

TABLE OF CONTENTS

	Page Number
LIST OF TABLES	ix
LIST OF FIGURES	xi
LIST OF ACRONYMS AND ABBREVIATIONS	xii
ACKNOWLEDGEMENTS	xv
PREFACE	xix
EXECUTIVE SUMMARY	xxiii
1.0 Introduction and Rationale for the Proposed Use of <i>In Vitro</i> Test Methods to Identify Ocular Corrosives and Severe Irritants.....	1-1
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 BCOP Test Method.....	1-7
1.2 Scientific Basis for the BCOP Test Method.....	1-7
1.2.1 Purpose and Mechanistic Basis of the BCOP Test Method.....	1-7
1.2.2 Similarities and Differences of Modes of Action Between the BCOP Test Method and Ocular Irritancy in Humans and/or Rabbits	1-8
1.2.2.1 The Mammalian Eye: Common Anatomy of the Human, Rabbit, and Bovine Eye.....	1-8
1.2.2.2 Differences Between Human, Rabbit, and Bovine Eyes	1-9
1.2.2.3 The <i>In Vivo</i> Rabbit Eye Test Method.....	1-10
1.2.2.4 Comparison of BCOP Test Method with the <i>In Vivo</i> Rabbit Eye Test Method.....	1-13
1.2.3 Intended Range of Substances Amenable to the BCOP Test Method and/or Limits of the BCOP Test Method.....	1-15
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 Proposed BCOP Test Method	1-17
1.3.3 Similarities and Differences in the Endpoints Measured in the Proposed Test Method and the <i>In Vivo</i> Reference Test Method...	1-17
1.3.4 Use of Proposed Test Method in Overall Strategy of Hazard or Safety Assessment	1-18
1.4 Validation of the <i>In Vitro</i> BCOP Test Method.....	1-18
1.5 Search Strategies and Selection of Citations for the BCOP BRD	1-20
2.0 BCOP Test Method Protocol Components.....	2-1
2.1 Overview of How the BCOP Test Method is Conducted	2-1
2.2 Description and Rationale for the Test Method Components	2-2
2.2.1 Materials, Equipment, and Supplies Needed	2-3

2.2.1.1	Bovine Eyes: Source, Collection/Handling and Quality	2-3
2.2.1.2	Instrument to Measure Light Transmission Through the Cornea.....	2-6
2.2.1.3	Instrument to Evaluate Permeability.....	2-7
2.2.1.4	Organ Culture Media.....	2-7
2.2.1.5	Solvents	2-8
2.2.1.6	Incubation Apparatus	2-8
2.2.1.7	Corneal Holder.....	2-8
2.2.2	Dose-Selection Procedures, Including the Need for Any Dose Range-Finding Studies or Acute Toxicity Data Prior to Conducting a Study	2-9
2.2.3	Endpoints Measured	2-10
2.2.4	Duration of Exposure	2-11
2.2.4.1	Pre-Exposure Preparations	2-11
2.2.4.2	Effects of Residual Equilibration Medium in the Test Substance Chamber	2-12
2.2.4.3	Test Substance Exposure Volume	2-12
2.2.4.4	Concentration Tested	2-12
2.2.4.5	Application of Test Substance to Bovine Cornea.....	2-13
2.2.4.6	Test Substance Exposure Duration	2-13
2.2.4.7	Post-Exposure Incubation.....	2-14
2.2.5	Known Limits of Use	2-14
2.2.6	Nature of the Response Assessed.....	2-15
2.2.6.1	Corneal Opacity	2-15
2.2.6.2	Permeability.....	2-15
2.2.6.3	Histology	2-15
2.2.7	Appropriate Controls and the Basis for Their Selection	2-15
2.2.7.1	Negative Controls	2-15
2.2.7.2	Positive Controls.....	2-16
2.2.7.3	Solvent Control.....	2-16
2.2.7.4	Benchmark Substances.....	2-17
2.2.8	Acceptable Ranges for Recommended Control Responses and the Basis for the Acceptable Ranges.....	2-17
2.2.8.1	Negative/Solvent Controls.....	2-17
2.2.8.2	Positive Controls.....	2-17
2.2.8.3	Benchmark Substances.....	2-18
2.2.9	Nature of the Data to be Collected and the Methods Used for Data Collection	2-18
2.2.9.1	Corneal Opacity	2-18
2.2.9.2	Permeability.....	2-18
2.2.9.3	Histology	2-18
2.2.10	Type of Media in Which Data Are Stored.....	2-19
2.2.11	Measures of Variability	2-19
2.2.12	Statistical or Nonstatistical Methods Used to Analyze the Resulting Data.....	2-19

2.2.13	Decision Criteria and the Basis for the Prediction Model Used to Classify a Test Chemical as a Severe Eye Irritant.....	2-20
2.2.14	Information and Data that Will be Included in the Study Report and Availability of Standard Forms for Data Collection and Submission	2-21
2.3	Basis for Selection of the Test Method System.....	2-23
2.4	Proprietary Components.....	2-23
2.5	Basis for the Number of Replicate and Repeat Experiments	2-24
2.5.1	Sample Replicates	2-24
2.5.2	Experimental Replicates	2-24
2.6	Compliance with Good Laboratory Practice Guidelines.....	2-24
2.7	Study Acceptance Criteria.....	2-24
3.0	Substances Used for Validation of the BCOP Test Method.....	3-1
3.1	Rationale for the Chemicals or Products Selected for Use	3-1
3.1.1	Gautheron et al. (1994)	3-1
3.1.2	Balls et al. (1995)	3-2
3.1.3	Swanson et al. (1995)	3-2
3.1.4	Gettings et al. (1996)	3-3
3.1.5	Casterton et al. (1996).....	3-3
3.1.6	Southee (1998)	3-3
3.1.7	Swanson and Harbell (2000).....	3-4
3.1.8	Bailey et al. (2004)	3-4
3.2	Rationale for the Number of Substances Tested.....	3-4
3.3	Chemicals or Products Evaluated	3-4
3.3.1	Gautheron et al. (1994)	3-5
3.3.2	Balls et al. (1995)	3-6
3.3.3	Swanson et al. (1995)	3-6
3.3.4	Gettings et al. (1996)	3-7
3.3.5	Casterton et al. (1996).....	3-7
3.3.6	Southee (1998)	3-7
3.3.7	Swanson and Harbell (2000).....	3-7
3.3.8	Bailey et al. (2004)	3-7
3.4	Coding Procedures Used in the Validation Studies	3-7
3.4.1	Gautheron et al. (1994)	3-7
3.4.2	Balls et al. (1995)	3-7
3.4.3	Swanson et al. (1995)	3-8
3.4.4	Gettings et al. (1996)	3-8
3.4.5	Casterton et al. (1996).....	3-8
3.4.6	Southee (1998)	3-8
3.4.7	Swanson and Harbell (2000).....	3-8
3.4.8	Bailey et al. (2004)	3-8
4.0	<i>In Vivo</i> Reference Data Used for an Assessment of Test Method Accuracy.....	4-1
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.....	4-1
4.1.3	Current <i>In Vivo</i> Ocular Irritancy Classification Systems	4-7
4.2	Detailed Reference Data Used to Assess <i>In Vitro</i> Test Method Accuracy	4-8
4.3	<i>In Vivo</i> Classification Criteria Used for BRD Analysis.....	4-11
4.3.1	GHS Classification Rules Used for BRD Analysis.....	4-11
4.3.2	EPA Classification Rules Used for BRD Analysis.....	4-13
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.....	4-15
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	4-17
5.0	BCOP Test Method Data and Results	5-1
5.1	Description of the BCOP Test Method Protocols Used to Generate Data.....	5-1
5.2	Availability of Copies of Original Data Used to Evaluate Accuracy and Reliability.....	5-2
5.3	Description of the Statistical Approaches Used to Evaluate the Resulting Data	5-3
5.4	Summary of Results.....	5-5
5.4.1	Gautheron et al. (1994).....	5-5
5.4.2	Balls et al. (1995).....	5-6
5.4.3	Swanson et al. (1995).....	5-6
5.4.4	Gettings et al. (1996).....	5-6
5.4.5	Casterton et al. (1996)	5-6
5.4.6	Southee (1998).....	5-7
5.4.7	Swanson and Harbell (2000).....	5-7
5.4.8	Bailey et al. (2004).....	5-7
5.5	Use of Coded Chemicals and Compliance with GLP Guidelines	5-7
5.6	Lot-to-lot Consistency of Test Substances.....	5-8
5.7	Availability of Data for External Audit	5-8
6.0	BCOP Test Method Accuracy.....	6-1
6.1	Accuracy of the BCOP Test Method	6-1
6.1.1	GHS Classification System: BCOP Test Method Accuracy	6-3
6.1.1.1	Discordant Results According to the GHS Classification System.....	6-5
6.1.2	EPA Classification System: BCOP Test Method Accuracy	6-8
6.1.2.1	Discordant Results According to the EPA Classification System.....	6-9
6.1.3	EU Classification System: BCOP Test Method Accuracy	6-12

6.1.3.1	Discordant Results According to the EU Classification System	6-14
6.2	Accuracy of the BCOP Test Method for Identifying Ocular Corrosives and Severe Irritants – Summary of Results	6-16
6.2.1	Discordance Among Chemical Classes	6-16
6.2.2	Discordance Among Physical or Chemical Properties of Interest.	6-16
7.0	BCOP Test Method Reliability	7-1
7.1	Selection Rationale for the Substances Used to Evaluate the Reliability of the BCOP Test Method	7-1
7.2	Analyses of Repeatability and Reproducibility	7-2
7.2.1	Assessment of Intralaboratory Repeatability and Reproducibility..	7-2
7.2.1.1	Southee (1998).....	7-2
7.2.1.2	Data from Dr. Joseph Sina (Merck)	7-8
7.2.1.3	Data from Dr. John Harbell (IIVS) for Gettings et al. (1996).....	7-8
7.2.1.4	Data from Dr. Freddy Van Goethem for Gautheron et al. 1994	7-8
7.2.2	Evaluation of Interlaboratory Reproducibility	7-12
7.2.2.1	Interlaboratory Reproducibility of Hazard Classification Category Using the GHS Classification System	7-13
7.2.2.2	Interlaboratory Reproducibility of Hazard Classification Category Using the EPA Classification System	7-13
7.2.2.3	Interlaboratory Reproducibility of Hazard Classification Category Using the EU Classification System	7-16
7.2.2.4	Common Chemical or Product Classes Among Test Substances with Discordant Interlaboratory Results...	7-18
7.2.2.5	Interlaboratory Reproducibility Based on Coefficient of Variation Analysis of <i>In Vitro</i> Scores ..	7-18
7.2.3	Additional Analyses of Interlaboratory Reproducibility	7-22
7.3	Historical Positive and Negative Control Data.....	7-25
7.4	Summary.....	7-26
8.0	BCOP Test Method Data Quality	8-1
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
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)	9-2
9.1.2	Bruner et al. (1998)	9-2
9.1.3	Cassidy and Stanton (1997)	9-3
9.1.4	Chamberlain et al. (1997)	9-4
9.1.5	Cooper et al. (2001)	9-6
9.1.6	Gautheron et al. (1992)	9-7
9.1.7	Gautheron et al. (1994)	9-7
9.1.8	Jones et al. (2001)	9-9
9.1.9	Rachui et al. (1994)	9-9
9.1.10	Rougier et al. (1994)	9-9
9.1.11	Sina et al. (1995)	9-10
9.1.12	Ubels et al. (1998)	9-10
9.1.13	Ubels et al. (2000)	9-11
9.1.14	Ubels et al. (2002)	9-11
9.1.15	Ubels et al. (2004)	9-11
9.1.16	Vanparys et al. (1993)	9-12
9.1.17	1997 Bovine Corneal Opacity and Permeability Technical Workshop	9-13
9.1.18	Review Articles on the BCOP Assay	9-14
9.1.19	Poster Presentations	9-14
9.2	Other Scientific Reports Received in Response to a <i>Federal Register</i> Notice	9-17
9.2.1	S.C. Johnson & Son, Inc.	9-17
9.2.2	L'OREAL	9-18
9.2.3	IIVS	9-19
9.2.4	Johnson & Johnson Pharmaceutical Research and Development ...	9-19
10.0	Animal Welfare Considerations (Refinement, Reduction and Replacement)..	10-1
10.1	How the BCOP Test Method Will Refine, Reduce, or Replace Animal Use	10-1
10.2	Requirement for Use of Animals	10-1
11.0	Practical Considerations	11-1
11.1	Transferability of the BCOP 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	BCOP Test Method Training Considerations	11-2
11.2.1	Required Level of Training and Expertise Needed to Conduct the BCOP Test Method	11-2
11.3	Cost Considerations	11-3
11.4	Time Considerations	11-3
12.0	References	12-1

13.0	Glossary	13-1
Appendix A	Publicly Available Protocols for the BCOP Test Method.....	A-1
A1	INVITTOX Protocol 98. The Bovine Corneal Opacity and Permeability Assay – Method of Gautheron.....	A-3
A2	INVITTOX Protocol 124. Bovine Corneal Opacity and Permeability Assay (BCOP) - SOP of Microbiological Associates, Ltd., United Kingdom	A-11
A3	Table of BCOP Protocols from the Reviewed Literature	A-33
Appendix B	Characterization of the Substances Tested in the BCOP Test Method..	B-1
B1	Chemical and Product Classes of Substances Tested in the BCOP Assay ...	B-3
B2	Components of Formulations Tested in Gettings et al. (1996).....	B-13
B3	Components of Formulations Tested in Swanson et al. (1995).....	B-17
B4	Components of Formulations Tested in Swanson and Harbell (2001)	B-23
Appendix C	<i>In Vitro</i> Data for Substances Tested in the BCOP Assay.....	C-1
C1	BCOP Data Sorted by Reference.....	C-3
C2	BCOP Data Sorted by Substance Name.....	C-29
Appendix D	Comparison of <i>In Vivo</i> and <i>In Vitro</i> Ocular Irritancy Classifications ...	D-1
D1	BCOP Data Sorted by Reference.....	D-3
D2	BCOP Data Sorted by Substance Name.....	D-29
Appendix E	Intralaboratory Coefficient of Variation (CV) Analysis of BCOP.....	E-1
E1	BCOP Data from Southee (1998)	E-3
E2	BCOP Data from Dr. Joseph Sina.....	E-17
E3	BCOP Data from Dr. Freddy Van Goethem	E-27
Appendix F	Interlaboratory Correlation Coefficients from the EC/HO Validation Study (Balls et al., 1995).....	F-1
Appendix G	Additional BCOP Studies Received in Response to <i>Federal Register</i> Notices (Vol. 69, No. 57, pp. 13859-13861) and (Vol. 70, No. 38, pp. 9661-9662)	G-1
G1	Dataset Received from S.C. Johnson & Son, Inc. in Support of Cuellar et al. (2004) Poster Presentation.....	G-3
G2	Dataset Received from S.C. Johnson & Son, Inc. in Support of Cuellar et al. (2002) Poster Presentation.....	G-43
G3	Dataset Received from S.C. Johnson & Son, Inc. in Support of Gran et al. (2003) Poster Presentation.....	G-61
G4	Dataset Received from L'OREAL Advanced Research for an In-house Porcine Corneal Opacity and Permeability Assay	G-91
G5	Supporting Analyses Received from IIVS for Gettings et al. (1996) Study	G-101

G6	Dataset Received from Johnson & Johnson Pharmaceutical Research and Development – A Division of Janssen Pharmaceutica N.V. (Laboratory No. 9 in Gautheron et al. 1994).....	G-191
G7	Dataset Received from Johnson & Johnson Pharmaceutical Research and Development – A Division of Janssen Pharmaceutica N.V. (BCOP Tests With Young vs. Old Corneas).....	G-251