive EPA and GHS classification were included. A total of 122 substances were included in the analysis. Among these substances, 99/122 (81%) are identified as ocular irritants (i.e., EPA Category I, II, or III) by the EPA system. NICEATM compared the EPA ocular hazard classification of these substances with the classification that would be assigned by the GHS

system. As indicated in **Table 4-3**, using the GHS criteria would result in no hazard labeling for 33% (33/99) of the ECETOC substances that are identified as ocular hazards by EPA. This includes 76% (31/41) of currently labeled EPA Category III irritants (those causing eye injuries persisting for 24 hours to 7 days) that would not require hazard labeling using the GHS (see **Table 4-4** and **Annex VI**). There were no substances labeled as ocular hazards by the GHS that were not also labeled as hazards by the EPA (**Table 4-4**).

Table 4-3 OECD Database: Substances Classified in the U.S. as Ocular Hazards Using the EPA Hazard Category Criteria, but Not Classified as Ocular Hazards by GHS Classification Criteria

EPA Category I, II, or III	GHS Hazard Classification	No. of Substances
99	1	36/99 (36%)
	2A	22/99 (22%)
	2B	8/99 (8%)
	NC	33/99 (33%)

Abbreviations: EPA = U.S. Environmental Protection Agency; GHS = UN Globally Harmonized System; NC = Not Classified; No. = number; OECD = Organisation for Economic Co-operation and Development; UN = United Nations.

Table 4-4 OECD Database: Comparison of Substances Classified Using Each EPA and GHS Eye Hazard Category

EPA Classification	No. of Substances	GHS Classification			
		1	2A	2B	NC
EPA I	36	35/36 (97%)	1/36 (3%)	0/36 (0%)	0/36 (0%)
EPA II	22	1/22 (4%)	18/22 (82%)	1/22 (4%)	2/22 (9%)
EPA III	41	0/41 (0%)	3/41 (7%)	7/41 (17%)	31/41 (76%)
EPA IV	23	0/23 (0%)	0/23 (0%)	0/23 (0%)	23/23 (100%)

Abbreviations: EPA = U.S. Environmental Protection Agency; GHS = UN Globally Harmonized System; NC = Not Classified; No. = number; OECD = Organisation for Economic Co-operation and Development; UN = United Nations.

A closer look at the individual rabbit eye test data for the 33 EPA eye irritants that would not be labeled using GHS criteria reveals that 39% (13/33) of these substances produced corneal opacity and/or corneal ulceration, including seven (21% [7/33]) that extended beyond 48 hours after test substance administration (**Table 4-5**). A total of twelve substances produced iritis (visible evidence of tissue damage inside the eye), six of which also produced corneal opacity.

Table 4-5 ECETOC Database: Responses, Frequency, and Severity of Ocular Lesions Among 33 Substances Classified in the U.S. as Ocular Hazards Using the EPA Hazard Category Criteria, but Not Classified as Ocular Hazards by the Proposed HCS and Current GHS Classification Criteria

In Vivo Finding	No. of Substances	No. of Substances Where More than One Animal Exhibited the *In Vivo* Finding[1]
Corneal opacity/ulceration score ≥ 1	13/33 (39%)	8/13 (62%)
CO score ≥ 1: duration of 48 hours or more	7/33 (21%)	6/7 (86%)
CR or CC score ≥ 2	28/33 (85%)	20/28 (71%)
CR or CC score ≥ 2: duration of 72 hours or more	6/33 (18%)	2/6 (33%)
Iritis: visible inflammation inside the eye	12/33 (36%)	6/12 (50%)
Iritis: visible inflammation inside the eye; duration of 48 hours or more	2/33 (6%)	1/2 (50%)

Abbreviations: CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; EPA = U.S. Environmental Protection Agency; GHS = UN Globally Harmonized System; HCS = OSHA Hazard Communication Standard; OSHA = U.S. Occupational Safety and Health Administration; No. = number; UN = United Nations.

[1] The total number of animals in each test ranged from 3 to 6.

5.0 Summary of Analyses

These results from two independent databases of Draize rabbit eye test results are consistent and indicate that a significantly greater proportion of substances causing eye irritation, including some substances producing eye injuries lasting more than seven days (EPA Category II), will not be labeled using the GHS criteria. Taken together, these data indicate that the GHS hazard classification criteria will significantly reduce eye hazard labeling compared to that provided by current HCS/FHSA regulations. *Of greatest concern is that the proposed HCS and current GHS classification criteria will not identify many substances as eye irritants that produce significant ocular damage, including extended corneal opacity, which can result in visual impairment and internal ocular inflammation.*

6.0 Possible Options for GHS Hazard Categories for Classification and Labeling of Reversible Eye Irritation

Paragraph 1.1.1.6 of the GHS states that during the development of the GHS, "the requirements of [the U.S., Canada, EU, and] other countries were also examined as the work developed, but the primary task was to find ways to adopt the best aspects of these existing systems and develop a harmonized approach. This work was done based on agreed principles of harmonization that were adopted early in the process: (a) the level of protection offered to workers, consumers, the general public and the environment should not be reduced as a result of harmonizing the classification and labeling systems..." (UN 2007).

The current GHS criteria for classification of reversible ocular irritants (Category 2) involve scoring 3-animal tests for eye lesions (corneal opacity, iritis, conjunctival redness and chemosis)

on days 1, 2, and 3 (see also **Table 6-1**). A mean score is calculated for each animal using the three daily observation scores. Category 2 is assigned to those substances that induce any of the following *mean* animal scores in at least *two* animals: corneal opacity or iritis ≥1 or conjunctival redness or chemosis ≥ 2 that persists beyond 7 days but reverses within 21 days. Any substances not meeting this requirement would not be labeled as ocular hazards. An optional category (2B) is also provided for regulatory authorities to subcategorize Category 2 eye irritants as mild irritants if positive responses reverse by day 7.

Given the large number of substances that are labeled as eye hazards by current U.S. regulatory classification systems (EPA and FHSA) but that are not labeled as eye hazards by the current GHS classification system, NICEATM and ICCVAM performed technical analyses to support three optional GHS hazard categories that would achieve the GHS principle stated above. Countries and regulatory authorities could then choose to adopt the optional categories as necessary in order to not reduce the protection compared to the current level of protection afforded by the respective national or agency classification regulations. Each of the three proposals below provide classification criteria for a 3-animal test that will provide the same level of hazard labeling as current EPA, FHSA, and HCS hazard classification regulations. The proposals are as follows:

- **Proposal #1** (**Table 6-1**):
  Current GHS Category 2 is unchanged. An optional Category 3 is included for those countries that need such a category to maintain the current level of hazard labeling.
  - Assign Category 3 based on positive ocular lesions obtained in any animal at any time point.
    Category 3A: Any lesions that reverse within 21 days.
    Category 3B: Any lesions that reverse within 7 days.
- **Proposal #2 (Table 6-2):**
  Current GHS Category 2A is unchanged. Current GHS Category 2B criteria are changed based on ocular lesions that appear in at least one animal at any time point and that reverse within 21 days.
  - Use an optional Category 2C when ocular lesions in Category 2B reverse within 7 days.
- **Proposal #3 (see Table 6-3):**
  Current GHS Category 2A and 2B are modified.
  - Assign category based on ocular lesions obtained in at least one animal at any of the three time points.

Table 6-4 compares these three proposals to the current GHS hazard categories. In conclusion, each of these three proposals will provide GHS classification criteria that can be used to maintain the same level of labeling and protection afforded by current EPA and FHSA hazard criteria regulations.

Table 6-1 Proposal #1 – Add an Optional Category 3

Category	Current GHS Criteria	Proposal #1
2A	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 that reverses within 21 days	Same as current GHS
2B (optional)	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 that reverses within 7 days	Same as current GHS
3A (optional)	—	≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 which fully reverses within 21 days
3B (optional)	—	≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 7 days

Abbreviations: CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; GHS = UN Globally Harmonized System; IR = iritis.

[1] Mean values are calculated over 24 to 72 hours.

Table 6-2 Proposal #2 – Modify the Optional Category 2B and Add Another Optional Category

Category	Current GHS Criteria	Proposal #2
2A	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 21 days	Same as current GHS
2B (optional)	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 7 days	≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 at any time which reverses within 21 days
2C (optional)	—	>1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 7 days

Abbreviations: CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; GHS = UN Globally Harmonized System; IR = iritis.

[1] Mean values are calculated over 24 to 72 hours.

Table 6-3 Proposal #3 – Categorize Based on Individual Animal Scores Instead of Mean Animal Scores

Category	Current GHS Criteria	Proposal #3
2A	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 that reverses within 21 days	≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 at any time which reverses within 21 days
2B (optional)	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 that reverses within 7 days	≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 at any time which reverses within 7 days

Abbreviations: CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; GHS = UN Globally Harmonized System; IR = iritis.

[1] Mean values are calculated over 24 to 72 hours.

Table 6-4 Comparison of Current GHS Categories to Possible Optional Categories

Category	Current GHS	Proposal #1	Proposal #2	Proposal #3
2A	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 that reverses within 21 days	Same as current GHS	Same as current GHS	≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 at any time which reverses within 21 days
2B (optional)	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 that reverses within 7 days	Same as current GHS	≥ 1 animals with CO or IR ≥ 1 or CC or CR ≥ 2 at any time which reverses within 21 days	≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 at any time which reverses within 7 days
2C (optional)	—	—	>1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 7 days	—
3A (optional)	—	≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 that fully reverses within 21 days	—	—
3B (optional)	—	≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 that reverses within 7 days	—	—

Abbreviations: CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; GHS = UN Globally Harmonized System; IR = iritis.

[1] Mean values are calculated over 24 to 72 hours.

7.0 References

CPSC. 2003. Test for Eye Irritation. 16 CFR 1500.42.

DeSousa DJ, Rouse AA, Smolon WJ. 1984. Statistical consequences of reducing the number of rabbits utilized in eye irritation testing: data on 67 petrochemicals. Toxicol Appl Phamacol 76:234-242.

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

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. EPA737-B-96-0001. Washington, DC:U.S. Environmental Protection Agency.

Federal Hazardous Substances Act (FHSA). 2005. Public Law 86-613.

OECD. 2002. Test Guideline 405, Acute Eye Irritation/corrosion [adopted April 24, 2002]. In: OECD Guidelines for Testing of Chemicals. Paris:Organisation for Economic Co-operation and Development.

Springer JA, Chambers WA, Green S, Gupta KC, Hill RN, Hurley PM, et al. 1993. Number of animals for sequential testing. Food Chem Toxicol 31:105-109.

Talsma DM, Leach CL, Hatoum NS, Gibbons RD, Roger J-C, Garvin PJ. 1988. Reducing the number of rabbits in the Draize eye irritancy test: a statistical analysis of 155 studies conducted over 6 years. Fund App Toxicol 10:146-153.

United Nations. 2007. Globally Harmonized System of Classification and Labelling of Chemicals (GHS), 3rd rev ed. New York:United Nations.

This page intentionally left blank

Annex I

OECD Series on Testing and Assessment – Number 14:
Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries (**ENV/JM/MONO(99)4)**

An electronic version of this document can be obtained at
http://www.olis.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono%2899%294

This page intentionally left blank

Annex II

Grades for Ocular Lesions

This page intentionally left blank

Grades for Ocular Lesions[9]

Cornea **Score**

Opacity: Degree of density (area most dense taken for reading). No ulceration or opacity 0

Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible *1

Easily discernible translucent area, details of iris slightly obscured *2

Nacrous area, no details or iris visible, size of pupil barely discernible *3

Opaque cornea, iris not discernible through the opacity *4

Iris

Normal 0

Markedly deepened rugae, congestion, swelling moderate circumcorneal hyperemia, or injection, any 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 or all of these) *2

Conjunctivae

Redness (refers to palpebral and bulbar conjunctivae, excluding cornea and iris).

Blood vessels normal 0

Some blood vessels definitely hyperemic (injected) 1

Diffuse, crimson color, individual vessels not easily discernible *2

Diffuse beefy red *3

Chemosis (refers to lids and/or nictitating membranes)

No swelling 0

Any swelling above normal (includes nictitating membranes) 1

Obvious swelling with partial eversion of lids *2

Swelling with lids about half closed *3

Swelling with lids more than half-closed *4

*Starred figures indicate positive grades.

[9] Reproduced from EPA (1998).

This page intentionally left blank

Annex III

Consideration of the Minimum Number of Animals with Positive Eye Injury Responses Required for Classification of a Chemical as an Eye Irritation Hazard

This page intentionally left blank

1.0 Summary

Current regulations under the U.S. Federal Hazardous Substances Act (FHSA) specify a classification system based on using up to three sequential tests for each substance, with six animals used per test. Decisions on further sequential testing are based on the number of positive responses observed in each test. However, current best practices for eye irritation/corrosion testing involve sequential testing of up to three animals. Therefore, an analysis was undertaken to determine the most appropriate minimum number of positive animals that should be required for FHSA eye hazard labeling based on results from a 3-animal test. The analysis compared three different classification strategies and the frequency at which each would identify substances as ocular irritants. The different response rates and the resulting classifications that would be assigned by each strategy were also compared. These analyses indicate that using a criterion of at least one positive animal in a 3-animal test as the basis for classifying an eye irritation hazard would be considered at least as protective as the current FHSA testing requirements and criteria that use 6 to 18 animals. Accordingly, a proposal is presented that includes classification criteria for a 3-animal test that will provide the same or a more protective level of hazard labeling as current FHSA requirements, while using up to 83% fewer animals.

2.0 Introduction

Physical trauma or chemical burns due to contact with workplace or household products or chemicals result in about 125,000 household eye injuries each year and approximately 2,000 job-related eye injuries per day that require medical treatment.[10,11] In order to provide warnings to consumers and workers of the potential for chemicals and products to cause eye injuries, regulatory authorities require ocular safety testing to determine if substances may cause eye damage. Testing results are then used for hazard classification and labeling of eye injury potential according to relevant national and/or international classification systems. These classification systems are intended to warn users of the potential for substances to cause eye injuries, the precautions necessary to avoid injuries, and the immediate first-aid procedures that should be followed in the case of an accidental exposure.

The guidelines for classification of ocular irritation hazard potential for substances regulated under the Federal Hazardous Substances Act (FHSA 2005) are described in 16 CFR 1500.42 (CPSC 2003). The FHSA system is based on the severity of effects for each endpoint (i.e., corneal ulceration and opacity, conjunctival redness and swelling, iritis) that occur during the first 72 hours following test substance administration with observations recorded at 24, 48, and 72 hours (**Table J-III-1**).

[10] Available at: http://www.geteyesmart.org/eyesmart/injuries/home.cfm
[11] Available at: http://www.cdc.gov/niosh/topics/eye/

Table J-III-1 FHSA Classification System (16 CFR 1500.42)

Positive Response for a Single Rabbit[1] (≥1 of the following at 24, 48, and/or 72 hours)	***In Vivo* Effect**
Corneal ulceration (other than a fine stippling) Corneal opacity ≥1 Iritis ≥1 Conjunctival swelling and/or redness ≥2	First Test - If ≥4/6 animals are positive, the test is positive. If ≤1 animal is positive, the test is negative. If 2/6 or 3/6 animals are positive, the test is repeated using a different group of six animals. Second Test - If ≥3/6 animals are positive, the test is positive. If 0/6 are positive, the test is negative. If 1/6 or 2/6 are positive, the test is repeated using a different group of six animals. Third Test - Should a third test be needed, the test is positive if ≥1/6 animals are positive. If 0/6 are positive, the test is negative.

Abbreviations: CC = conjunctival chemosis; CFR = U.S. Code of Federal Regulations; CO = corneal opacity; CR = conjunctival redness; FHSA = U.S. Federal Hazardous Substances Act; IR = iritis.

For the FHSA Classification System (2005), the testing guidelines and associated regulations are included in 16 CFR 1500.42 (CPSC 2003).

At least three animals per test (1-animal screen for corrosive/severe irritants permitted). Maximum score in any animal used for classification.

[1] The following scores are considered positive: CO or IR ≥1 or CR or CC ≥2. Therefore, CO and IR scores of 0 or CR and CC scores ≤1 are considered negative.

Current best practices for eye irritation/corrosion testing involve sequential testing of up to three animals (e.g., OECD Test Guideline 405 [OECD 2002]), given that statistical analyses demonstrated that results from rabbit eye tests using only three animals consistently agreed with the outcome of a 6-animal test (DeSousa et al. 1984; Springer et al. 1993; Talsma et al. 1988). However, as indicated in **Table J-III-1**, the current FHSA regulations for ocular hazard classification and labeling are based on using up to three sequential tests for each substance, with six animals used per test. Decisions on further sequential testing are based on the number of positive responses in each test. Therefore, there is a need to develop criteria for hazard classification and labeling under the FHSA that could be applied to results from a 3-animal test, while providing the same level of protection achieved by the more extensive testing strategy. Accordingly, an analysis was undertaken to determine the most appropriate minimum number of positive animals that should be required for FHSA eye hazard labeling if only a 3-animal test is used.

3.0 Methods

In order to determine the optimal number of positive animals that would require FHSA hazard classification and labeling, the current FHSA requirements were evaluated to determine the minimum number of animals that would be required under the sequential testing strategy to assign a definitive classification (**Table J-III-2**). The weakest possible response that is considered positive by the FHSA classification system is 22% (2/6+1/6+1/6 or 4/18). However, it is possible for an even higher positive response rate, 28% (3/6+2/6+0/6 or 5/18), to be considered negative according to the FHSA system (see **Table J-III-2**). Ideally, a classification system should not produce such internal inconsistencies. For this evaluation, the current sequential testing strategy used to assign an FHSA classification, which could use up to 18 animals, is designated as Strategy 1.

Because all of the Draize eye test data used in the NICEATM analyses are from studies that used no more than six animals, NICEATM also evaluated a potential criterion where a minimum of one positive out of three animals (i.e., ≥33% positive animals) would be required to assign an irritant classification. For this evaluation, the ≥1/3 threshold is designated as Strategy 2.

Table J-III-2 Number of Animals Required to Assign an Irritant Classification According to the Current FHSA Requirements[1]

Positive Test Criteria for "Irritant" Classification	Positive Animals	Positive Animals	Positive Animals	Positive Animals	Positive Animals	Positive Animals
First Test	≥4/6	2/6 or 3/6	3/6	3/6	2/6	2/6
Second Test	-	≥3/6	2/6	1/6	2/6	1/6
Third Test	-	-	≥1/6	≥1/6	≥1/6	≥1/6
Minimum Number of Positive Animals for Irritant Classification	**4/6 (67%)**	**5/12 (42%)**	**6/18 (33%)**	**5/18 (28%)**	**5/18 (28%)**	**4/18 (22%)**
Maximum Number of Positive Animals for Not Labeled Classification	**1/6 (17%)**	**2/12 (17%)**	**5/18 (28%)**	**4/18 (22%)**	**4/18 (22%)**	**3/18 (17%)**

Abbreviation: FHSA = U.S. Federal Hazardous Substances Act.

[1] For the FHSA Classification System (2005), the testing guidelines and associated regulations are included in 16 CFR 1500.42 (CPSC 2003).

By comparison, the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2007) is based on a 3-animal test in which at least 67% (2/3) of animals tested must produce a positive response[12] in order to assign an irritant classification (i.e., GHS Category 2). Therefore, a threshold of 2/3 (67%) is designated as Strategy 3 but is based on the same criterion as Strategies 1 and 2, that a positive is based on a positive response at any of the three observation points, rather than the mean of the response over all three time points as currently required by the GHS classification system.

4.0 Results

In order to compare the three strategies with regard to the frequency at which each strategy would identify substances as ocular irritants, NICEATM compared a number of different response rates and the resulting classification that would be assigned by each strategy. As indicated in **Table J-III-3**, Strategy 3 will identify far fewer irritants than either Strategy 1 (current FHSA requirements) or Strategy 2. For example, if half of all animals tested with a given substance produce a positive response, on average, then Strategy 3 has only a 50% chance of classifying that substance an eye irritant, compared with 88% for Strategy 1 or Strategy 2.

[12] Based on mean values for each test animal calculated from grading at 24, 48, and 72 hours following test substance administration.

Table J-III-3 Percentage of Substances That Would Be Labeled as Ocular Irritants Based on Three Different Evaluation Strategies

Underlying Response Rate	Percentage of Substances That Would Be Labeled as Ocular Irritants		
	Strategy 1 Current FHSA[1]	Strategy 2 ≥1/3 positive animals	Strategy 3 ≥2/3 positive animals
20%	20.4%	48.8%	10.4%
40%	72.6%	78.4%	35.2%
50%	87.9%	87.5%	50.0%
75%	>99%	98.4%	84.3%

[1] For the U.S. Federal Hazardous Substances Act Classification System (2005), the testing guidelines and associated regulations are included in 16 CFR 1500.42 (CPSC 2003).

To illustrate the calculations summarized in **Table J-III-3**, suppose that, on average, 20% of all animals tested will produce a positive response. Using the current FHSA requirements, a negative classification could require either the first, second, or third tests. Based on the binomial distribution, the likelihood of observing 0/6, 1/6, 2/6, 3/6, or >3/6 positives is 0.262, 0.393, 0.246, 0.082, and 0.017, respectively. The probability that the first test will produce a negative classification is simply the sum of the likelihood of observing 0/6 and 1/6 positive responses or 0.655. Thus, 65.5% of the time, no further testing would be necessary, and the substance would not be labeled.

A second test would be needed if the first test positive outcome rate was either 2/6 or 3/6 (likelihood = 0.328). The second test would result in a negative classification if 0/6 positive responses were observed, making the likelihood of a negative classification by the second test (0.328)(0.262) or 0.086 (8.6%).

The third test would be needed if the second test showed 1/6 or 2/6 positives responses, which would occur with a likelihood of 0.639. Then the third test would produce a negative classification if 0/6 positive responses were observed. Thus, the likelihood that a negative classification will result from the third test is simply (0.328)(0.639)(0.262) or 0.055 (5.5%). Adding these three probabilities results in the overall likelihood of a negative classification of 0.655+0.086+0.055 or 0.796 (79.6%), and thus the likelihood of a positive classification by subtraction is 1-0.796 or 0.204 (20.4%; see **Table J-III-3**).

These calculations are much simpler for Strategies 2 and 3. The likelihood of a positive classification using Strategy 2 is just 1 minus the likelihood of observing 0/3 positives or 1-(0.8)(0.8)(0.8) or 0.488 (48.8%). For Strategy 3, a positive response rate of 1/3 (likelihood = 0.384) would also lead to a negative classification, making the overall likelihood of a positive classification for Strategy 3: 0.488-0.384 or 0.104 (10.4%).

Three important results are evident from **Table J-III-3**:

- Even though it uses fewer animals, Strategy 2 is more powerful than the current FHSA requirements for detecting positive response rates of 20% to 40%.
- Strategy 3 has low power in all cases considered.
- Strategy 2 is the only strategy that always regards a single positive outcome as indicating an irritant response.

For example, the current FHSA requirements may have as many as five animals showing a positive response, yet the substance is still not considered an irritant (**Table J-III-2**), whereas

Strategy 3 considers a positive response rate of 33% (1/3) to not be indicative of an irritant response.

5.0 Discussion

Given that many national and international ocular safety testing guidelines now require only three animals, it is unlikely that users are conducting ocular safety tests as described in the current FHSA requirements. Thus, an update to these hazard classification guidelines appears in order. These analyses can be used to establish criteria that are needed to maintain the same level of eye hazard classification and labeling as the current FHSA criteria using a 3-animal test. The results detailed here indicate that the minimum number of animals with a positive response in a 3-animal test required for eye hazard classification and labeling that would be considered at least equivalent to the current FHSA requirements is one of three positives (Strategy 2), rather than two of three positives (Strategy 3).

It should also be emphasized that Strategy 3 approximates the GHS classification system with one important exception: it assumes that any positive response at any time point is used for a positive animal. In contrast, the GHS classification system uses mean values for each test animal calculated from grading at 24, 48, and 72 hours following test substance administration. Therefore, the criteria for a positive response under the proposed GHS system require an even higher threshold for identifying an irritant than does Strategy 3. One can assume that the actual differences between Strategy 1 or 2 and Strategy 3, developed based on mean calculations, are even greater than presented in **Table J-III-3**. For this reason, the criteria for a positive animal response provided in the current FHSA eye hazard regulations (i.e., a positive score at any time point during the observation period) are preferred for any revised classification system, rather than a mean value calculated from three time points (as in the GHS system).

Applying these rules to revised FHSA requirements will substantially reduce the number of animals required to assign a definitive classification for ocular hazard potential of substances and materials that are regulated under the FHSA classification system. Creating hazard classification criteria that are based on a 3-animal test, rather than the currently required sequential 6-animal test that could require up to 18 animals, would have an immediate impact on reducing the number of animals required for ocular safety testing by up to six-fold.

This page intentionally left blank

Annex IV

Nature, Duration, and Severity of Ocular Lesions for 31 ECETOC Substances that are EPA Irritants (Category I, II, or III) or FHSA Irritants but Not Classified as Ocular Hazards by GHS Classification Criteria

This page intentionally left blank

Test Substance	EPA Cat.[1]	FHSA-33%[2]	FHSA HCS[3]	Animal Number[4]	Maximum Observed Draize Lesion Score[5] and Duration[6] Last day positive score present (Last day any lower score present)							
					CO	Day	IR	Day	CR	Day	CC	Day
Ethyl thioglycolate	III	Irritant	Irritant	1	3	3 (5)	1	6	3	2 (6)	2	2 (3)
				2	1	1	1	1	2	1 (2)	2	1
				3	0	-	0	-	0	-	0	-
Sodium lauryl sulfate (3%)	III	Irritant	Irritant	1	2	2	0	-	2	2 (3)	2	2
				2	2	1	1	1	2	1 (2)	2	1
				3	2	1	0	-	2	1 (3)	2	1
				4	1	1	0	-	2	2 (3)	1	(1)
				5	0	-	0	-	2	1	1	(1)
				6	0	-	0	-	1	(1)	1	(1)
Glycidyl methacrylate	III	Irritant	Irritant	1	2	3	1	3	3	1 (3)	4	1 (3)
				2	1	1	1	1	2	1 (2)	1	1
				3	1	1	0	-	2	1 (2)	1	(2)
Ethyl acetate	III	Irritant	Irritant	1	2	1	0	-	2	1 (3)	1	(2)
				2	1	1	1	1	2	1 (3)	1	(2)
				3	1	1	0	-	2	1 (3)	1	(2)
				4	1	1	0	-	1	(3)	1	(1)
2,2-Dimethyl-3-pentanol	III	Irritant	Irritant	1	2	2 (3)	0	-	1	(4)	1	(2)
				2	1	2	0	-	1	(2)	1	(1)
				3	0	-	0	-	1	(2)	0	-
Tetraaminopyrimidine sulfate	III	Irritant	Irritant	1	2	1	0	-	2	1 (3)	1	(1)
				2	1	1	0	-	2	1 (3)	1	(1)
				3	0	-	0	-	1	(3)	1	(1)

Test Substance	EPA Cat.[1]	FHSA-33%[2]	FHSA HCS[3]	Animal Number[4]	Maximum Observed Draize Lesion Score[5] and Duration[6] Last day positive score present (Last day any lower score present)							
					CO	Day	IR	Day	CR	Day	CC	Day
Cellosolve acetate	III	Irritant	Irritant	1	2	2 (3)	0	-	2	2 (7)	2	2 (3)
				2	0	-	0	-	2	1 (3)	1	(2)
				3	0	-	0	-	2	1 (2)	1	(1)
				4	0	-	0	-	1	(3)	0	-
Methyl amyl ketone	III	Irritant	Irritant	1	1	1 (3)	1	1	2	2 (3)	2	1 (3)
				2	1	1	1	1	2	1 (7)	2	1 (3)
				3	1	1	0	-	2	1 (3)	2	1 (3)
				4	0	-	0	-	1	(1)	1	(3)
Fomesafen, acid form (solid)	III	Irritant	Irritant	1	1	3	0	-	2	2 (3)	1	(7)
				2	1	2	1	1	2	2 (3)	2	1 (3)
				3	0	-	1	1	1	(7)	1	(2)
				4	0	-	0	-	2	1 (3)	1	(1)
				5	0	-	0	-	2	2 (3)	2	1 (3)
				6	0	-	0	-	1	(1)	0	-
n-Butyl acetate	III	Irritant	Inconcl	1	1	1	0	-	1	(7)	1	(1)
				2	1	1	0	-	1	(3)	1	(1)
				3	0	-	0	-	1	(3)	1	(1)
				4	0	-	0	-	1	(3)	0	-

Test Substance	EPA Cat.[1]	FHSA-33%[2]	FHSA HCS[3]	Animal Number[4]	Maximum Observed Draize Lesion Score[5] and Duration[6] Last day positive score present (Last day any lower score present)							
					CO	Day	IR	Day	CR	Day	CC	Day
Cetylpyridinium bromide (0.1%)	III	Irritant	Need 2nd test	1	1	1	0	-	0	-	0	-
				2	1	1	0	-	0	-	0	-
				3	0	-	0	-	1	(1)	0	-
				4	0	-	0	-	1	(2)	0	-
				5	0	-	0	-	1	(2)	0	-
				6	0	-	0	-	0	-	0	-
Myristyl myristate	III	Irritant	Irritant	1	1	1	2	2	2	1	0	-
				2	0	-	1	2	1	(1)	0	-
				3	0	-	0	-	3	2	1	(2)
Allyl methacrylate	III	Irritant	Need 2nd test	1	0	-	1	1	1	(3)	1	(1)
				2	0	-	0	-	2	1 (3)	1	(1)
				3	0	-	0	-	1	(3)	1	(1)
				4	0	-	0	-	1	(2)	1	(1)
				5	0	-	0	-	1	(2)	1	(1)
				6	0	-	0	-	1	(1)	0	-
Trichloroacetic acid (3%)	III	Irritant	Need 2nd test	1	0	-	0	-	2	1 (7)	2	2 (7)
				2	0	-	0	-	2	1 (7)	1	(7)
				3	0	-	0	-	2	2 (3)	1	(2)
				4	0	-	0	-	1	(3)	0	-
				5	0	-	0	-	1	(1)	0	-
				6	0	-	0	-	1	(1)	0	-

Test Substance	EPA Cat.[1]	FHSA-33%[2]	FHSA HCS[3]	Animal Number[4]	Maximum Observed Draize Lesion Score[5] and Duration[6] Last day positive score present (Last day any lower score present)							
					CO	Day	IR	Day	CR	Day	CC	Day
cis-Cyclo-octene	III	Irritant	Irritant	1	0	-	0	-	2	1 (3)	0	-
				2	0	-	0	-	2	1 (3)	0	-
				3	0	-	0	-	2	1 (3)	0	-
				4	0	-	0	-	2	1 (2)	0	-
				5	0	-	0	-	1	(2)	0	-
				6	0	-	0	-	2	1 (3)	0	-
N,N-Dimethylguanidine sulfate	III	Irritant	Irritant	1	0	-	0	-	2	2 (4)	0	-
				2	0	-	0	-	2	4	1	(2)
				3	0	-	0	-	2	3 (4)	1	(4)
Toluene	III	Irritant	Irritant	1	0	-	0	-	2	3	2	2 (3)
				2	0	-	0	-	1	(7)	1	(3)
				3	0	-	0	-	2	2 (7)	2	2 (3)
				4	0	-	0	-	2	1 (3)	2	1 (3)
2,4-Difluoronitrobenzene	III	Irritant	Need 2nd test	1	0	-	0	-	2	3 (7)	1	(3)
				2	0	-	0	-	2	3	2	3
				3	0	-	0	-	1	3	0	-
				4	0	-	0	-	1	(3)	0	-
				5	0	-	0	-	1	(3)	0	-
				6	0	-	0	-	1	(3)	0	-

Test Substance	EPA Cat.[1]	FHSA-33%[2]	FHSA HCS[3]	Animal Number[4]	Maximum Observed Draize Lesion Score[5] and Duration[6] Last day positive score present (Last day any lower score present)							
					CO	Day	IR	Day	CR	Day	CC	Day
Tween 20	III	Irritant	Inconcl	1	0	-	0	-	2	1(3)	1	(1)
				2	0	-	0	-	2	1 (3)	1	(1)
				3	0	-	0	-	1	(3)	0	-
				4	0	-	0	-	1	(1)	0	-
1,5-Hexadiene	III	Irritant	Need 2nd test	1	0	-	0	-	2	1 (3)	1	(1)
				2	0	-	0	-	1	(3)	1	(1)
				3	0	-	0	-	1	(3)	1	(1)
				4	0	-	0	-	2	1 (3)	1	(1)
				5	0	-	0	-	1	1	0	-
				6	0	-	0	-	1	(1)	0	-
Triton X-100 (1%)	III	Irritant	Need 2nd test	1	0	-	0	-	2	1 (2)	0	-
				2	0	-	0	-	2	1	0	-
				3	0	-	0	-	0	-	0	-
				4	0	-	0	-	0	-	0	-
				5	0	-	0	-	0	-	0	-
				6	0	-	0	-	0	-	0	-
1,5-Dibromopentane	III	Irritant	Inconcl	1	0	-	0	-	2	1 (2)	1	(1)
				2	0	-	0	-	1	(1)	1	(2)
				3	0	-	0	-	1	(1)	0	-
1,4-Dibromobutane	III	Irritant	Inconcl	1	0	-	0	-	2	1	2	1
				2	0	-	0	-	1	(1)	1	(1)
				3	0	-	0	-	0	-	0	-

Test Substance	EPA Cat.[1]	FHSA-33%[2]	FHSA HCS[3]	Animal Number[4]	Maximum Observed Draize Lesion Score[5] and Duration[6] Last day positive score present (Last day any lower score present)							
					CO	Day	IR	Day	CR	Day	CC	Day
Thiodiglycol	III	Irritant	Inconcl	1	0	-	0	-	2	1 (2)	1	(1)
				2	0	-	0	-	1	(2)	1	(2)
				3	0	-	0	-	0	-	0	-
iso-Myristyl alcohol	III	Irritant	Inconcl	1	0	-	0	-	1	(3)	2	1 (2)
				2	0	-	0	-	1	(1)	1	(1)
				3	0	-	0	-	0	-	0	-
Ethyl trimethyl acetate	III	NL	NL	1	1	3	0	-	1	(3)	1	(3)
				2	0	-	0	-	1	(2)	1	(2)
				3	0	-	0	-	1	(2)	1	(2)
				4	0	-	0	-	1	(2)	0	-
				5	0	-	0	-	1	(1)	0	-
				6	0	-	0	-	0	-	0	-
1,5-Dimethyl cyclo-octadiene	III	NL	NL	1	1	2	0	-	1	(3)	0	-
				2	0	-	0	-	1	(7)	0	-
				3	0	-	0	-	1	(1)	0	-
				4	0	-	0	-	1	(1)	0	-
				5	0	-	0	-	1	(1)	0	-
				6	0	-	0	-	1	(1)	0	-
Methyl isobutyl ketone	III	NL	Inconcl	1	1	1	0	-	2	1 (2)	0	-
				2	0	-	0	-	1	(3)	0	-
				3	0	-	0	-	1	(2)	1	(1)
				4	0	-	0	-	1	(2)	1	(1)

Test Substance	EPA Cat.[1]	FHSA-33%[2]	FHSA HCS[3]	Animal Number[4]	Maximum Observed Draize Lesion Score[5] and Duration[6] Last day positive score present (Last day any lower score present)							
					CO	Day	IR	Day	CR	Day	CC	Day
Styrene	III	NL	Inconcl	1	1	1	0	-	1	(7)	1	(3)
				2	0	-	0	-	1	(3)	1	(1)
				3	0	-	0	-	1	(2)	1	(1)
				4	0	-	0	-	1	(1)	1	(1)
Methyl cyclopentane	III	NL	NL	1	0	-	0	-	2	2 (3)	1	(2)
				2	0	-	0	-	1	(2)	0	-
				3	0	-	0	-	1	(2)	0	-
				4	0	-	0	-	1	(2)	0	-
				5	0	-	0	-	1	(2)	0	-
				6	0	-	0	-	1	(2)	0	-

Abbreviations: Cat. = category; CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; EPA = U.S. Environmental Protection Agency; FHSA = Federal Hazardous Substances Act; HCS = FHSA Hazard Communication Standard; Inconcl = Inconclusive; IR = iritis; NL = Not Labeled (as irritant).

[1] Substances classified using the EPA hazard classification based on the EPA Label Review Manual (EPA 2003).

[2] Substances classified based on the FHSA HCS using a proportionality rule of 33% for studies with fewer than six animals.

[3] Current FHSA HCS classification (16 CFR 1500.42).

[4] The animal number represents the sequence of lesion severity in a study from most severe to least severe where CO>IR>CR>CC and does not correlate to the animal number used in the study report.

[5] Maximum score observed in the Draize rabbit eye test.

[6] Duration of lesions is expressed as the last day in which an FHSA positive score of CO or IR ≥1 or CR or CC ≥2 was present and the last day in which any lower lesion score was present and in parentheses the last day in which any lower lesion score was present.

This page intentionally left blank

Annex V

OECD Database: Comparison of Percent Irritant Classification Using EPA, FHSA, and GHS Classification Systems

This page intentionally left blank

Classification System/ Category	NICEATM DRD Analyses (2010) Number of Substances Classified (%)	Number of Substances Excluded from NICEATM Database	Rationale for Exclusion	OECD DRD Analyses (1999) Number of Substances Classified (%)	Number of Substances Excluded from OECD DRD Database	Rationale for Exclusion
EPA I	41/130 (32)	14	Reversibility of positive lesions not determined	34/139 (25)	5 *NOTE: All are EPA Category IV in NICEATM analyses (no positive animals any test)*	N = 4 3-animal tests N = 1 4-animal tests
EPA II	22/130 (17)			42/139 (30)		
EPA III	44/130 (34)			47/139 (34)		
EPA I, II, or III	107/130 (82)			123/139 (89)		
GHS 1	38/125 (30)	19	Reversibility of positive lesions not determined	39/143 (27)	1 *NOTE: GHS Not Classified in NICEATM analyses (no positive animals any test)*	N = 1 4-animal tests
GHS 2A	22/125 (18)			31/143 (22)		
GHS 2B	9/125 (7)			12/143 (8)		
GHS 1, 2A, or 2B	69/125 (55)			82/143 (57)		
FHSA Irritant (33% threshold)	112/142 (79)	2	N = 2 1-animal tests	-	-	-
FHSA Irritant (16 CFR 1500.42)	102/131 (78)	13	N = 2 1-animal tests N = 11 (inconclusive)	83/106 (78)	38	N = 2 1-animal tests N = 25 3-animal tests N = 4 4-animal tests N = 7 6-animal tests (inconclusive)

Abbreviations: DRD = Detailed Review Document; EPA = U.S. Environmental Protection Agency; FHSA = Federal Hazardous Substances Act; GHS = Globally Harmonized System; NICEATM = National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods; OECD = Organisation for Economic Co-operation and Development; N = number.

This page intentionally left blank

Annex VI

Nature, Duration, and Severity of Ocular Lesions for 33 OECD DRD Substances that are EPA Irritants (Category I, II, or III) or FHSA Irritants but Not Classified as Ocular Hazards by GHS Classification Criteria

Test Substance	EPA Category[1]	FHSA-33%[2]	FHSA HCS[3]	Animal Number[4]	Maximum Observed Draize Lesion Score[5] and Duration[6] Last day positive score present (Last day any lower score present)							
					CO	Day	IR	Day	CR	Day	CC	Day
PROD-00125	III	Irritant	Irritant	1	2	2	1	1	2	1 (3)	2	1 (3)
				2	2	2	0	-	2	2 (3)	1	(1)
				3	0	0	1	1	2	1 (3)	1	(2)
				4	0	-	0	-	2	1 (3)	1	(2)
				5	0	-	0	-	2	1 (2)	1	(2)
				6	0	-	0	-	2	1 (2)	1	(1)
PROD-00215	III	Irritant	Irritant	1	2	2 (3)	0	-	2	2 (7)	2	2 (3)
				2	0	-	0	-	2	1 (3)	1	(2)
				3	0	-	0	-	2	1 (2)	1	(1)
				4	0	-	0	-	1	(3)	0	-
PROD-00214	III	Irritant	Irritant	1	2	1	0	-	2	1 (3)	1	(2)
				2	1	1	1	1	2	1 (3)	1	(2)
				3	1	1	0	-	2	1 (3)	1	(2)
				4	1	1	0	-	1	(3)	1	(1)
PROD-00094	II	Irritant	Needs 2nd test	1	2	1 (2)	0	-	1	(1)	0	-
				2	1	7	0	-	1	(2)	1	(1)
				3	1	4	0	-	0	-	0	-
				4	0	-	0	-	0	-	0	-
				5	0	-	0	-	0	-	1	(1)
				6	0	-	0	-	0	-	0	-
PROD-00143	III	Irritant	Irritant	1	1	3	1	3	1	(7)	2	2 (7)
				2	1	3	1	2	2	2 (7)	2	1 (3)
				3	1	3	1	3	1	(7)	2	2 (3)
				4	1	2	1	2	2	2 (7)	2	1 (7)
				5	0	-	1	2	1	(7)	1	(7)
				6	0	-	1	1	1	(3)	1	(2)

Test Substance	EPA Category[1]	FHSA-33%[2]	FHSA HCS[3]	Animal Number[4]	Maximum Observed Draize Lesion Score[5] and Duration[6] Last day positive score present (Last day any lower score present)							
					CO	Day	IR	Day	CR	Day	CC	Day
PROD-00129	III	Irritant	Irritant	1	1	3	0	-	2	3	2	3
				2	1	2	0	-	2	2 (3)	2	2 (3)
				3	1	2	0	-	2	2 (3)	2	1 (3)
				4	1	1	0	-	2	2 (3)	2	1 (2)
				5	0	-	0	-	2	1 (3)	2	1 (3)
				6	0	-	0	-	2	1 (3)	1	(3)
PROD-00091	III	Irritant	Irritant	1	1	4	0	-	2	2 (3)	1	(4)
				2	1	2	1	1	2	2 (3)	2	1 (4)
				3	0	-	1	1	1	(7)	1	(2)
				4	0	-	0	-	2	2 (4)	2	1 (2)
				5	0	-	0	-	2	1 (3)	1	(1)
				6	0	-	0	-	1	(1)	0	-
PROD-00056	III	IrritantI	Inconcl	1	1	2	1	2	1	(1)	0	-
				2	1	2	0	-	0	-	0	-
				3	0	-	0	-	1	(2)	0	-
				4	0	-	0	-	0	-	0	-
PROD-00213	III	Irritant	Inconcl	1	1	1	0	-	1	(7)	1	(1)
				2	1	1	0	-	1	(3)	1	(1)
				3	0	-	0	-	1	(3)	0	-
				4	0	-	0	-	1	(3)	1	(1)
PROD-00134	II	Irritant	Irritant	1	1	1	0	-	2	7 (10)	2	1 (2)
				2	0	-	0	-	2	1 (7)	2	1 (3)
				3	0	-	0	-	2	1 (7)	2	1 (2)

Test Substance	EPA Category[1]	FHSA-33%[2]	FHSA HCS[3]	Animal Number[4]	Maximum Observed Draize Lesion Score[5] and Duration[6] Last day positive score present (Last day any lower score present)							
					CO	Day	IR	Day	CR	Day	CC	Day
PROD-00120	III	Irritant	Irritant	1	1	1	0	-	1	(2)	1	(2)
				2	0	-	0	-	2	1 (7)	1	(7)
				3	0	-	0	-	2	1 (7)	1	(2)
				4	0	-	0	-	2	1 (2)	1	(2)
				5	0	-	0	-	2	1 (7)	1	(2)
				6	0	-	0	-	1	(2)	1	(1)
PROD-00093	III	Irritant	Needs 2nd test	1	1	1	0	-	1	(2)	2	1 (2)
				2	0	-	1	1	2	1 (2)	3	1 (2)
				3	0	-	0	-	1	(2)	1	(1)
				4	0	-	0	-	1	(2)	1	(1)
				5	0	-	0	-	1	(2)	0	-
				6	0	-	0	-	1	(1)	1	(1)
PROD-00105	III	Irritant	Needs 2nd test	1	0	-	1	1	2	2 (3)	3	2 (3)
				2	0	-	1	1	2	1 (3)	1	(1)
				3	0	-	1	1	1	(2)	1	(1)
				4	0	-	0	-	1	(1)	1	(1)
				5	0	-	0	-	1	(1)	0	-
				6	0	-	0	-	1	(1)	0	-
PROD-00075	III	Irritant	Irritant	1	0	-	1	1	1	(1)	0	-
				2	0	-	1	1	1	(1)	0	-
				3	0	-	1	1	1	(1)	0	-
				4	0	-	0	-	2	1 (2)	0	-
				5	0	-	0	-	1	(1)	0	-
				6	0	-	0	-	1	(1)	0	-

Test Substance	EPA Category[1]	FHSA-33%[2]	FHSA HCS[3]	Animal Number[4]	Maximum Observed Draize Lesion Score[5] and Duration[6] Last day positive score present (Last day any lower score present)							
					CO	Day	IR	Day	CR	Day	CC	Day
PROD-00103	III	Irritant	Irritant	1	0	-	1	1	3	1 (3)	2	1 (2)
				2	0	-	1	1	2	2 (3)	2	1 (3)
				3	0	-	0	-	3	1 (3)	1	(2)
				4	0	-	0	-	2	1 (2)	1	(1)
				5	0	-	0	-	1	(3)	2	1 (2)
				6	0	-	0	-	1	(3)	2	1 (2)
PROD-00064	III	Irritant	Irritant	1	0	-	1	1	1	(3)	4	1 (7)
				2	0	-	0	-	1	(3)	2	4
				3	0	-	0	-	1	(3)	2	2 (3)
				4	0	-	0	-	1	(2)	2	3
				5	0	-	0	-	1	(2)	2	2
				6	0	-	0	-	1	(1)	2	1
PROD-00118	III	Irritant	Irritant	1	0	-	1	1	3	1 (4)	3	1 (4)
				2	0	-	0	-	2	2 (4)	3	2 (4)
				3	0	-	0	-	2	1 (2)	2	1 (2)
				4	0	-	0	-	1	(2)	2	2 (4)
				5	0	-	0	-	1	(1)	1	(4)
				6	0	-	0	-	1	(1)	1	(2)
PROD-00119	III	Irritant	Inconcl	1	0	-	1	1	1	(1)	0	-
				2	0	-	0	-	1	(1)	0	-
				3	0	-	0	-	1	(1)	0	-
PROD-00124	III	Irritant	Irritant	1	0	-	0	-	2	2 (7)	2	2 (7)
				2	0	-	0	-	2	2 (4)	3	1 (4)
				3	0	-	0	-	2	2 (3)	1	(2)
				4	0	-	0	-	2	1 (2)	2	1 (2)
				5	0	-	0	-	1	(3)	1	(2)
				6	0	-	0	-	1	(2)	1	(2)

Test Substance	EPA Category[1]	FHSA-33%[2]	FHSA HCS[3]	Animal Number[4]	Maximum Observed Draize Lesion Score[5] and Duration[6] Last day positive score present (Last day any lower score present)							
					CO	Day	IR	Day	CR	Day	CC	Day
PROD-00132	III	Irritant	Irritant	1	0	-	0	-	2	2 (3)	0	-
				2	0	-	0	-	2	1 (7)	1	(1)
				3	0	-	0	-	2	1 (7)	1	(1)
				4	0	-	0	-	2	1 (3)	1	(1)
				5	0	-	0	-	1	(2)	1	(2)
				6	0	-	0	-	1	(1)	1	(1)
PROD-00109	III	Irritant	Needs 2nd test	1	0	-	0	-	2	2 (3)	2	2 (3)
				2	0	-	0	-	2	1 (3)	3	1 (2)
				3	0	-	0	-	2	1(3)	2	1 (3)
				4	0	-	0	-	1	(2)	1	(2)
				5	0	-	0	-	1	(1)	1	(1)
				6	0	-	0	-	1	(1)	1	(1)
PROD-00218	III	Irritant	Needs 2nd test	1	0	-	0	-	2	2 (3)	1	(2)
				2	0	-	0	-	2	1 (7)	2	2 (7)
				3	0	-	0	-	2	1 (7)	1	(7)
				4	0	-	0	-	1	(3)	0	-
				5	0	-	0	-	1	(1)	0	-
				6	0	-	0	-	1	(1)	0	-
PROD-00131	III	Irritant	Irritant	1	0	-	0	-	2	2 (4)	2	1 (2)
				2	0	-	0	-	2	1 (4)	1	(1)
				3	0	-	0	-	2	1 (3)	2	1 (2)
PROD-00082	III	Irritant	Needs 2nd test	1	0	-	0	-	2	1 (2)	1	(1)
				2	0	-	0	-	2	1 (2)	1	(2)
				3	0	-	0	-	1	(2)	1	(1)
				4	0	-	0	-	1	(1)	0	-
				5	0	-	0	-	0	-	0	-
				6	0	-	0	-	0	-	0	-

Test Substance	EPA Category[1]	FHSA-33%[2]	FHSA HCS[3]	Animal Number[4]	Maximum Observed Draize Lesion Score[5] and Duration[6] Last day positive score present (Last day any lower score present)							
					CO	Day	IR	Day	CR	Day	CC	Day
PROD-00108	III	Irritant	Irritant	1	0	-	0	-	2	1	2	1 (3)
				2	0	-	0	-	2	1	2	1
				3	0	-	0	-	1	(1)	2	1
				4	0	-	0	-	1	(1)	2	1
				5	0	-	0	-	1	(2)	1	(7)
				6	0	-	0	-	1	(3)	2	2 (3)
PROD-00150	III	Irritant	Inconcl	1	0	-	0	-	2	2 (3)	1	(1)
				2	0	-	0	-	1	(2)	0	-
				3	0	-	0	-	1	(2)	0	-
PROD-00212	III	NL	NL	1	1	3	0	-	1	(3)	1	(3)
				2	0	-	0	-	1	(2)	1	(2)
				3	0	-	0	-	1	(2)	1	(2)
				4	0	-	0	-	1	(2)	0	-
				5	0	-	0	-	1	(1)	0	-
				6	0	-	0	-	0	-	0	-
PROD-00133	III	NL	Needs 2nd test	1	0	-	0	-	2	1 (3)	1	(1)
				2	0	-	0	-	1	(4)	1	(1)
				3	0	-	0	-	1	(2)	2	1 (2)
				4	0	-	0	-	1	(2)	1	(1)
				5	0	-	0	-	1	(1)	1	(1)
				6	0	-	0	-	1	(1)	1	(1)
PROD-00084	III	NL	NL	1	0	-	0	-	2	1	0	-
				2	0	-	0	-	1	(2)	0	-
				3	0	-	0	-	0	-	0	-
				4	0	-	0	-	0	-	0	-
				5	0	-	0	-	0	-	0	-
				6	0	-	0	-	0	-	0	-

Test Substance	EPA Category[1]	FHSA-33%[2]	FHSA HCS[3]	Animal Number[4]	Maximum Observed Draize Lesion Score[5] and Duration[6] Last day positive score present (Last day any lower score present)							
					CO	Day	IR	Day	CR	Day	CC	Day
PROD-00107	III	NL	NL	1	0	-	0	-	2	1 (7)	2	1 (2)
				2	0	-	0	-	1	(7)	0	-
				3	0	-	0	-	1	(3)	0	-
				4	0	-	0	-	1	(3)	0	-
				5	0	-	0	-	1	(1)	1	(1)
				6	0	-	0	-	1	(1)	0	-
PROD-00117	III	NL	NL	1	0	-	0	-	0	-	0	-
				2	0	-	0	-	2	1	0	-
				3	0	-	0	-	1	(2)	0	-
				4	0	-	0	-	1	(1)	0	-
				5	0	-	0	-	1	(1)	0	-
				6	0	-	0	-	1	(1)	0	-
PROD-00121	III	NL	NL	1	0	-	0	-	1	(7)	0	-
				2	0	-	0	-	1	(3)	2	1 (2)
				3	0	-	0	-	1	(1)	0	-
				4	0	-	0	-	0	-	0	-
				5	0	-	0	-	0	-	0	-
				6	0	-	0	-	0	-	0	-
PROD-00128	III	NL	NL	1	0	-	0	-	0	-	1	(1)
				2	0	-	0	-	0	-	2	1 (2)
				3	0	-	0	-	0	-	1	(2)
				4	0	-	0	-	0	-	1	(1)
				5	0	-	0	-	0	-	1	(1)
				6	0	-	0	-	0	-	1	(1)

Abbreviations: CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; DRD = Detailed Review Document; EPA = U.S. Environmental Protection Agency; FHSA = Federal Hazardous Substances Act; HCS = FHSA Hazard Communication Standard; Inconcl = Inconclusive; IR = iritis; OECD = Organisation for Economic Co-operation and Development; NL = NL (as irritant).

[1] Substances classified using the EPA hazard classification based on the EPA Label Review Manual (EPA 2003).

[2] Substances classified based on the FHSA HCS using a proportionality rule of 33% for studies with fewer than six animals.

[3] Current FHSA HCS classification (16 CFR 1500.42).

[4] The animal number represents the sequence of lesion severity in a study from most severe to least severe where CO>IR>CR>CC and does not correlate to the animal number used in the study report.

[5] Maximum score observed in the Draize rabbit eye test.

[6] Duration of lesions is expressed as the last day in which an FHSA positive score of CO or IR ≥1 or CR or CC ≥2 was present and the last day in which any lower lesion score was present.

This page intentionally left blank

Annex VII

Representative Rabbit Eye Test Data Used in the NICEATM Evaluation of the GHS Classification System

Recent analyses reveal that a significant percentage of substances classified and labeled as eye irritation hazards by current U.S. hazard classification regulations[13] will not be classified and labeled as eye hazards using the United Nations Globally Harmonized System for the Classification and Labelling of Chemicals (GHS) eye irritation criteria (UN 2007). To evaluate if and to what extent using the GHS classification criteria might not identify substances as eye irritation hazards that are currently classified as eye irritation hazards by the GHS and EPA criteria, NICEATM evaluated 149 rabbit eye irritation test studies obtained from a publicly available database (ECETOC 1998). Within this database, a total of 31 substances that would require hazard labeling as eye irritants using the EPA classification criteria were "Not Classified" for eye irritation using the GHS classification criteria. Of these 31 substances, 17 produced corneal opacity and/or corneal ulceration, including seven that also produced iritis. Eight of these substances produced corneal opacity that extended beyond 48 hours after test substance administration. A representative set of data from seven Draize rabbit eye tests are provided on the pages that follow.

[13] The following lesions/scores are considered positive and therefore used to assign an EPA or FHSA irritant category: corneal opacity or iritis score ≥ 1 or conjunctival swelling or redness score ≥ 2.

This page intentionally left blank

NICEATM–ICCVAM
In Vivo
2,2-Dimethyl-3-pentanol

[Chemical intermediate used in the manufacture of flame retardants and lubricants. 2,2-Dimethyl-3-pentanol is not on the OECD or the EPA HPV chemical list.]

CASRN:	3970-62-5	**Number of Animals:**	3
Data Source:	ECETOC	**Data ID:**	Technical Report No. 48 (2); June 1998
Data Page:	59	**Study ID:**	32
Testing Lab:		**Study Date:**	
Species/Strain:	RABBIT	**Study Class:**	IN VIVO
Concentration:	100%	**Amount:**	0.1 ml
pH:		**Purity:**	97%
Substance Source:	Aldrich	**MMAS:**	8.3
Product Class:		**Chemical Class:**	ALCOHOL
EPA:	Category III	**EU:**	Not labeled
GHS:	Not labeled	**FHSA:**	Irritant (2/3 pos animals)
Physical Form:			

Animal Number 1

			HR 1	Day 1	Day 2	Day 3	Day 4	Day 7
Cornea	Opacity	**A**	2	2	2	1	0	0
	Area Involved	**B**	1	1	1	1	0	0
Iris		**C**	0	0	0	0	0	0
Conjunctiva	Redness	**D**	1	1	1	1	1	0
	Chemosis	**E**	2	1	1	0	0	0
	Discharge	**F**	2	0	0	0	0	0
EPA: Category III	**EU:** Not labeled		**GHS:** Cat2B		**FHSA:** Irritant			
Notes:								

Animal Number 2

			HR 1	Day 1	Day 2	Day 3	Day 4	Day 7
Cornea	Opacity	**A**		1	1	0	0	0
	Area Involved	**B**	1	1	1	0	0	0
Iris		**C**	0	0	0	0	0	0
Conjunctiva	Redness	**D**	1	1	1	0	0	0
	Chemosis	**E**	2	1	0	0	0	0
	Discharge	**F**	3	0	0	0	0	0
EPA: Category III	**EU:** Not labeled		**GHS:** Not labeled		**FHSA:** Irritant			
Notes: Corneal Opacity - 1 Hour - Dulling of cornea								

Animal Number 3

			HR 1	Day 1	Day 2	Day 3	Day 4	Day 7
Cornea	Opacity	**A**		0	0	0	0	0
	Area Involved	**B**	2	0	0	0	0	0
Iris		**C**	0	0	0	0	0	0
Conjunctiva	Redness	**D**	1	1	1	0	0	0
	Chemosis	**E**	2	0	0	0	0	0
	Discharge	**F**	2	0	0	0	0	0
EPA: Category IV **EU:** Not labeled **GHS:** Not labeled **FHSA:** Not labeled								
Notes: Corneal Opacity - 1 Hour - Dulling of cornea								

NICEATM–ICCVAM
In Vivo
Ethyl acetate

[Chemical used in automobile and household paints and surface coatings, paint thinners and glazes, pharmaceutical preparations, flavors and perfume essences, flexible packaging (e.g. aluminum foil and plastic films), contact cement, manufacturing of adhesives, cleaning fluids, inks, nail polish and removers, coated papers, liquid bandages, explosives, artificial leather, and photographic film. Ethyl acetate is an OECD HPV chemical with greater than 1,011 kilotons produced worldwide in 1998 and 118 kilotons produced in the U.S. in 1997.]

CASRN:	141-78-6	**Number of Animals:**	4
Data Source:	ECETOC	**Data ID:**	Technical Report No 48(2); June 1998
Data Page:	24	**Study ID:**	5
Testing Lab:		**Study Date:**	
Species/Strain:	RABBIT	**Study Class:**	IN VIVO
Concentration:	100%	**Amount:**	0.1 ml
pH:		**Purity:**	99%
Substance Source:	Fisher	**MMAS:**	15
Product Class:		**Chemical Class:**	ESTER
EPA:	Category III	**EU:**	Not labeled
GHS:	Not labeled	**FHSA:**	Irritant (4/4 pos animals)
Physical Form:			

Animal Number 1

			Day 1	Day 2	Day 3	Day 7
Cornea	Opacity	**A**	2	0	0	0
	Area Involved	**B**	1	0	0	0
Iris		**C**	0	0	0	0
Conjunctiva	Redness	**D**	2	1	1	0
	Chemosis	**E**	1	1	0	0
	Discharge	**F**	1	0	0	0
EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant						
Notes:						

Animal Number 2

			Day 1	Day 2	Day 3	Day 7
Cornea	Opacity	**A**	1	0	0	0
	Area Involved	**B**	1	0	0	0
Iris		**C**	1	0	0	0
Conjunctiva	Redness	**D**	2	1	1	0
	Chemosis	**E**	1	1	0	0
	Discharge	**F**	1	0	0	0
EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant						
Notes:						

Animal Number 3

			Day 1	Day 2	Day 3	Day 7
Cornea	Opacity	**A**	1	0	0	0
	Area Involved	**B**	1	0	0	0
Iris		**C**	0	0	0	0
Conjunctiva	Redness	**D**	2	1	1	0
	Chemosis	**E**	1	1	0	0
	Discharge	**F**	1	0	0	0
EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant						
Notes:						

Animal Number 4

			Day 1	Day 2	Day 3	Day 7
Cornea	Opacity	**A**	1	0	0	0
	Area Involved	**B**	1	0	0	0
Iris		**C**	0	0	0	0
Conjunctiva	Redness	**D**	1	1	1	0
	Chemosis	**E**	1	0	0	0
	Discharge	**F**	1	0	0	0
EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant						
Notes:						

NICEATM–ICCVAM
In Vivo
Ethyl thioglycolate

[Thioglycolic salts and esters are widely used as hair straighteners, in hair dyes and colorings, and in the manufacture of food flavoring concentrates. Thioglycolic acid and its derivatives are widely used in the fields of PVC stabilizers, down-hole acidizing, corrosion inhibition in the oil field industry, manufacturing of pharmaceuticals, agrochemicals and dyes, shrink-resistant treatment of wool, fabric dying, and leather processing. Ethyl thioglycolate is also used as a depilitory agent. Ethyl thioglycolate is not on the OECD HPV chemical list.]

CASRN:	623-51-8	**Number of Animals:**	3
Data Source:	ECETOC	**Data ID:**	Technical Report No. 48 (2); June 1998
Data Page:	222	**Study ID:**	229
Testing Lab:		**Study Date:**	
Species/Strain:	RABBIT	**Study Class:**	IN VIVO
Concentration:	100%	**Amount:**	0.1 ml
pH:		**Purity:**	99.1%
Substance Source:	Sigma	**MMAS:**	24.67
Product Class:		**Chemical Class:**	ESTER, SULFUR COMPOUND, ORGANIC, ALCOHOL
EPA:	Category III	**EU:**	Not labeled
GHS:	Not labeled	**FHSA:**	Irritant (2/3 pos animals)
Physical Form:			

Animal Number 1

			HR 1	Day 1	Day 2	Day 3	Day 5	Day 6	Day 7
Cornea	Opacity	**A**	0	1	0	0			
	Area Involved	**B**	0	2	0	0			
Iris		**C**	0	1	0	0			
Conjunctiva	Redness	**D**	1	2	1	0			
	Chemosis	**E**	2	2	0	0			
	Discharge	**F**		1	0	0			

EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant

Notes: Discharge - 1 Hr - Evaluation obscured by residual test substance

Animal Number 2

			HR 1	Day 1	Day 2	Day 3	Day 5	Day 6	Day 7
Cornea	Opacity	**A**	2	2	3	3	2	0	0
	Area Involved	**B**	4	3	1	1	1	0	0
Iris		**C**	1	1	1	1	1	1	0
Conjunctiva	Redness	**D**	1	3	3	2	1	1	0
	Chemosis	**E**	3	2	2	1	0	0	0
	Discharge	**F**		2	1	0	0	0	0

EPA: Category III **EU:** R36(posCO),R36(posI),R36(posCR) **GHS:** Cat2B **FHSA:** Irritant

Notes: Discharge - 1 Hr - Evaluation obscured by residual test substance; Day 1 - White purulent discharge; score of 2 assigned

Animal Number 3

			HR 1	Day 1	Day 2	Day 3	Day 5	Day 6	Day 7
Cornea	Opacity	**A**	0	0	0	0			
	Area Involved	**B**	0	0	0	0			
Iris		**C**	0	0	0	0			
Conjunctiva	Redness	**D**	0	0	0	0			
	Chemosis	**E**	0	0	0	0			
	Discharge	**F**	0	0	0	0			

EPA: Category IV **EU:** Not labeled **GHS:** Not labeled **FHSA:** Not labeled

Notes:

NICEATM–ICCVAM
In Vivo
Glycidyl methacrylate

[Chemical used in the production of polymer coatings and finishes, adhesives, plastics, and elastomers. Consumer exposure is unlikely (Dow Chemical Co.), but workers potentially might be exposed during manufacturing operations. Glycidyl methacrylate is an OECD HPV chemical with 3,000 tons/year produced in Japan.]

CASRN:	106-91-2	**Number of Animals:**	3
Data Source:	ECETOC	**Data ID:**	Technical Report No 48(2); June 1998
Data Page:	47	**Study ID:**	23
Testing Lab:		**Study Date:**	
Species/Strain:	RABBIT	**Study Class:**	IN VIVO
Concentration:	100%	**Amount:**	0.1 ml
pH:		**Purity:**	>99%
Substance Source:	Elf Atochem	**MMAS:**	28
Product Class:		**Chemical Class:**	ETHER
EPA:	Category III	**EU:**	Not labeled
GHS:	Not labeled	**FHSA:**	Irritant (3/3 pos animals)
Physical Form:			

Animal Number: 1

			HR 1	Day 1	Day 2	Day 3	Day 7
Cornea	Opacity	**A**		1	2	2	0
	Area Involved	**B**	4	4	2	2	0
Iris		**C**	1	1	1	1	0
Conjunctiva	Redness	**D**	2	3	2	2	0
	Chemosis	**E**	3	4	2	2	0
	Discharge	**F**	3	3	1	0	0

EPA: Category III **EU:** R36(posI),R36(posCC) **GHS:** Cat2B **FHSA:** Irritant

Notes: Corneal Opacity - 1 Hr - Dulling of the cornea

Animal Number 2

			HR 1	Day 1	Day 2	Day 3	Day 7
Cornea	Opacity	**A**		1	0	0	
	Area Involved	**B**	4	2	0	0	
Iris		**C**	1	0	0	0	
Conjunctiva	Redness	**D**	2	2	1	0	
	Chemosis	**E**	2	1	1	0	
	Discharge	**F**	3	0	0	0	
EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant							
Notes: Corneal Opacity - 1 Hr - Dulling of the cornea							

Animal Number 3

			HR 1	Day 1	Day 2	Day 3	Day 7
Cornea	Opacity	**A**		1	0	0	
	Area Involved	**B**	4	2	0	0	
Iris		**C**	1	1	0	0	
Conjunctiva	Redness	**D**	2	2	1	0	
	Chemosis	**E**	2	1	0	0	
	Discharge	**F**	2	1	0	0	
EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant							
Notes: Corneal Opacity - 1 Hr - Dulling of the cornea							

NICEATM–ICCVAM
In Vivo
Myristyl myristate

[Wax base ingredient used in personal care products and cosmetics including cleansing and moisturizing creams, blushes, rouges, eye shadow, eyeliner, and eyebrow pencils, makeup, hair shampoos and conditioners, suntan products, bath products, cuticle softeners, shaving creams, skin and baby lotions, perfumes and deodorants/anti-perspirants. Myristyl myristate is an OECD High Production Volume (HPV) chemical, which indicates that the production volume is over 1,000 tons/year worldwide, although no specific information is available. However, it is not listed as an EPA HPV chemical indicating that production volume is not over 500 tons/year in the U.S.]

CASRN:	3234-85-3	**Number of Animals:**	3
Data Source:	ECETOC	**Data ID:**	Technical Report No. 48 (2); June 1998
Data Page:	117	**Study ID:**	162
Testing Lab:		**Study Date:**	
Species/Strain:	RABBIT	**Study Class:**	IN VIVO
Concentration:	100%	**Amount:**	0.1 ml (87 mg)
pH:		**Purity:**	
Substance Source:	DS Industries ApS	**MMAS:**	7.7
Product Class:		**Chemical Class:**	ESTER
EPA:	Category III	**EU:**	Not labeled
GHS:	Not labeled	**FHSA:**	Irritant (3/3 pos animals)
Physical Form:			

Animal Number 1

			HR 1	Day 1	Day 2	Day 3
Cornea	Opacity	**A**	0	0	0	0
	Area Involved	**B**	0	0	0	0
Iris		**C**	1	0	0	0
Conjunctiva	Redness	**D**	1	1	3	0
	Chemosis	**E**	1	0	1	0
	Discharge	**F**	0	0		0
EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant						
Notes:						

Animal Number 2

			HR 1	Day 1	Day 2	Day 3
Cornea	Opacity	**A**	0	0	0	0
	Area Involved	**B**	0	0	0	0
Iris		**C**	1	0	1	0
Conjunctiva	Redness	**D**	1	1		0
	Chemosis	**E**	1		0	0
	Discharge	**F**	0	0	0	0
EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant						
Notes:						

Animal Number 3

			HR 1	Day 1	Day 2	Day 3
Cornea	Opacity	**A**	0	1	0	0
	Area Involved	**B**	0	0		0
Iris		**C**	1	0	2	0
Conjunctiva	Redness	**D**	1	2	0	0
	Chemosis	**E**	1	0	0	0
	Discharge	**F**	1	0	0	0
EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant						
Notes:						

NICEATM–ICCVAM
In Vivo
Sodium lauryl sulfate

[Ingredient in personal care products including shampoos and conditioners, soaps, toothpastes, mouthwashes, hair colorants, skin powders and cleansers, body washes, and shaving creams and in cleaning products including floor cleaners, vegetable washes, tub, tile, shower and toilet bowl cleaners, fabric glues, silver cleaners, soap-scum removers, general purpose cleaning sprays, oven cleaners, carpet cleaners, stain removers, and adhesives. It is an OECD HPV chemical with over 10,000 tons/year produced in Germany.]

CASRN:	151-21-3	**Number of Animals:**	6
Data Source:	ECETOC	**Data ID:**	Technical Report No. 48 (2); June 1998
Data Page:	174	**Study ID:**	201
Testing Lab:		**Study Date:**	
Species/Strain:	RABBIT	**Study Class:**	IN VIVO
Concentration:	3.0%	**Amount:**	0.1 ml
pH:		**Purity:**	98 %
Substance Source:	Sigma	**MMAS:**	16
Product Class:		**Chemical Class:**	SALT, ORGANIC, CARBOXYLIC ACID, SALT
EPA:	Category III	**EU:**	Not labeled
GHS:	Not labeled	**FHSA:**	Irritant (5/6 pos animals)
Physical Form:			

Animal Number 1

			Day 1	Day 2	Day 3	Day 7
Cornea	Opacity	**A**	1	0	0	0
	Area Involved	**B**	1	0	0	0
Iris		**C**	0	0	0	0
Conjunctiva	Redness	**D**	2	2	1	0
	Chemosis	**E**	1	0	0	0
	Discharge	**F**	1	1	0	0
EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant						
Notes:						

Animal Number 2

			Day 1	Day 2	Day 3	Day 7
Cornea	Opacity	**A**	2	0	0	
	Area Involved	**B**	1	0	0	
Iris		**C**	1	0	0	
Conjunctiva	Redness	**D**	2	1	0	
	Chemosis	**E**	2	0	0	
	Discharge	**F**	2	1	0	
EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant						
Notes:						

Animal Number 3

			Day 1	Day 2	Day 3	Day 7
Cornea	Opacity	**A**	2	0	0	0
	Area Involved	**B**	1	0	0	0
Iris		**C**	0	0	0	0
Conjunctiva	Redness	**D**	2	1	1	0
	Chemosis	**E**	2	0	0	0
	Discharge	**F**	2	1	0	0
EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant						
Notes:						

Animal Number 4

			Day 1	Day 2	Day 3	Day 7
Cornea	Opacity	**A**	0	0	0	
	Area Involved	**B**	0	0	0	
Iris		**C**	0	0	0	
Conjunctiva	Redness	**D**	2	0	0	
	Chemosis	**E**	1	0	0	
	Discharge	**F**	1	0	0	
EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant						
Notes:						

Animal Number 5

			Day 1	Day 2	Day 3	Day 7
Cornea	Opacity	**A**	2	2	0	0
	Area Involved	**B**	1	1	0	0
Iris		**C**	0	0	0	0
Conjunctiva	Redness	**D**	2	2	1	0
	Chemosis	**E**	2	2	0	0
	Discharge	**F**	1	1	0	0
EPA: Category III **EU:** Not labeled **GHS:** Cat2B **FHSA:** Irritant						
Notes:						

Animal Number 6

			Day 1	Day 2	Day 3	Day 7
Cornea	Opacity	**A**	0	0	0	
	Area Involved	**B**	0	0	0	
Iris		**C**	0	0	0	
Conjunctiva	Redness	**D**	1	0	0	
	Chemosis	**E**	1	0	0	
	Discharge	**F**	1	0	0	
EPA: Category IV **EU:** Not labeled **GHS:** Not labeled **FHSA:** Not labeled						
Notes:						

NICEATM–ICCVAM
In Vivo
Tetraaminopyrimidine sulfate

[Raw material used in the preparation of hair dyes and as a chemical intermediate. Tetraaminopyrimidine sulfate is not on the OECD HPV chemical list.]

CASRN:	5392-28-9	**Number of Animals:**	3
Data Source:	ECETOC	**Data ID:**	Technical Report No. 48 (2); June 1998
Data Page:	122	**Study ID:**	167
Testing Lab:		**Study Date:**	
Species/Strain:	RABBIT	**Study Class:**	IN VIVO
Concentration:	100%	**Amount:**	100 mg
pH:		**Purity:**	97%
Substance Source:	Aldrich	**MMAS:**	10.3
Product Class:		**Chemical Class:**	AMINE, HETEROCYCLE, SULFUR COMPOUND, ORGANIC
EPA:	Category III	**EU:**	Not labeled
GHS:	Not labeled	**FHSA:**	Irritant (2/3 pos animals)
Physical Form:			

Animal Number 1

			HR 1	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14
Cornea	Opacity	**A**	1	2	0	0	0	0	0
	Area Involved	**B**	1	1	0	0	0	0	0
Iris		**C**	0	0	0	0	0	0	0
Conjunctiva	Redness	**D**	2	2	1	1	0	0	0
	Chemosis	**E**	2	1	0	0	0	0	0
	Discharge	**F**	1	0	0	0	0	0	0

EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant

Notes:

Animal Number 2

			HR 1	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14
Cornea	Opacity	**A**	1	1	0	0	0	0	0
	Area Involved	**B**	1	1	0	0	0	0	0
Iris		**C**	0	0	0	0	0	0	0
Conjunctiva	Redness	**D**	2	2	1	1	0	0	0
	Chemosis	**E**	2	1	0	0	0	0	0
	Discharge	**F**	1	0	0	0	0	0	0
EPA: Category III **EU:** Not labeled **GHS:** Not labeled **FHSA:** Irritant									
Notes:									

Animal Number 3

			HR 1	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14
Cornea	Opacity	**A**	1	0	0	0	0	0	0
	Area Involved	**B**	1	0	0	0	0	0	0
Iris		**C**	0	0	0	0	0	0	0
Conjunctiva	Redness	**D**	1	1	1	1	0	0	0
	Chemosis	**E**	2	1	0	0	0	0	0
	Discharge	**F**	1	0	0	0	0	0	0
EPA: Category IV **EU:** Not labeled **GHS:** Not labeled **FHSA:** Not labeled									
Notes:									

This page intentionally left blank

Appendix K

ECVAM Scientific Advisory Committee (ESAC)

Statement on the Scientific Validity of Cytotoxicity/Cell-Function Based *In Vitro* Assays for Eye Irritation Testing

This page intentionally left blank

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
In vitro methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

STATEMENT ON THE SCIENTIFIC VALIDITY OF CYTOTOXICITY/CELL-FUNCTION BASED IN VITRO ASSAYS FOR EYE IRRITATION TESTING

At its 31st meeting, held on 7 and 8 July, 2009 at the European Centre for the Validation of Alternative Methods (ECVAM), Ispra, Italy, the non-Commission members of the ECVAM Scientific Advisory Committee (ESAC)[1] unanimously endorsed the following statement:

The replacement of traditional animal-based test methods by alternative ones should ideally be obtained by one-to-one replacements: to keep the testing regime simple and economical one single alternative method should, wherever feasible, be sufficient to generate data of equal or better quality than the traditional test.

However, in the case of eye irritation it is currently generally accepted that, in the foreseeable future, no single *in vitro* eye irritation test will be able to replace the *in vivo* Draize eye test to predict across the full range of irritation for different chemical classes. However, strategic combinations of several alternative test methods within a (tiered) testing strategy may be able to replace the Draize eye test.

A possible conceptual framework for such a (tiered) testing strategy has been developed within an ECVAM workshop (Ref. 1). The framework is based on alternative eye irritation methods that vary in their capacity to detect either severe irritant substances (EU R41; GHS 'Category 1') or substances considered non-irritant (EU 'Non-Classified'; GHS 'No Category'). According to this framework the entire range of irritancy may be resolved by arranging tests in a tiered strategy that may be operated from either end: to detect first severe irritants and resolve absence of irritancy ("Top-Down Approach") or to proceed inversely, starting with the identification of non-irritants first ("Bottom-Up Approach"). Mild irritancy will be resolved in a last tier in both approaches.

To evaluate the scientific validity of possible building blocks of such a test strategy and to assess their possible placement within a Bottom-Up and Top-Down Approach, ECVAM has undertaken a retrospective validation study of four cell-based *in vitro* methods.

The test methods evaluated were:

a. Cytosensor Microphysiometer (INVITTOX Protocols 97 and 102 modified)[2]
b. Fluorescein Leakage (INVITTOX Protocols 71, 82, 86 and120);
c. Neutral Red Release (INVITTOX Protocol 54 and PREDISAFE™);
d. Red Blood Cell haemolysis (INVITTOX Protocols 37 and 99),

The four test methods, including ten protocol variations, were subjected to independent, expert review with respect to their use to either

[1] Details can be found in the PRP report
[2] Invittox protocols can be downloaded from ECVAM's database service on Alternative Methods to Animal Experimentation, DBALM: http://ecvam-dbalm.jrc.ec.europa.eu

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
In vitro methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

a) initiate a Bottom-Up Approach, for consideration for regulatory use to identify non-irritants (EU: 'Non Classified'; GSH: 'No Category'; EPA: 'Category IV') from all other classes as part of a tiered testing strategy, or
b) to initiate a Top-Down Approach, to identify ocular corrosives and severe irritants (EU R41, GHS 'Category 1', and EPA 'Category I') from all other classes as part of a tiered testing strategy.

In the absence of internationally agreed performance criteria for either approach, the PRP of the ESAC applied the following criteria:

- any test used to initiate a Top-Down Approach must balance specificity and sensitivity to correctly identify a substantial proportion of severe irritants, with a false positive rate that would not lead to the over-classification of an unreasonable number of materials of lower ocular irritancy potential – an over-classification rate (false positives) of <10% was considered acceptable
- any test used to initiate a Bottom-Up Approach should ideally give no false negatives with respect to human safety, and no false negative should be produced by high-moderate or severe irritants.

Following independent ESAC peer review of this retrospective validation study and considering the potential test strategies in which the tests may be used, the ESAC concluded the following:

1. CYTOSENSOR MICROPHYSIOMETER TEST METHOD

The Cytosensor Microphysiometer test method can be used for two of the three EU and GHS classification categories used for the endpoint of ocular irritation:

A. The **Cytosensor Microphysiometer test method (INVITTOX Protocol 102 modified)** is considered to have been scientifically validated and to be ready for consideration for regulatory use as an initial step within a **Top-Down Approach** to identify ocular corrosives and severe irritants (EU R41, GHS Category 1, and EPA Category I) from all other classes for the chemical applicability domain of water-soluble chemicals (substances and mixtures).

B. Furthermore, the **Cytosensor Microphysiometer test method (INVITTOX Protocol 102 modified)** is considered to have been scientifically validated and to be ready for consideration for regulatory use as an initial step within a **Bottom-Up Approach** to identify non-irritants (EU:NC; GHS: NC; EPA: cat IV) from all other classes only for water-soluble surfactants and water-soluble surfactant-containing mixtures.

C. On the basis of a thorough evaluation of the data compiled in the course of the ECVAM validation study, the ESAC concludes that the **Cytosensor Microphysiometer** test method does NOT correctly identify moderate and mild ocular irritants (EU: R36; GHS: Cat 2A/B; EPA: Cat II/III). Therefore, the test method can only be employed to make decisions on two of the three categories of the eye irritation classification scheme (see A and B). Consequently, ESAC does NOT recommend this test method as a full replacement method. It should be noted in this context that the **Top-Down and Bottom-Up Approach** foresees the theoretical possibility of a *default* mild/moderate categorization (e.g. EU R36 or GHS Cat 2) of all those substances neither identified as ocular corrosives and severe irritants (see A) nor as "non-

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
In vitro methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

classified" substances (see B) in the first two tiers of the strategy. However, the test method's high false negative rate (9-55%) when initiating a top-down approach and high false positive rate (50-69%) when initiating a bottom-up approach exclude the possibility to use the method for default categorization. The test methods can thus not be considered a full-replacement method on its own using the Top-Down and Bottom-Up approach.

Although these recommendations are based on the evaluation of data sets obtained using specific hard- and software, it is anticipated that other Cytosensor Microphysiometer equipment and software may become available with either equivalent or better performance and will need to be efficiently validated. Depending on the similarity of new equipment with respect to the validated one, this may be performed as a *Similar Method Validation* ('me-too') or an *Update Validation*. ESAC therefore recommends the development of Performance Standards for the Cytosensor Microphysiometer test method.

The current chemical applicability domain is limited: whilst in some cases this might be increased by expanding the data set of studied compounds, the test method is not amenable to testing non-water soluble solids, suspensions, or viscous materials.

2. FLUORESCEIN LEAKAGE TEST METHOD

The **Fluorescein Leakage test method (INVITTOX Protocol 71)** is considered to have been scientifically validated and to be ready for consideration for regulatory use as an initial step within a **Top-Down Approach** to identify ocular corrosives and severe irritants (EU R41, GSH Category 1, and EPA Category I) from all other classes for water-soluble chemicals (substances and mixtures).

Additional testing and further refinement, in particular with respect to variability and definition of the applicability domain, by expanding the dataset of tested chemicals and direct comparison with *in vivo* data is recommended and should be kept under review.

With regard to the

- Neutral Red Release (INVITTOX Protocol 54 and PREDISAFE™);
- Fluorescein Leakage (INVITTOX Protocols 82, 86 and120);
- Red Blood Cell haemolysis (INVITTOX Protocols 37 and 99),

ESAC considers that the available evidence is insufficient[3] to support a recommendation that they are ready for consideration for regulatory use.

Similarly, the available evidence for Fluorescein Leakage INVITTOX Protocol 71 does not support a recommendation for its use to initiate a Bottom-Up Approach for regulatory use.

[3] Details can be found in the PRP report

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
In vitro methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

This statement takes account of the dossiers prepared for peer review; the views of independent experts of the ESAC Peer Review Panel (PRP) who evaluated the dossiers against defined validation criteria as well as supplementary submissions made by the Validation Management Group.

In agreement with common practice upon completion of a validation study, ESAC recommends the development of Performance Standards for the Cytosensor Microphysiometer and the Fluorescein Leakage assays to allow the validation of *similar test methods* or *modifications of the validated test methods* based on pre-defined evaluation and acceptance criteria.

Joachim Kreysa

Head of Unit

In vitro methods Unit

European Centre for the Validation of Alternative Methods

Ispra, 10th July 2009

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
In vitro methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

153 **REFERENCECS**

154

155 1. Scott, L. et al. (2009) A proposed eye irritation testing strategy to reduce and replace
156 in vivo studies using Bottom-Up and Top-Down approaches. Toxicol In Vitro. May
157 31. *[Epub ahead of print]*

158

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
In vitro methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

The ESAC was established by the European Commission, and is composed of nominees from the EU Member States, industry, academia and animal welfare organisations, together with representatives of the relevant Commission services.

This statement was endorsed by the following members of the ESAC:

Ms Argelia Castaño(Spain)
Ms Maija Dambrova (Latvia)
Ms Alison Gray (ESTIV)
Ms Katalin Horvath (Hungary)
Ms Dagmar Jírová (Czech Republic)
Mr Roman Kolar (Eurogroup for Animals)
Ms Elisabeth Knudsen (Denmark - acting as moderator at the meeting)
Mr Manfred Liebsch (Germany)
Mr Gianni Dal Negro (EFPIA)
Mr. Walter Pfaller (Austria)
Mr Tõnu Püssa (Estonia)
Mr Dariusz Sladowski (Poland)
Mr Jon Richmond (UK)
Ms Vera Rogiers (ECOPA)
Mr Michael Ryan (Ireland)
Ms Annalaura Stammati (Italy)
Mr Jan van der Valk (The Netherlands)
Mr Carl Westmoreland (COLIPA)
Mr Timo Ylikomi (Finland)

The following Commission Services and Observer Organisations were involved in the consultation process, but not in the endorsement process itself:

Commission services
Mr Joachim Kreysa (DG JRC, Head of In vitro methods Unit/ECVAM, chairman)
Mr Claudius Griesinger (DG JRC, ESAC secretariat)
Ms Susanne Hoke (DG ENTR)
Ms Susanna Louhimies (DG ENV)
Mr Juan Riego Sintes (DG JRC)

The following observers were present
Mr Hajime Kojima (JaCVAM)
Mr William Stokes (NICEATM)
Ms Marilyn Wind (ICCVAM)