TABLE OF CONTENTS

APPENDICES ..... **vi**
LIST OF TABLES ..... **vii**
LIST OF FIGURES ..... **ix**
LIST OF ACRONYMS AND ABBREVIATIONS ..... **x**
ACKNOWLEDGEMENTS ..... **xiii**
PREFACE ..... **xvii**
EXECUTIVE SUMMARY ..... **xxi**

1.0 Introduction and Rationale for the Proposed Use of *In Vitro* Test Methods to Identify Ocular Corrosives and Severe Irritants **1-1**
1.1 Introduction 1-1
1.1.1 Historical Background of *In Vitro* Ocular Irritation/Corrosion Test Methods and Rationale for Their Development 1-1
1.1.2 Peer Reviews of the ICE Test Method 1-6
1.2 Scientific Basis for the ICE Test Method 1-6
1.2.1 Purpose and Mechanistic Basis of the ICE Test Method 1-6
1.2.2 Similarities and Differences of Modes and Mechanisms of Action Between the ICE Test Method and Ocular Irritancy in Humans and/or Rabbits 1-7
1.2.2.1 The Mammalian Eye: Common Anatomy of the Human and Rabbit Eye 1-7
1.2.2.2 Differences Between Human and Rabbit Eyes 1-9
1.2.2.3 The *In Vivo* Rabbit Eye Test Method 1-9
1.2.2.4 Differences Between the Chicken and Mammalian Eye 1-13
1.2.2.5 Comparison of the ICE Test Method with the *In Vivo* Rabbit Eye Test Method 1-13
1.2.3 Intended Range of Substances Amenable to the ICE Test Method and/or Limits of the ICE Test Method 1-14
1.3 Regulatory Rationale and Applicability 1-15
1.3.1 Current Regulatory Testing Requirements and ICCVAM Prioritization Criteria 1-15
1.3.2 Intended Uses of the ICE Test Method 1-16
1.3.3 Similarities and Differences in the Endpoints Measured in the ICE and the *In Vivo* Reference Test Method 1-16
1.3.4 Use of Proposed Test Method in Overall Strategy of Hazard or Safety Assessment 1-17
1.4 Validation of the ICE Test Method 1-17
1.5 Search Strategies and Selection of Citations for the ICE BRD 1-19

2.0 ICE Test Method Protocol Components **2-1**
2.1 Overview of How the ICE Test Method is Conducted 2-1
2.2 Description and Rationale for the Test Method Components 2-1
2.2.1 Materials, Equipment, and Supplies Needed 2-2
2.2.1.1 Sources of Chicken Eyes 2-2

2.2.1.2 Preparation of the Eyes 2-2
2.2.1.3 ICE Experimental Setup 2-3
2.2.2 Dose-Selection Procedures, Including the Need for Any Dose Range-Finding Studies or Acute Toxicity Data Prior to Conducting a Study 2-4
2.2.3 Endpoints Measured 2-4
2.2.4 Duration of Exposure 2-5
2.2.4.1 Quantity of Test Substance Applied 2-5
2.2.4.2 Application of the Test Substance 2-5
2.2.5 Known Limits of Use 2-5
2.2.6 Nature of the Response Assessed 2-6
2.2.6.1 Data to be Collected 2-6
2.2.7 Appropriate Controls and the Basis for their Selection 2-6
2.2.7.1 Negative Controls 2-6
2.2.7.2 Positive Controls 2-6
2.2.7.3 Solvent Controls 2-6
2.2.7.4 Benchmark Controls 2-6
2.2.8 Acceptable Range of Control Responses and the Basis for the Acceptable Ranges 2-7
2.2.8.1 Negative Controls 2-7
2.2.8.2 Positive Controls 2-7
2.2.8.3 Solvent Controls 2-7
2.2.8.4 Benchmark Controls 2-7
2.2.9 Nature of the Data to be Collected and the Methods Used for Data Collection 2-7
2.2.9.1 Corneal Swelling 2-7
2.2.9.2 Corneal Opacity 2-8
2.2.9.3 Fluorescein Retention 2-8
2.2.9.4 Morphological Effects 2-8
2.2.9.5 Methods for Collection of Numerical Data 2-8
2.2.10 Type of Media in Which Data Are Stored 2-9
2.2.11 Measures of Variability 2-9
2.2.12 Statistical or Non-Statistical Methods Used to Analyze the Resulting Data 2-10
2.2.12.1 Irritation Index 2-10
2.2.13 Decision Criteria and the Basis for the Prediction Model Used to Classify a Test Chemical 2-11
2.2.13.1 Interpretation of Endpoint Scores 2-11
2.2.13.2 Defining the Irritancy Classification 2-12
2.2.14 Information and Data that Will Be Included in the Study Report and Availability of Standard Forms for Data Collection and Submission 2-13
2.3 Basis for Selection of the Test System 2-14
2.4 Proprietary Components 2-15
2.5 Basis for Number of Replicate and Repeat Experiments 2-16
2.5.1 Within Experiment Replicates 2-16

2.5.2 Experimental Replicates 2-16
2.6 Compliance with Good Laboratory Practice 2-16
2.7 Study Acceptance Criteria....2-16

3.0 Substances Used For Validation of the ICE Test Method....3-1
3.1 Rationale for the Substances or Products Selected for Use....3-1
3.1.1 Prinsen and Koëter (1993) 3-1
3.1.2 Balls et al. (1995) 3-1
3.1.3 Prinsen (1996) 3-2
3.1.4 Prinsen (2000) 3-2
3.1.5 Prinsen (2005) 3-2
3.2 Rationale for the Number of Substances Tested....3-2
3.3 Chemicals or Products Evaluated 3-2
3.3.1 Prinsen and Koëter (1993) 3-4
3.3.2 Balls et al. (1995) 3-5
3.3.3 Prinsen (1996) 3-5
3.3.4 Prinsen (2000) 3-5
3.3.5 Prinsen (2005) 3-5
3.4 Coding Procedures Used in the Validation Studies 3-5
3.4.1 Prinsen and Koëter (1993) 3-5
3.4.2 Balls et al. (1995) 3-5
3.4.3 Prinsen (1996) 3-6
3.4.4 Prinsen (2000) 3-6
3.4.5 Prinsen (2005) 3-6

4.0 *In Vivo* Reference Data Used for an Assessment Of ICE Test Method Accuracy 4-1
4.1 Description of Protocol Used to Generate *In Vivo* Data 4-1
4.1.1 Draize Rabbit Eye Test....4-1
4.1.2 Current *In Vivo* Ocular Irritation Test Method Protocols....4-1
4.1.3 Current *In Vivo* Ocular Irritancy Classification Systems....4-7
4.2 Detailed Reference Data Used to Assess *In Vitro* Test Method Accuracy....4-8
4.3 *In Vivo* Classification Criteria Used for Analysis....4-10
4.3.1 GHS Classification Rules Used for BRD Analysis....4-10
4.3.2 EPA Classification Rules Used for BRD Analysis 4-12
4.3.3 EU Classification Rules Used for BRD Analysis 4-12
4.4 Availability of Original Records for the *In Vivo* Reference Data....4-13
4.5 *In Vivo* Data Quality....4-13
4.6 Availability and Use of Toxicity Information from the Species of Interest....4-14
4.7 Information About Accuracy and Reliability of the *In Vivo* Test Method 4-15
4.7.1 Information About the Accuracy of the *In Vivo* Test Method 4-15
4.7.2 Information About the Reliability of the *In Vivo* Test Method....4-16

5.0 ICE Test Method Data And Results 5-1
5.1 Description of the ICE Test Method Protocols Used to Generate Data 5-1
5.2 Availability of Copies of Original Data Used to Evaluate the Accuracy and Reliability 5-1
5.3 Description of the Statistical Approaches Used to Evaluate the Resulting Data 5-1
5.4 Summary of Results 5-4
5.4.1 Prinsen and Koëter (1993) 5-4
5.4.2 Balls et al. (1995) 5-4
5.4.3 Prinsen (1996) 5-4
5.4.4 Prinsen (2000) 5-4
5.4.5 Prinsen (2005) 5-4
5.5 Use of Coded Chemicals and Compliance with GLP Guidelines 5-5
5.6 Lot-to-lot Consistency of Test Substances 5-5
5.7 Availability of Data for External Audit 5-5

6.0 ICE Test Method Accuracy 6-1
6.1 Accuracy of the ICE Test Method 6-1
6.1.1 GHS Classification System: ICE Test Method Accuracy 6-3
6.1.1.1 Prinsen and Koëter (1993) 6-3
6.1.1.2 Balls et al. (1995) 6-3
6.1.1.3 Prinsen (1996) 6-5
6.1.1.4 Prinsen (2005) 6-5
6.1.1.5 Entire Data Set 6-5
6.1.1.6 Discordant Results According to the GHS Classification System 6-5
6.1.2 EPA Classification System: ICE Test Method Accuracy 6-7
6.1.2.1 Prinsen and Koëter (1993) 6-8
6.1.2.2 Balls et al. (1995) 6-8
6.1.2.3 Prinsen (1996) 6-10
6.1.2.4 Prinsen (2005) 6-10
6.1.2.5 Entire Data Set 6-10
6.1.2.6 Discordant Results According to the EPA Classification System 6-10
6.1.3 EU Classification System: ICE Test Method Accuracy 6-11
6.1.3.1 Prinsen and Koëter (1993) 6-12
6.1.3.2 Balls et al. (1995) 6-12
6.1.3.3 Prinsen (1996) 6-12
6.1.3.4 Prinsen (2000) 6-12
6.1.3.5 Prinsen (2005) 6-14
6.1.3.6 Entire Data Set 6-14
6.1.3.7 Discordant Results According to the EU Classification System 6-14
6.2 Accuracy of the ICE Test Method for Identifying Ocular Corrosives and Severe Irritants - Summary of Results 6-14

6.2.1 Discordance Among Chemical Classes 6-15
6.2.2 Discordance Among Physical or Chemical Properties of Interest 6-16

7.0 ICE Test Method Reliability 7-1
7.1 Selection Rationale for the Substances Used to Evaluate the Reliability of the ICE Test Method 7-1
7.2 Analyses of Repeatability and Reproducibility 7-2
7.2.1 Quantitative and Qualitative Assessments of Intralaboratory Repeatability 7-2
7.2.2 Quantitative and Qualitative Assessments of Intralaboratory Reproducibility 7-2
7.2.3 Assessment of Interlaboratory Reproducibility 7-5
7.2.3.1 Interlaboratory Reproducibility Based on *In Vitro* Irritancy Classification 7-5
7.2.3.2 Interlaboratory Reproducibility of Hazard Classification Category Using the GHS Classification System 7-5
7.2.3.3 Interlaboratory Reproducibility of Hazard Classification Category Using the EPA Classification System 7-7
7.2.3.4 Interlaboratory Reproducibility of Hazard Classification Category Using the EU Classification System 7-9
7.2.3.5 Common Chemical or Product Classes Among Test Substances with Discordant Interlaboratory Results Using the GHS Classification System 7-10
7.2.4 Coefficient of Variation Analysis 7-10
7.2.5 Additional Analysis of Interlaboratory Reproducibility 7-16
7.3 Historical Positive and Negative Control Data 7-16
7.4 Summary of Results 7-18

8.0 Test Method Data Quality 8-1
8.1 Adherence to National and International GLP Guidelines 8-1
8.1.1 Prinsen and Koëter (1993) 8-1
8.1.2 Balls et al. (1995) 8-1
8.1.3 Prinsen (1996) 8-1
8.1.4 Prinsen (2000) 8-2
8.1.5 Prinsen (2005) 8-2
8.2 Data Quality Audits 8-2
8.3 Impact of Deviations from GLP Guidelines 8-2
8.4 Availability of Laboratory Notebooks or Other Records 8-3
8.5 Need for Data Quality 8-3

9.0 Other Scientific Reports And Reviews 9-1
9.1 Reports in the Peer Reviewed Literature 9-1
9.1.1 Balls et al. (1995) 9-1
9.1.2 Chamberlain et al. (1997) 9-3
9.1.3 Prinsen (1996) 9-4

9.2 Data Received in Response to the ICCVAM *Federal Register* Notice or from Study Authors 9-5
9.2.1 Proctor and Gamble (P&G) Submission from Drs. Daniel Marsman and Karen Acuff 9-5

10.0 Animal Welfare Considerations (Refinement, Reduction and Replacement) .. 10-1
10.1 How the ICE Test Method Will Refine, Reduce, or Replace Animal Use 10-1
10.2 Requirement for the Use of Animals 10-1

11.0 Practical Considerations 11-1
11.1 Transferability of the ICE Test Method 11-1
11.1.1 Facilities and Major Fixed Equipment Required to Conduct the ICE Test Method 11-1
11.1.2 Availability of Other Necessary Equipment and Supplies 11-2
11.2 ICE Test Method Training Considerations 11-2
11.2.1 Required Level of Training and Expertise Needed to Conduct the ICE Test Method 11-2
11.3 Cost Considerations 11-2
11.4 Time Considerations 11-3

12.0 References 13-1

13.0 Glossary 14-1

Appendix A Publicly Available Protocols for the ICE Test Method A-1
A1 INVITTOX Protocol 80. The Chicken Enucleated Eye Test (CEET) – Method of Prinsen A-3
A2 Table of ICE Protocols A-19

Appendix B Chemical and Product Class Information for the Substances Tested in the ICE Test Method B-1

Appendix C *In Vitro* Data for Substances Tested in the ICE Test Method C-1
C1 ICE Data Sorted by Reference C-3
C2 ICE Data Sorted by Subtance Name C-11

Appendix D *In Vivo* and *In Vitro* Comparison of Ocular Irritancy Classification D-1
D1 ICE Data Sorted by Reference D-3
D2 ICE Data Sorted by Substance Name D-11

Appendix E Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al., 1995) E-1