

## TABLE OF CONTENTS

<b>LIST OF TABLES .....</b>	<b>viii</b>
<b>LIST OF FIGURES .....</b>	<b>xi</b>
<b>LIST OF ACRONYMS AND ABBREVIATIONS .....</b>	<b>xii</b>
<b>ACKNOWLEDGEMENTS .....</b>	<b>xix</b>
<b>PREFACE .....</b>	<b>xxi</b>
<b>EXECUTIVE SUMMARY .....</b>	<b>xxv</b>

<b>1.0</b>	<b>Introduction and Rationale for the Proposed Use of <i>In Vitro</i> Test Methods to Identify Ocular Corrosives and Severe Irritants.....</b>	<b>1-1</b>
1.1	Introduction .....	1-1
1.1.1	Historical Background of <i>In Vitro</i> Ocular Irritation/Corrosion Test Methods and Rationale for Their Development .....	1-1
1.1.2	Peer Reviews of the IRE Test Method .....	1-6
1.2	Scientific Basis for the IRE Test Method.....	1-6
1.2.1	Purpose and Mechanistic Basis of the IRE Test Method.....	1-6
1.2.2	Similarities and Differences of Modes and Mechanisms of Action Between the IRE 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 <i>In Vivo</i> Rabbit Eye Test Method.....	1-10
1.2.2.4	Comparison of the IRE Test Method with the <i>In Vivo</i> Rabbit Eye Test Method.....	1-13
1.2.3	Intended Range of Substances Amenable to the IRE Test Method and/or Limits of the IRE Test Method.....	1-14
1.3	Regulatory Rationale and Applicability.....	1-14
1.3.1	Current Regulatory Testing Requirements and ICCVAM Prioritization Criteria.....	1-14
1.3.2	Intended Uses of the IRE Test Method.....	1-16
1.3.3	Similarities and Differences in the Endpoints Measured in the Proposed Test Method and <i>In Vivo</i> Reference Test Method .....	1-16
1.3.4	Use of Proposed Test Method in Overall Strategy of Hazard or Safety Assessment .....	1-16
1.4	Validation of the IRE Test Method.....	1-17
1.5	Search Strategies and Selection of Citations for the IRE BRD.....	1-17
1.6		
<b>2.0</b>	<b>IRE Test Method Protocol Components.....</b>	<b>2-1</b>
2.1	Overview of How the IRE 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 Rabbit Eyes.....	2-2
2.2.1.2	Quality of Eyes.....	2-3
2.2.1.3	Preparation of the Eyes.....	2-3

2.2.1.4	IRE Experimental Setup.....	2-4
2.2.2	Dose-Selection Procedures, Including the Need for Any Dose Ranging Studies or Acute Toxicity Data Prior to Conducting a Study .....	2-4
2.2.3	Endpoints Measured .....	2-4
2.2.3.1	Corneal Opacity .....	2-5
2.2.3.2	Corneal Swelling.....	2-5
2.2.3.3	Fluorescein Penetration/Retention .....	2-5
2.2.3.4	Assessment of Epithelial Integrity .....	2-5
2.2.3.5	Additional Endpoints .....	2-6
2.2.4	Duration of Exposure .....	2-6
2.2.4.1	Pre-exposure Preparations .....	2-6
2.2.4.2	Test Substance Exposure Duration .....	2-6
2.2.4.3	Application and Amount of Test Substance.....	2-6
2.2.4.4	Number of Eyes Required per Test Substance and Controls .....	2-7
2.2.4.5	Concentration of Test Substance .....	2-7
2.2.5	Known Limits of Use .....	2-7
2.2.6	Nature of the Response Assessed.....	2-8
2.2.7	Appropriate Controls and the Basis for their Selection.....	2-8
2.2.7.1	Negative Controls .....	2-8
2.2.7.2	Solvent/Vehicle Controls .....	2-8
2.2.7.3	Positive Controls.....	2-8
2.2.7.4	Benchmark Controls.....	2-9
2.2.8	Acceptable Range of Control Responses and the Basis for the Acceptable Ranges .....	2-9
2.2.8.1	Negative/Solvent Controls.....	2-9
2.2.8.2	Positive Controls.....	2-9
2.2.8.3	Benchmark Controls.....	2-9
2.2.9	Nature of the Data to be Collected and the Methods Used for Data Collection.....	2-10
2.2.9.1	Corneal Opacity and Area of Involvement.....	2-10
2.2.9.2	Corneal Thickness and Calculation of Corneal Swelling.....	2-10
2.2.9.3	Fluorescein Penetration .....	2-11
2.2.9.4	Evaluation of Corneal Epithelial Integrity .....	2-12
2.2.9.5	Overall Scoring System for Identification of a Severe Irritant.....	2-12
2.2.10	Types of Media in which Data are Stored .....	2-13
2.2.11	Measures of Variability .....	2-13
2.2.12	Statistical or Nonstatistical Methods Used to Analyze the Resulting Data.....	2-14
2.2.13	Decision Criteria and the Basis for the Prediction Model Used to Classify a Test Chemical as a Severe Eye Irritant .....	2-14

2.2.14	Information and Data that Will Be Included in the Study Report and Availability of Standard Forms for Data Collection and Submission.....	2-14
2.3	Basis for Selection of the Test Method System.....	2-16
2.4	Proprietary Components.....	2-16
2.5	Basis for Number of Replicate and Repeat Experiments.....	2-16
2.6	Compliance with Good Laboratory Practice .....	2-18
2.7	Study Acceptance Criteria.....	2-18
<b>3.0</b>	<b>Substances Used For Validation of the IRE Test Method.....</b>	<b>3-1</b>
3.1	Rationale for the Substances or Products Selected for Use.....	3-1
3.1.1	CEC Collaborative Study (1991) .....	3-1
3.1.2	Balls et al. (1995) .....	3-2
3.1.3	Gettings et al. (1996) .....	3-2
3.1.4	Guerriero et al. (2004) .....	3-3
3.2	Rationale for the Number of Substances Tested.....	3-3
3.3	Chemicals or Products Evaluated .....	3-3
3.4	Coding Procedures Used in the Studies .....	3-5
3.4.1	CEC Collaborative Study (1991) .....	3-5
3.4.2	Balls et al. (1995) .....	3-5
3.4.3	Gettings et al. (1991, 1996).....	3-5
3.4.4	Guerriero et al. (2004) .....	3-5
<b>4.0</b>	<b><i>In Vivo</i> Reference Data Used for an Assessment Of Test Method</b>	
	<b>Accuracy .....</b>	<b>4-1</b>
4.1	Description of Protocol Used to Generate <i>In Vivo</i> Data .....	4-1
4.1.1	Draize Rabbit Eye Test.....	4-1
4.1.2	Current <i>In Vivo</i> Ocular Irritation Test Method Protocols.....	4-2
4.1.3	Current <i>In Vivo</i> Ocular Irritancy Classification Systems.....	4-3
4.2	Detailed Reference Data Used to Assess <i>In Vitro</i> Test Method Accuracy.....	4-9
4.3	<i>In Vivo</i> Classification Criteria Used for BRD Analysis.....	4-10
4.3.1	GHS Classification Rules Used for BRD Analysis.....	4-11
4.3.2	EPA Classification Rules Used for BRD Analysis .....	4-12
4.3.3	EU Classification Rules Used for BRD Analysis .....	4-13
4.4	Availability of Original Records for the <i>In Vivo</i> Reference Data.....	4-14
4.5	<i>In Vivo</i> Data Quality.....	4-14
4.6	Availability and Use of Toxicity Information from the Species of Interest.....	4-14
4.7	Information About Accuracy and Reliability of the <i>In Vivo</i> Test Method .....	4-16
4.7.1	Information About the Accuracy of the <i>In Vivo</i> Test Method .....	4-16
4.7.2	Information About the Reliability of the <i>In Vivo</i> Test Method .....	4-16

<b>5.0</b>	<b>IRE Test Method Data And Results .....</b>	<b>5-1</b>
5.1	Description of the IRE Test Method Protocols Used to Generate Data .....	5-1
5.1.1	CEC Collaborative Study (1991) .....	5-1
5.1.2	Balls et al. (1995).....	5-1
5.1.3	Gettings et al. (1996).....	5-2
5.1.4	Guerrero et al. (2004).....	5-2
5.2	Data Obtained to Evaluate the Accuracy and Reliability of the IRE Test Method.....	5-2
5.3	Description of the Statistical Approaches Used to Evaluate the Resulting Data .....	5-3
5.3.1	CEC Collaborative Study (1991) .....	5-3
5.3.2	Balls et al. (1995).....	5-3
5.3.3	Gettings et al. (1996).....	5-3
5.3.4	Guerrero et al. (2004).....	5-3
5.4	Summary of Results.....	5-4
5.5	Use of Coded Chemicals and Compliance with GLP Guidelines .....	5-4
5.6	Description of “Lot-to-lot” Consistency, Time Frame of Studies and Testing Laboratories .....	5-4
5.6.1	CEC Collaborative Study (1991).....	5-5
5.6.2	Balls et al. (1995).....	5-5
5.6.3	Gettings et al. (1996).....	5-5
5.6.4	Guerrero et al. (2004).....	5-5
5.7	Availability of Data for External Audit, if Requested .....	5-6
<b>6.0</b>	<b>IRE Test Method Accuracy.....</b>	<b>6-1</b>
6.1	Accuracy of the IRE Test Method .....	6-1
6.1.1	GHS Classification System: IRE Test Method Accuracy .....	6-3
6.1.1.1	Balls et al. (1995).....	6-4
6.1.1.2	Gettings et al (1996).....	6-4
6.1.1.3	Guerrero et al. (2004).....	6-4
6.1.1.4	Expanded Data Set.....	6-6
6.1.1.5	Pooled Data Set.....	6-6
6.1.1.6	Discordant Results According to the GHS Classification System .....	6-6
6.1.1.7	Expanded Data Set.....	6-6
6.1.1.8	Pooled Data Set.....	6-6
6.1.2	EPA Classification System: IRE Test Method Accuracy.....	6-10
6.1.2.1	Balls et al. (1995).....	6-10
6.1.2.2	Gettings et al. (1996).....	6-10
6.1.2.3	Guerrero et al. (2004).....	6-10
6.1.2.4	Expanded Data Set.....	6-12
6.1.2.5	Pooled Data Set.....	6-12
6.1.2.6	Discordant Results According to the EPA Classification System .....	6-12
6.1.3	EU Classification System: IRE Test Method Accuracy .....	6-15

6.1.3.1	CEC Collaborative Study (1991).....	6-15
6.1.3.2	Balls et al. (1995).....	6-17
6.1.3.3	Gettings et al (1996).....	6-17
6.1.3.4	Guerrero et al. (2004).....	6-17
6.1.3.5	Expanded Data Set.....	6-17
6.1.3.6	Pooled Data Set.....	6-17
6.1.3.7	Discordant Results According to the EU Classification System.....	6-17
6.2	Accuracy of the IRE Test Method for Identifying Ocular Corrosives and Severe Irritants - Summary of Results.....	6-21
6.2.1	Discordance Among Chemical Classes.....	6-21
6.2.2	Discordance Among Physical or Chemical Properties Of Interest.....	6-22
<b>7.0</b>	<b>IRE Test Method Reliability.....</b>	<b>7-1</b>
7.1	Selection Rationale for the Substances Used to Evaluate the Reliability of the IRE 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.....	7-3
7.2.3.1	Interlaboratory Reproducibility of Hazard Classification Category Using the GHS Classification System.....	7-3
7.2.3.2	Interlaboratory Reproducibility of Hazard Classification Category Using the EPA Classification System.....	7-5
7.2.3.3	Interlaboratory Reproducibility of Hazard Classification Category Using the EU Classification System.....	7-6
7.2.3.4	Common Chemical or Product Classes Among Substances with Discordant Interlaboratory Results Using the GHS, EPA, and EU Classification Systems.....	7-9
7.2.4	Coefficient of Variation Analysis.....	7-10
7.2.4.1	CEC Collaborative Study (1991).....	7-10
7.2.4.2	Balls et al. (1995).....	7-12
7.2.5	Additional Analysis of Interlaboratory Reproducibility.....	7-17
7.3	Historical Positive and Negative Control Data.....	7-17
7.4	Conclusions.....	7-19
<b>8.0</b>	<b>□ Test Method Data Quality.....</b>	<b>8-1</b>
8.1	Adherence to National and International GLP Guidelines.....	8-1
8.2	Data Quality Audits.....	8-1

8.3	Impact of Deviations from GLP Guidelines .....	8-2
8.4	Availability of Laboratory Notebooks or Other Records.....	8-2
8.5	Need for Data Quality.....	8-2
<b>9.0</b>	<b>Other Scientific Reports And Reviews .....</b>	<b>9-1</b>
9.1	Summaries of IRE Data from Published and Unpublished Studies .....	9-1
9.1.1	Balls et al. (1995).....	9-1
9.1.2	Chamberlain et al. (1997).....	9-2
9.1.3	Cooper et al. (2001).....	9-4
9.1.4	Gettings et al. (1996).....	9-5
9.1.5	Guerrero et al. (2004).....	9-6
9.1.6	Jacobs and Martens (1990).....	9-6
9.1.7	Jacobs and Martens (1989).....	9-7
9.1.8	Jacobs and Martens (1988).....	9-7
9.1.9	Jones et al. (2001).....	9-8
9.1.10	Koëter and Prinsen (1985).....	9-8
9.1.11	Lewis et al. (1994).....	9-10
9.1.12	Price and Andrews.....	9-10
9.1.13	Whittle et al. (1992).....	9-11
9.1.14	York et al. (1982).....	9-12
9.2	Data Received in Response to the ICCVAM <i>Federal Register</i> Notice or from Study Authors .....	9-12
9.2.1	Jacobs and Martens (January 1987).....	9-12
9.2.2	Jacobs and Martens (May 1987).....	9-13
9.2.3	Proctor and Gamble (P&G) Submission from Drs. Daniel Marsman and Karen Acuff .....	9-13
	9.2.3.1 Summary of P&G Confocal Ocular Test Method .....	9-13
	9.2.3.2 P&G Data .....	9-14
<b>10.0</b>	<b>Animal Welfare Considerations.....</b>	<b>10-1</b>
10.1	How the IRE Test Method Will Refine, Reduce, or Replace Animal Use.....	10-1
10.2	Requirement for the Use of Animals .....	10-1
<b>11.0</b>	<b>Practical Considerations .....</b>	<b>11-1</b>
11.1	Transferability of the IRE Test Method.....	11-1
11.1.1	Facilities and Major Fixed Equipment.....	11-1
11.1.2	General Availability of Other Necessary Equipment and Supplies.....	11-1
11.2	Training Considerations.....	11-2
11.2.1	Required Level of Training and Expertise Needed to Conduct the IRE Test Method.....	11-2
11.3	Cost Considerations .....	11-3
11.4	Time Considerations .....	11-3

<b>12.0</b>	<b>References .....</b>	<b>12-1</b>
<b>13.0</b>	<b>Glossary .....</b>	<b>13-1</b>
<b>Appendix A</b>	<b>Publicly Available Protocols for the IRE Test Method.....</b>	<b>A-1</b>
A1	INVITTOX Protocol 85. The Rabbit Enucleated Eye Test Method of Dr. Lesley Earl.....	A-3
A2	Table of IRE Protocols from the Reviewed Literature .....	A-13
<b>Appendix B</b>	<b>Chemical and Product Class Information for the Substances Tested in the IRE Test Method.....</b>	<b>B-1</b>
B1	Chemical and Product Class Information for the Substances Tested in the IRE Test Method. ....	B-3
B2	Table of Formulation Components in Gettings et al. (1996).....	B-13
<b>Appendix C</b>	<b><i>In Vitro</i> Data for Substances Tested in the IRE Test Method .....</b>	<b>C-1</b>
C1	Balls et al. (1995) Data Sorted by Substance Name .....	C-3
C2	Guerriero et al. (2004) Data Sorted by Substance Name .....	C-13
C3	Gettings et al. (1996) - Data Sorted by Substance Name.....	C-21
C4	CEC (1991) - Data Sorted by Reference.....	C-27
<b>Appendix D</b>	<b><i>In Vivo</i> and <i>In Vitro</i> Comparison of Ocular Irritancy Classification .....</b>	<b>D-1</b>
D1	Data Sorted by Reference.....	D-3
D2	Data Sorted by Substance.....	D-11
<b>Appendix E</b>	<b>Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al. 1995) .....</b>	<b>E-1</b>