

# **OECD GUIDELINE FOR THE TESTING OF CHEMICALS**

## **Draft Proposal for a New Guideline: The Isolated Chicken Eye (ICE) Test Method for Identifying Ocular Corrosives and Severe Irritants<sup>1</sup>**

### **INTRODUCTION**

1. The Isolated Chicken Eye (ICE) test method is an *in vitro* test method that can be used, under certain circumstances and with specific limitations, to identify ocular corrosives and severe irritants<sup>1,2</sup> (i.e., U.S. Environmental Protection Agency [EPA] Category 1, European Union [EU] R41, the United Nations [UN] Globally Harmonized System of Classification and Labelling of Chemicals [GHS] Category 1). While it is not considered valid as a complete replacement for the rabbit eye test, the ICE is recommended for use as part of a tiered testing strategy for regulatory classification and labeling within a specific applicability domain (1)(2). Substances that are positive in this assay and considered an ocular corrosive or severe irritant after a weight-of-evidence decision will not need to be tested in animals. A substance that tests negative would need to be tested *in vivo* using an accepted test guideline (i.e., OECD Test Guideline 405 (3) or EPA OPPTS 870.1000 (4)) or *in vitro* using an adequately validated test method<sup>3</sup>.

2. The purpose of this Test Guideline is to describe the procedures used to evaluate the potential ocular corrosivity or severe irritancy of a test substance as measured by its ability to induce toxicity in an enucleated chicken eye. Toxic effects to the cornea are measured by: 1) a qualitative assessment of opacity, 2) a qualitative assessment of damage to epithelium based on application of fluorescein to the eye (fluorescein retention), 3) a quantitative measurement of increased thickness (swelling), and 4) a qualitative evaluation of macroscopic morphological damage to the surface. The corneal opacity, swelling, and damage assessments following exposure to a test substance are assessed individually and then combined to derive an Eye Irritancy Classification. A histopathological assessment can be included on a case-by-case basis to aid in the classification of test substances as a corrosive/severe irritant.

3. The focus of this Test Guideline is on the use of the ICE test method for the detection of ocular corrosives and severe irritants, as defined by the EPA (5), EU (6), and GHS (7). Ocular irritants that induce lesions that resolve in less than 21 days, as well as nonirritants, have been tested using the ICE test method. However, the accuracy and

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<sup>1</sup> For the purpose of this Test Guideline, severe irritants are defined as those that induce ocular lesions that persist in the rabbit for at least 21 days after administration.

<sup>2</sup> EPA Category 1 = Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days (5); EU R41 = Production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application (6); GHS Category 1 = Production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application (7).

<sup>3</sup> The *in vitro* test method must be able to correctly identify an ICE false negative (i.e., a misclassified corrosive or severe irritant), irritants that induce ocular damage that resolves within 21 days, and non-irritants.

32 reliability of the ICE test method for these categories, as defined by the EPA (5), EU (6),  
33 and GHS (7), have not been formally evaluated.

34 4. Definitions are provided in **Annex I**.

## 35 **INITIAL CONSIDERATIONS AND LIMITATIONS**

36 5. This Test Guideline is based on the recommended Interagency Coordinating  
37 Committee on the Validation of Alternative Methods (ICCVAM) ICE test method  
38 protocol (**Annex II**), which was developed following an international independent  
39 scientific peer review of the validation status and scientific validity of the ICE test  
40 method (1, 2, 8), with contributions from the European Centre for the Validation of  
41 Alternative Methods (ECVAM), the Japanese Center for the Validation of Alternative  
42 Methods (JaCVAM), and the ICE test method developer, TNO Quality of Life  
43 Department of Toxicology and Applied Pharmacology (TNO). The ICCVAM ICE test  
44 method protocol was developed with information obtained from published protocols, as  
45 well as the current protocol used by TNO (9)(10)(11)(12)(13).

46 6. The identified limitations for this method are based on the false negative rates that  
47 are observed for certain chemical (i.e., alcohols and surfactants) and physical (i.e., solids)  
48 classes (see paragraph 48) (1). When substances within these chemical and physical  
49 classes are excluded from the database, the accuracy of ICE across the EU, EPA, and  
50 GHS classification systems is substantially improved (1). Furthermore, the current  
51 validation database did not allow for an adequate evaluation of some chemical or product  
52 classes (e.g., formulations). However, investigators could consider this test method for  
53 testing such substances, whereby a positive result, as part of a weight-of-evidence  
54 decision, could be accepted for hazard classification purposes.

55 7. All procedures with chicken eyes should follow the institution's applicable  
56 regulations and procedures for handling of human or animal-derived materials, which  
57 include, but are not limited to, tissues and tissue fluids. Universal laboratory precautions  
58 are recommended.

59 8. Histopathology data have not been formally evaluated for use in this assay. A  
60 histopathological assessment can be included on a case-by-case basis to aid in the  
61 classification of test substances as a corrosive/severe irritant. Histopathology may also be  
62 potentially useful when a more complete characterization of damage is needed. Users are  
63 encouraged to preserve tissues and prepare histopathology specimens that can be used to  
64 develop a database and decision criteria that may further improve the accuracy of this test  
65 method. The OECD Guidance Document on Histopathological Preparation and  
66 Evaluation of Tissues from *In Vitro* Ocular Toxicity Test Methods (to be provided)  
67 includes detailed procedures on the collection of histopathology specimens and  
68 information on where to submit specimens and resulting histopathology data.

69 9. A limitation of the test method is that, although it takes into account some of the  
70 ocular effects evaluated in *in vivo* rabbit ocular irritancy tests and to some degree their  
71 severity, it does not consider all of types of ocular damage assessed *in vivo* (i.e.,

72 conjunctival and iridal injuries), nor does it allow for assessing the potential for systemic  
73 toxicity associated with this route of exposure. Although the reversibility of corneal  
74 lesions cannot be evaluated *per se* in the ICE test method, it has been proposed, based on  
75 rabbit eye studies, that an assessment of the initial depth of corneal injury can be used to  
76 distinguish between irreversible and reversible effects (14).

77 10. Efforts are ongoing to expand the applicability domain of the ICE test method for  
78 identifying ocular corrosives and severe irritants, and to characterize its usefulness and  
79 limitations for identifying non-severe irritants and nonirritants. Users are encouraged to  
80 submit data and histopathology specimens generated according to this Test Guideline to  
81 national and international validation organizations (i.e., the European Centre for the  
82 Validation of Alternative Methods, the Japanese Center for the Validation of Alternative  
83 Methods, or the U.S. National Toxicology Program Interagency Center for the Evaluation  
84 of Alternative Toxicological Methods). These data may also be used to evaluate possible  
85 future use of the ICE test method for the identification of non-severe irritants and  
86 nonirritants.

## 87 **PRINCIPLE OF THE TEST**

88 11. The ICE test method is an organotypic model that provides short-term  
89 maintenance of the chicken eye in an isolated system. In this test method, damage by the  
90 test substance is assessed by determination of corneal swelling, opacity, and fluorescein  
91 retention. While the latter two parameters involve a qualitative assessment, analysis of  
92 corneal swelling provides for a quantitative assessment. Each measurement is either  
93 converted into a quantitative score used to calculate an overall Irritation Index, or  
94 assigned a qualitative categorization that is used to assign an *in vitro* ocular corrosivity  
95 and severe irritancy classification. Either of these outcomes can then be used to predict  
96 the *in vivo* ocular corrosivity and severe irritation potential of a test substance.

97 12. During an ICE study, a test substance is applied to the corneas of enucleated  
98 chicken eyes, isolated from chickens processed for human consumption. Within two  
99 hours after death, chicken heads are transported from the slaughterhouse to the laboratory  
100 and eyes dissected. After dissection, the eyes are placed in a superfusion apparatus, where  
101 isotonic saline is applied to the cornea, at a rate of two to three drops per minute, through  
102 a steel tube attached to a peristaltic pump. Substances are applied as a single dose (0.03  
103 mL for liquids, 0.03 g for solids) for 10 seconds. Corneal reactions are measured at  
104 regular intervals up to four hours post-treatment, while fluorescein retention is evaluated  
105 at 30 minutes post-treatment only. Mean values for each parameter (corneal swelling,  
106 opacity, and fluorescein retention) are determined and the maximum mean values<sup>4</sup> of  
107 these measurements are used for hazard classification purposes using established decision  
108 criteria (see DECISION CRITERIA).

### 109 **Source of Chicken Eyes**

110 13. Historically, eyes collected from chickens (breed not specified) obtained from a

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<sup>4</sup> For each endpoint, the mean value for the three eyes per test group is recorded for each time point and the largest mean value is used for classification.

111 slaughterhouse where they are killed for human consumption have been used for this  
112 assay, eliminating the need for laboratory animals.

### 113 **Age of Source Animals**

114 14. Although a controlled study to evaluate the optimum chicken age has not been  
115 conducted, the age and weight of the chickens used historically in this test method are  
116 that of spring chickens traditionally processed by a poultry slaughterhouse (i.e.,  
117 approximately seven weeks old, 2.5-3.0 kg). However, unpublished studies on adult  
118 chickens show no significant differences in results (Prinsen, personal communication).

### 119 **Collection and Transport of Eyes to the Laboratory**

120 15. A local source of chickens close to the laboratory should be located so that their  
121 heads can be transferred from the slaughterhouse to the laboratory and processed within  
122 two hours after death (Prinsen, personal communication). Heads should be removed  
123 immediately after sedation of the animals, usually by electric shock, and incision of the  
124 neck for bleeding.

125 16. Because eyes are dissected in the laboratory, the intact heads are transported from  
126 the slaughterhouse at ambient temperature in plastic boxes humidified with towels  
127 moistened with isotonic saline.

### 128 **Selection Criteria for Eyes Used in the ICE**

129 17. Eyes that have high baseline fluorescein staining (i.e.,  $> 0.5$ ) or corneal opacity  
130 score (i.e.,  $> 0.5$ ) after they are enucleated are rejected.

131 18. Each treatment group (test article, concurrent negative control, concurrent  
132 positive control) consists of a minimum of three eyes.

133 **PROCEDURE** (see **Annex II** for a detailed protocol for the ICE test method)

### 134 **Preparation of the Eyes**

135 19. The eyelids are carefully excised, taking care not to damage the cornea. Corneal  
136 integrity is quickly assessed with a drop of 2% (w/v) sodium fluorescein applied to the  
137 corneal surface for a few seconds, and then rinsed with isotonic saline. Fluorescein-  
138 treated eyes are then examined with a slit-lamp microscope to ensure that the cornea is  
139 undamaged (i.e., fluorescein retention and corneal opacity scores  $< 0.5$ ).

140 20. If undamaged, the eye is further dissected from the skull, taking care not to  
141 damage the cornea. The eyeball is pulled from the orbit by holding the nictitating  
142 membrane firmly with surgical forceps, and the eye muscles are cut with a bent, blunt-  
143 tipped scissor. It is important to avoid causing corneal damage due to excessive pressure  
144 (i.e., compression artifacts).

145 21. When the eye is removed from the orbit, a visible portion of the optic nerve

146 should be left attached. Once removed from the orbit, the eye is placed on an absorbent  
147 pad and the nictitating membrane and other connective tissue are cut away.

148 22. The enucleated eye is mounted in a stainless steel clamp with the cornea  
149 positioned vertically. The clamp is then transferred to a chamber of the superfusion  
150 apparatus<sup>5</sup>. The clamps should be positioned in the superfusion apparatus such that the  
151 entire cornea is supplied with the isotonic saline drip. The chambers of the superfusion  
152 apparatus should be temperature controlled at  $32 \pm 1.5^\circ\text{C}$ .

153 23. After being placed in the superfusion apparatus, the eyes are again examined with  
154 a slit-lamp microscope to ensure that they have not been damaged during the dissection  
155 procedure. Corneal thickness should also be measured at this time at the corneal apex  
156 using the depth measuring device on the slit-lamp microscope. Eyes with: 1) a fluorescein  
157 retention score of  $> 0.5$ , 2) corneal opacity  $> 0.5$ , or 3) any additional signs of damage  
158 should be replaced. For eyes that are not rejected based on any of these criteria,  
159 individual eyes with a corneal thickness deviating more than 10% from the mean value  
160 for all eyes will be rejected. Users should be aware that slit-lamp microscopes could yield  
161 discordant corneal thickness measurements if the slit-width setting of the microscope is  
162 not properly calibrated.

163 24. Once all eyes have been examined and approved, the eyes are incubated for  
164 approximately 45 to 60 minutes to equilibrate them to the test system prior to dosing.  
165 Following the equilibration period, a zero reference measurement is recorded for corneal  
166 thickness and opacity to serve as a baseline (i.e., time = 0). The fluorescein score  
167 determined at dissection is used as the baseline measurement for that endpoint.

### 168 **Application of the Test Substance**

169 25. Immediately following the zero reference measurements, the eye (in its holder) is  
170 removed from the superfusion apparatus, placed in a horizontal position, and the test  
171 substance is applied to the cornea.

172 26. Liquid test substances are typically tested undiluted, but may be diluted if deemed  
173 necessary (e.g., as part of the study design). The preferred solvent for diluted substances  
174 is physiological saline. However, alternative solvents may also be used under controlled  
175 conditions, but the appropriateness of solvents other than physiological saline must be  
176 demonstrated.

177 27. Liquid test substances are applied to the cornea such that the entire surface of the  
178 cornea is evenly covered with the test substance; the standard volume is 0.03 mL.

179 28. Solid substances should be ground as finely as possible in a mortar and pestle, or  
180 comparable grinding tool. The powder is applied to the cornea such that the surface is  
181 uniformly covered with the test substance; the standard amount is 0.03 g.

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<sup>5</sup>The superfusion apparatus and the steel clamps are available for purchase from TNO Quality of Life, Department of Toxicology and Applied Pharmacology, Zeist, the Netherlands (<http://www.tno.nl>).

182 29. The test substance (liquid or solid) is applied for 10 seconds and then rinsed from  
183 the eye with isotonic saline (approximately 20 mL) at ambient temperature. The eye (in  
184 its holder) is subsequently returned to the superfusion apparatus in the original upright  
185 position.

#### 186 **Control Substances**

187 30. Concurrent negative or solvent/vehicle controls and positive controls should be  
188 included in each experiment.

189 31. When testing liquids at 100% or solids, physiological saline is used as the  
190 concurrent negative control in the ICE test method to detect non-specific changes in the  
191 test system, and to ensure that the assay conditions do not inappropriately result in an  
192 irritant response.

193 32. When testing diluted liquids, a concurrent solvent/vehicle control group is  
194 included in the ICE test method to detect non-specific changes in the test system, and to  
195 ensure that the assay conditions do not inappropriately result in an irritant response. As  
196 stated in paragraph 26, only a solvent/vehicle that has been demonstrated to have no  
197 adverse effects on the test system can be used.

198 33. A known ocular irritant is included as a concurrent positive control in each  
199 experiment to verify that an appropriate response is induced. As the ICE assay is being  
200 used in this test guideline to identify corrosive or severe irritants, the positive control  
201 should be a reference substance that induces a severe response in this test method.  
202 However, to ensure that variability in the positive control response across time can be  
203 assessed, the magnitude of the severe response should not be excessive. Sufficient *in*  
204 *vitro* data for the positive control should be generated such that a statistically defined  
205 acceptable range for the positive control can be calculated. If adequate historical ICE test  
206 method data are not available for a particular positive control, studies may need to be  
207 conducted to provide this information.

208 34. Benchmark substances are useful for evaluating the ocular irritancy potential of  
209 unknown chemicals of a specific chemical or product class, or for evaluating the relative  
210 irritancy potential of an ocular irritant within a specific range of irritant responses.

#### 211 **Endpoints Measured**

212 35. Treated corneas are evaluated at pretreatment and at 30, 75, 120, 180, and 240  
213 minutes ( $\pm$  5 minutes) after the post-treatment rinse. These time points provide an  
214 adequate number of measurements over the four-hour treatment period, while leaving  
215 sufficient time between measurements for the requisite observations to be made for all  
216 eyes.

217 36. The endpoints evaluated are corneal opacity, swelling, fluorescein retention, and  
218 morphological effects (e.g., pitting or loosening of the epithelium). All of the endpoints,  
219 with the exception of fluorescein retention (which is determined only at pretreatment and

220 30 minutes after test substance exposure) are determined at each of the above time points.  
 221 To maximize obtaining reproducible results, reference photographs for all subjective  
 222 endpoints (i.e., corneal opacity, fluorescein retention, morphological effects, and, if  
 223 conducted, histopathology) should be available.

224 37. It is recommended that digital photographs should be taken to document the  
 225 results obtained in regard to corneal opacity or other gross morphological effects, as well  
 226 as fluorescein staining.

227 38. After the final examination at four hours, eyes may be preserved in 4% neutral  
 228 buffered formaldehyde for possible histopathological examination.

229 39. Corneal swelling is determined from corneal thickness measurements made with  
 230 an optical pachymeter on a slit-lamp microscope. It is expressed as a percentage and is  
 231 calculated from corneal thickness measurements according to the following formula:

$$232 \left( \frac{\text{corneal thickness at time } t - \text{corneal thickness at time } = 0}{\text{corneal thickness at time } = 0} \right) \times 100$$

233 40. The mean percentage of corneal swelling for all test eyes is calculated for all  
 234 observation time points. Based on the highest mean score for corneal swelling, as  
 235 observed at any time point, an overall category score is then given for each test  
 236 substance.

237 41. Corneal opacity is calculated by using the area of the cornea that is most densely  
 238 opacified for scoring. The mean corneal opacity value for all test eyes is calculated for all  
 239 observation time points. Based on the highest mean score for corneal opacity, as observed  
 240 at any time point, an overall category score is then given for each test substance.

241	<u>Score</u>	<u>Observation</u>
242	0	No opacity
243	0.5	Very faint opacity
244	1	Scattered or diffuse areas; details of the iris are clearly visible
245	2	Easily discernible translucent area; details of the iris are slightly obscured
246	3	Severe corneal opacity; no specific details of the iris are visible; size of the
247		pupil is barely discernible
248	4	Complete corneal opacity; iris invisible

249 42. The mean fluorescein retention value for all test eyes is calculated for the 30-  
 250 minute observation time point only, which is used for the overall category score given for  
 251 each test substance.

252	<u>Score</u>	<u>Observation</u>
253	0	No fluorescein retention
254	0.5	Very minor single cell staining
255	1	Single cell staining scattered throughout the treated area of the cornea
256	2	Focal or confluent dense single cell staining

257 3 Confluent large areas of the cornea retaining fluorescein

258 43. Morphological effects include “pitting” of corneal epithelial cells, “loosening” of  
259 epithelium, “roughening” of the corneal surface and “sticking” of the test substance to the  
260 cornea. These findings can vary in severity and may occur simultaneously. The  
261 classification of these findings is subjective according to the interpretation of the  
262 investigator.

263 44. Collection and processing of tissues for histopathological assessments are  
264 encouraged to facilitate evaluation of this endpoint for potential inclusion in decision  
265 criteria that may improve the accuracy of the test method.<sup>6</sup> A histopathological  
266 assessment can be included on a case-by-case basis to aid in the classification of test  
267 substances as a corrosive/severe irritant. Effects on the epithelium (erosion, necrosis),  
268 stroma (“disorder of fibers” and presence of pyknotic nuclei [“few” or “several”]), and  
269 endothelium (necrosis) are typically examined, along with the integrity of Bowman's and  
270 Descemet's membrane, and are assigned a qualitative grade from very slight to severe.  
271 However, a standardized scoring scheme and associated decision criteria for  
272 histopathology remain to be developed for routine use in the ICE.

## 273 **DATA AND REPORTING**

### 274 **Evaluation and Interpretation of Results**

275 45. Results from corneal opacity, swelling, and fluorescein retention should be  
276 evaluated separately and also combined to generate an Irritancy Classification for each  
277 test substance.

### 278 **Decision Criteria**

279 46. Once each endpoint has been scored, irritancy categories can be assigned based  
280 on a predetermined range. The rationale for the values selected for each range is based on  
281 a logical subdivision of these values into the ocular irritancy categories of non, slight,  
282 moderate, or severe/corrosive. Interpretation of corneal thickness, opacity, and  
283 fluorescein retention using four irritancy categories is done according to the following  
284 scales:

Mean Corneal Swelling (%) <sup>1</sup>	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

<sup>6</sup> Individuals interested in collecting and using histopathology data in the ICE test method should consult the OECD Guidance Document on Histopathological Preparation and Evaluation of Tissues from *In Vitro* Ocular Toxicity Test Methods.

>26 to 32 (<75 min after treatment)	IV
>32	IV

285 <sup>1</sup>Corneal swelling scores only applicable if thickness is measured with a Haag-Streit BP900 slit-lamp  
 286 microscope with depth-measuring device no. I and slit-width setting at 9½. Slit-lamp microscopes and  
 287 depth-measuring devices generating other swelling ranges than approximately 0-60% should be calibrated  
 288 to generate a similar range or modify the scheme to accommodate the different categories.

Mean Maximum Opacity Score <sup>1</sup>	Category
0.0-0.5	I
0.6-1.5	II
1.6-2.5	III
2.6-4.0	IV

289 <sup>1</sup>For each eye, at each of the observation time points, corneal opacity is scored from 0 to 4, using the  
 290 following criteria: 0 = no opacity; 1 = scattered or diffuse areas, details of iris clearly visible; 2 = easily  
 291 discernible translucent area, details of iris slightly obscured; 3 = severe corneal opacity, no details of iris  
 292 visible, size of pupil barely discernible; 4 = complete corneal opacity, iris not visible.

Mean Fluorescein Retention Score at 30 minutes post-treatment <sup>1</sup>	Category
0.0-0.5	I
0.6-1.5	II
1.6-2.5	III
2.6-3.0	IV

293 <sup>1</sup>The fluorescein retention value of each eye is scored from 0 to 3 at 30 min post-dosing using the following  
 294 criteria: 0 = no fluorescein retention; 1 = small number of cells retaining fluorescein; 2 = individual cells  
 295 and areas of the cornea retaining fluorescein; 3 = large areas of the cornea retaining fluorescein.

296 47. The overall *in vitro* irritancy classification for a test substance is assessed by  
 297 reading the irritancy classification that corresponds to the combination of categories  
 298 obtained for corneal swelling, corneal opacity, and fluorescein retention and applying the  
 299 schemes presented.

300 <u>Classification</u>	300 <u>Combinations of the 3 Endpoints</u>
301 Corrosive/Severe Irritant	3 x IV
302	2 x IV, 1 x III
303	2 x IV, 1 x II*
304	2 x IV, 1 x I*
305	Corneal opacity ≥ 3 at 30 min (in at least 2 eyes)
306	Corneal opacity = 4 at any time point (in at least 2 eyes)
307	Severe loosening of the epithelium (in at least 1 eye)
308	*Combinations less likely to occur.

309 48. As stated in paragraph 1, if the test substance is not identified as an ocular  
 310 corrosive or severe irritant, additional testing should be conducted for classification and  
 311 labeling purposes. The ICE test method has an overall false negative rate of 41% (13/32)  
 312 to 50% (15/30) for the identification of ocular corrosives and severe irritants, when  
 313 compared to *in vivo* rabbit eye test method data classified according to the EPA (5), EU  
 314 (6), or GHS (7) classification systems. When substances within certain chemical (i.e.,

315 alcohols and surfactants) and physical (i.e., solids) classes are excluded from the  
316 database, the false negative rate is improved to 29% (2/7) to 33% (3/9) (1).

317 49. Even if an ocular corrosive or severe irritant classification is not obtained for a  
318 test substance, ICE data can be useful in conjunction with *in vivo* data or valid *in vitro*  
319 test data to further evaluate the usefulness and limitations of the ICE test method for  
320 identifying non-severe irritants and nonirritants. Therefore, it is recommended that the  
321 complete classification scheme of the ICE test method (i.e., corrosive/severe irritants,  
322 non-severe irritants, or nonirritants) be applied and that these data are reported in parallel  
323 with any other data obtained (i.e., from the *in vivo* rabbit eye test or an adequately  
324 validated *in vitro* test method). The remaining categories in the ICE test method  
325 classification scheme include the following proposed decision criteria:

326 Moderate Irritant	At least two category scores of III, or at most one category 327 of IV
328 Mild Irritant	At least two category scores of II, or at most one category 329 III
330 Non-Irritant	At most one category score of II

### 331 **Study Acceptance Criteria**

332 50. A test is considered acceptable if the concurrent negative or vehicle/solvent  
333 controls and the concurrent positive controls give an Irritancy Classification that falls  
334 within nonirritating and severely irritating/corrosive classes, respectively.

### 335 **Test Report**

336 51. The test report should include the following information, if relevant to the  
337 conduct of the study:

#### 338 Test and Control Substances

- 339 • Chemical name(s) such as the structural name used by the Chemical Abstracts  
340 Service (CAS), followed by other names, if known
- 341 • The CAS Registry Number (RN), if known
- 342 • Purity and composition of the substance or preparation (in percentage(s) by weight),  
343 to the extent this information is available
- 344 • Physicochemical properties such as physical state, volatility, pH, stability, chemical  
345 class, water solubility relevant to the conduct of the study
- 346 • Treatment of the test/control substances prior to testing, if applicable (e.g., warming,  
347 grinding)
- 348 • Stability, if known

#### 349 Information Concerning the Sponsor and the Test Facility

- 350 • Name and address of the Sponsor

- 351 • Name and address of the test facility
- 352 • Name and address of the Study Director
- 353 • Storage and transport conditions of eyes (e.g., date and time of eye collection, time  
354 interval prior to initiating testing)
- 355     Justification of the Test Method and Protocol Used
- 356     Test Method Integrity
- 357 • The procedure used to ensure the integrity (i.e., accuracy and reliability) of the test  
358 method over time (e.g., periodic testing of proficiency substances, use of historical  
359 negative and positive control data)
- 360     Criteria for an Acceptable Test
- 361 • Acceptable concurrent negative control ranges based on historical data
- 362 • Acceptable concurrent positive control ranges based on historical data
- 363 • If applicable, acceptable concurrent benchmark control ranges based on historical  
364 data
- 365     Test Conditions
- 366 • Description of test system used
- 367 • Slit-lamp microscope used (e.g., model)
- 368 • Calibration information and instrument settings for the slit-lamp used
- 369 • Information for the chicken eyes used, including statements regarding their quality
- 370 • Details of test procedure used
- 371 • Test substance concentration(s) used
- 372 • Description of any modifications of the test procedure
- 373 • Reference to historical data of the model (e.g., negative and positive controls,  
374 proficiency substances, benchmark substances)
- 375 • Description of evaluation criteria used
- 376     Results
- 377 • Description of other effects observed
- 378 • Digital photographs of the eye
- 379     Discussion of the Results
- 380     Conclusion
- 381     A Quality Assurance Statement for Good Laboratory Practice (GLP)-Compliant  
382 Studies

- 383 • This statement indicates all inspections made during the study, and the dates any  
384 results were reported to the Study Director. This statement also serves to confirm  
385 that the final report reflects the raw data.

386 If GLP-compliant studies are performed, then additional reporting requirements provided  
387 in the relevant guidelines (15)(16)(17)(18) should be followed.

## 388 **REFERENCES**

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448

**ANNEX I**

449

**DEFINITIONS**

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

455

456 **Cornea:** The transparent part of the front of the eyeball that covers the iris and pupil and  
457 admits light to the interior.

458

459 **Corneal opacity:** Measurement of the extent of opaqueness of the cornea following  
460 exposure to a test substance. Increased corneal opacity is indicative of damage to the  
461 cornea.

462

463 **Corneal swelling:** An objective measurement in the ICE test of the extent of distention  
464 of the cornea following exposure to a test substance. It is expressed as a percentage and is  
465 calculated from baseline (pre-dose) corneal thickness measurements and the thickness  
466 recorded at regular intervals after exposure to the test material in the ICE test. The degree  
467 of corneal swelling is indicative of damage to the corneal epithelium.

468

469 **False negative rate:** The proportion of all positive substances falsely identified by a  
470 test method as negative. It is one indicator of test method accuracy.

471

472 **False positive rate:** The proportion of all negative substances that are falsely identified  
473 by a test method as positive. It is one indicator of test method accuracy.

474

475 **Fluorescein retention:** A subjective measurement in the ICE test of the extent of  
476 fluorescein sodium that is retained by epithelial cells in the cornea following exposure to a  
477 test substance. The degree of fluorescein retention is indicative of damage to the corneal  
478 epithelium.

479

480 **Globally Harmonized System (GHS):** A classification system presented by the United  
481 Nations that provides (a) a harmonized criteria for classifying substances and mixtures  
482 according to their health, environmental and physical hazards, and (b) harmonized hazard  
483 communication elements, including requirements for labeling and safety data sheets.

484

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

491

492 **Hazard:** The potential for an adverse health or ecological effect. A hazard potential  
493 results only if an exposure occurs that leads to the possibility of an adverse effect being  
494 manifested.

495

496 **Negative control:** An untreated replicate containing all components of a test system.  
497 This sample is processed with test substance-treated samples and other control samples to  
498 determine whether the solvent interacts with the test system.

499

500 **Nonirritant:** Substances that are not classified as EPA Category I, II, or III; EU R41 or  
501 R36; or GHS Category 1, 2A, or 2B ocular irritants.

502

503 **Ocular corrosive:** (a) A substance that causes irreversible tissue damage to the eye. (b)  
504 Substances that are classified as GHS Category 1, EPA Category I, or EU R41 ocular  
505 irritants.

506

507 **Ocular irritant:** (a) A substance that produces a reversible change in the eye following  
508 application to the anterior surface of the eye; (b) Substances that are classified as EPA  
509 Category II or III; EU R36; or GHS Category 2A, or 2B ocular irritants.

510

511 **Ocular severe irritant:** (a) A substance that causes tissue damage in the eye following  
512 application to the anterior surface of the eye that is not reversible within 21 days of  
513 application or causes serious physical decay of vision. (b) Substances that are classified  
514 as GHS Category 1, EPA Category I, or EU R41 ocular irritants.

515

516 **Positive control:** A replicate containing all components of a test system and treated with  
517 a substance known to induce a positive response. To ensure that variability in the positive  
518 control response across time can be assessed, the magnitude of the severe response  
519 should not be excessive.

520

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

524

525 **Slit-lamp microscope:** An instrument used to directly examine the eye under the  
526 magnification of a binocular microscope by creating a stereoscopic, erect image. In the  
527 ICE test method, this instrument is used to view the anterior structures of the chicken eye  
528 as well as to objectively measure corneal thickness with a depth-measuring device  
529 attachment.

530

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

537

538 **Tiered testing:** A stepwise testing strategy where all existing information on a test  
539 substance is reviewed, in a specified order, using a weight of evidence process at each  
540 tier to determine if sufficient information is available for a hazard classification decision ,  
541 prior to progression to the next tier. If the irritancy potential of a test substance can be  
542 assigned based on the existing information, no additional testing is required. If the  
543 irritancy potential of a test substance cannot be assigned based on the existing  
544 information, a step-wise sequential animal testing procedure is performed until an  
545 unequivocal classification can be made.

546

547 **Validated test method:** An accepted test method for which validation studies have been  
548 completed to determine the relevance and reliability of this method for a specific  
549 proposed use.

550

551 **Weight-of-evidence:** The process of considering the strengths and weaknesses of various  
552 pieces of information in reaching and supporting a conclusion concerning the hazard  
553 potential of a substance.

554

**ANNEX II**

555

**ICCVAM RECOMMENDED PROTOCOL FOR THE  
ISOLATED CHICKEN EYE (ICE) TEST METHOD**

556

557 This proposed protocol for measuring corneal damage was developed following a  
558 comprehensive test method evaluation process conducted by ICCVAM, which included  
559 an international independent scientific peer review of the validation status and scientific  
560 validity of the BCOP (1)(2). It is based primarily on the current protocol used by Menk  
561 Prinsen, the original developer of the test method (3)(4)(5)(6)(7). Future studies using  
562 the ICE test method could include further characterization of the usefulness or limitations  
563 of the ICE in a weight of evidence approach for regulatory decision making. Users  
564 should be aware that the proposed test method protocol could be revised based on any  
565 additional optimization and/or validation studies that are conducted in the future.  
566 ICCVAM recommends that test method users consult the ICCVAM/NICEATM website  
567 (<http://iccvam.niehs.nih.gov/>) to ensure use of the most current test method protocol.

568

## 568 **1.0 PURPOSE AND APPLICABILITY**

569 The purpose of this protocol is to describe the procedures used to evaluate the potential  
570 ocular irritancy of a test substance as measured by its ability to induce toxicity in an  
571 enucleated chicken eye. Toxic effects are measured by: 1) qualitative assessment of  
572 corneal opacity; 2) qualitative measurement of increased retention of fluorescein dye  
573 within the eye (permeability); 3) quantitative measurement of increased corneal thickness  
574 (swelling); and 4) qualitative evaluation of macroscopic morphological damage to the  
575 corneal surface. The opacity, swelling, and permeability assessments following exposure  
576 to a test article are assessed individually and then combined to derive an Eye Irritancy  
577 Classification.

578 The focus of this protocol is on the use of the ICE test method for the detection of ocular  
579 corrosives and severe irritants, as defined by the U.S. Environmental Protection Agency  
580 (EPA)(8), the European Union (EU)(9), and in the United Nations Globally Harmonized  
581 System (GHS) of Classification and Labelling of Chemicals (10). Substances other than  
582 ocular corrosives and severe irritants (e.g., nonirritants and mild/moderate ocular  
583 irritants) have been tested using this protocol; however, the accuracy and reliability of the  
584 ICE test method have not yet been formally evaluated for the other classes of ocular  
585 irritancy defined by EPA (8), EU (9), and the GHS (10).

## 586 **2.0 SAFETY AND OPERATING PRECAUTIONS**

587 All procedures with chicken eyes should follow the institution's applicable regulations  
588 and procedures for handling of human or animal materials, which include, but are not  
589 limited to, tissues and tissue fluids. Universal laboratory precautions are recommended,  
590 including the use of laboratory coats, eye protection, and gloves. If available, additional  
591 precautions required for specific study substances should be identified in the Material  
592 Safety Data Sheet for that substance.

## 593 **3.0 MATERIALS, EQUIPMENT, AND SUPPLIES**

### 594 **3.1 Source of Chicken Eyes**

595 Spring chickens obtained from a local source (e.g., poultry slaughterhouse),  
596 approximately 7 weeks old, male or female, with a weight range of 2.5-3.0 kg (breed not  
597 specified)

### 598 **3.2 Equipment and Supplies**

- 599 • Custom superfusion apparatus (that will accommodate the eye holders)  
600 with a water pump for temperature control
- 601 • Dissection equipment (e.g., scissors and forceps)
- 602 • Electronic balance
- 603 • Eye holders (custom stainless steel clamps)
- 604 • Micropipettor and pipette tips
- 605 • Mortar and pestle

- 606 • Physiological saline
- 607 • Slit-lamp microscope with an optical pachymeter equipped with centering
- 608 lights
- 609 • Tissue paper
- 610 • Transportation chambers (humidified plastic boxes containing tissues
- 611 moistened with isotonic saline or water)
- 612 • Volumetric flasks
- 613 • Peristaltic pump for the saline drip onto the eye

### 614 **3.3 Solutions**

615 The manufacturer's recommendations with regard to storage temperature and shelf life of  
616 stock solutions should be followed. Assay solutions should be prepared volumetrically.

- 617 • Fluorescein sodium BP, 2% w/v (also available commercially)
- 618 • Isotonic saline (i.e., 0.9% NaCl)
- 619 • 4% neutral buffered formaldehyde

## 620 **4.0 TEST SUBSTANCE PREPARATION**

### 621 **4.1 Liquid Test Substances**

622 Liquid test substances are typically tested undiluted, but may be diluted if deemed  
623 necessary (e.g., as part of the study design). The preferred solvents for diluted substances  
624 are either deionized/distilled water or physiological saline. However, alternative solvents  
625 may also be used under controlled conditions, but the appropriateness of solvents other  
626 than deionized/distilled water or physiological saline must be demonstrated.

### 627 **4.2 Solid Test Substances**

628 Prior to testing, solid, particulate or granular test substances should be ground as finely as  
629 possible in a mortar and pestle.

## 630 **5.0 CONTROLS**

### 631 **5.1 Negative Controls**

632 Concurrent negative or solvent/vehicle controls and positive controls should be included  
633 in each experiment. When testing liquids at 100% or solids, physiological saline is used  
634 as the concurrent negative control in the ICE test method to detect non-specific changes  
635 in the test system, and to ensure that the assay conditions do not inappropriately result in  
636 an irritant response.

### 637 **5.2 Solvent/Vehicle Controls**

638 When testing diluted liquids, a concurrent solvent/vehicle control group is included in  
639 the ICE test method to detect non-specific changes in the test system, and to ensure that  
640 the assay conditions do not inappropriately result in an irritant response. Only a  
641 solvent/vehicle that has been demonstrated to have no adverse effects on the test system

642 can be used.

### 643 **Positive Controls**

644 A known ocular irritant is included as a concurrent positive control in each experiment to  
645 verify that an appropriate response is induced. As the ICE assay is being used in this test  
646 guideline to identify corrosive or severe irritants, the positive control should be a  
647 reference substance that induces a severe response in this test method. However, to  
648 ensure that variability in the positive control response across time can be assessed, the  
649 magnitude of the severe response should not be excessive. Sufficient *in vitro* data for the  
650 positive control should be generated such that a statistically defined acceptable range for  
651 the positive control can be calculated. If adequate historical ICE test method data are not  
652 available for a particular positive control, studies may need to be conducted to provide  
653 this information.

### 654 **5.3 Benchmark Controls**

655 Benchmark controls may be useful to demonstrate that the test method is functioning  
656 properly for detecting the ocular irritancy potential of chemicals of a specific chemical  
657 class or a specific range of responses, or for evaluating the relative irritancy potential of a  
658 ocular irritant. Appropriate benchmark controls should have the following properties:

- 659 • consistent and reliable source(s) for the chemical
- 660 • structural and functional similarity to the class of the substance being  
661 tested
- 662 • known physical/chemical characteristics
- 663 • supporting data on known effects in animal models
- 664 • known potency in the range of the desired response

## 665 **6.0 EXPERIMENTAL DESIGN**

### 666 **6.1 Collection and Transport Conditions of Chicken Eyes**

667 Heads of spring chickens should be obtained from a local source (e.g., poultry  
668 slaughterhouse). Heads should be cut off immediately after sedation of the animals by  
669 electric shock and incision of the neck for bleeding. Chicken heads may then be  
670 transported to the laboratory at ambient temperature in humidified plastic boxes (i.e.,  
671 sealed with tissues moistened with isotonic saline) within two hours after they are  
672 humanely killed. Once at the laboratory, the eyes may be dissected from each chicken  
673 head.

### 674 **6.2 Preparation of Eyes**

- 675 a. Carefully remove the eyelids without damaging the cornea. Place a drop  
676 of fluorescein sodium BP 2% w/v onto the corneal surface for 10-20  
677 seconds, and then immediately rinse the eye with 20 mL isotonic saline.  
678 Examine the fluorescein-treated cornea with a slit-lamp microscope to

- 679 ensure that the cornea is undamaged (i.e., fluorescein retention and corneal  
680 opacity scores  $\leq 0.5$ ).
- 681 b. If undamaged, further dissect the eye from the eye socket, taking care not  
682 to damage the corneal epithelium. When removing the eye from the orbit,  
683 a visible portion of the optic nerve should be left attached to the eye.
- 684 c. Once removed from the orbit, place the eye on an underpad and cut away  
685 the nictitating membrane and other connective tissue.
- 686 d. Mount the eyes in stainless steel clamps (one eye per clamp), with the  
687 cornea positioned vertically and then transfer each clamp to a chamber in  
688 the superfusion apparatus. The chambers of the superfusion apparatus  
689 should be temperature controlled at  $32 \pm 1.5^\circ\text{C}$  with a water pump.  
690 Position the clamp in the superfusion apparatus such that the entire cornea  
691 is supplied with isotonic saline from a bent stainless steel tube at a rate of  
692 0.10-0.15 mL/minute via a peristaltic pump.
- 693 e. After being placed in the superfusion apparatus, examine the eyes again  
694 with the slit-lamp microscope to ensure that they have not been damaged  
695 (i.e., no corneal opacity) during the dissection procedure. Corneal  
696 thickness should also be measured at this time at the corneal apex using  
697 the depth-measuring device on the slit-lamp microscope. Eyes with: 1) a  
698 corneal thickness deviating more than 10% from the mean value for the  
699 eyes, 2) a fluorescein retention score of  $> 0.5$ ) any additional signs of  
700 damage should be rejected as test eyes and replaced.
- 701 f. Once all eyes have been examined and approved, incubate eyes at  $32 \pm 1.5$   
702  $^\circ\text{C}$  for 45-60 minutes to equilibrate them to the test system prior to dosing.

### 703 6.3 Treatment Groups

704 Use a minimum of three eyes to be treated with each test substance (including both  
705 positive and negative controls).

### 706 6.4 Treatment of Eyes and Observations

#### 707 6.4.1 Dosing procedure

- 708 • After the equilibration period, record a zero reference measurement for  
709 corneal thickness and corneal opacity to serve as a baseline (i.e., time = 0).  
710 The fluorescein retention score determined at dissection is used as the  
711 baseline measurement.
- 712 • Immediately following the zero reference measurement, apply the test  
713 substance to the eye (see **Sections 6.4.1.1** and **6.4.1.2**).
- 714 • During the dosing procedure, remove the clamp holding the eye from the  
715 superfusion apparatus and place it on tissue paper with the cornea facing  
716 upwards.

717 • Apply the test material for a total of 10 seconds and then rinse the eye  
718 with  
719 20 ml isotonic saline at room temperature.

720 • After the rinse step, return the eye to the superfusion apparatus.

#### 721 6.4.1.1 *Liquid test substances*

722 Apply a liquid test substance at 0.03 mL with a micropipettor such that the entire surface  
723 of the cornea is covered with the test substance.

#### 724 6.4.1.2 *Solid test materials*

725 If necessary, grind solid test substances into a fine powder with a mortar and pestle, or  
726 comparable grinding tools. Apply 0.03 g of a solid test substance evenly over the entire  
727 surface of the cornea

### 728 6.4.2 Endpoint Observations

729 • Examine the control and test eyes at 30, 75, 120, 180, and 240 minutes ( $\pm$   
730 5 minutes) after treatment using the criteria and scoring system as  
731 indicated in **Section 6.4.2.1**.

732 • Corneal opacity, corneal thickness, and any morphological effects should  
733 be evaluated at each time point, while fluorescein retention is determined  
734 only at the 30 minute time point.

735 • After the final (240 minutes) examination, immerse all eyes in 4% neutral  
736 buffered formaldehyde for preservation for possible histopathological  
737 examination (if necessary).

738 • To maximize the likelihood of obtaining reproducible results, reference  
739 photographs for all subjective endpoints (i.e., corneal opacity, fluorescein  
740 retention, morphological effects, histopathology) should be readily  
741 available.

#### 742 6.4.2.1 *Criteria and Scoring System*

743 The following criteria and scoring system are applied for the assessment of possible  
744 effects:

745 a. Corneal swelling is expressed as a percentage and is calculated according  
746 to the following formula:

$$747 \left( \frac{\text{corneal thickness at time } t - \text{corneal thickness at time } = 0}{\text{corneal thickness at time } = 0} \right) \times 100$$

748 The mean percentage of swelling for all test eyes is calculated for all observation time  
749 points. Based on the highest mean score for corneal swelling, as observed at any time  
750 point, an overall category score is then given for each test substance.

751 b. Corneal opacity is calculated by using the area of the cornea that is most  
752 densely opacified for scoring.

	<u>Score</u>	<u>Observation</u>
753		
754	0	No opacity
755	0.5	Very faint opacity
756	1	Scattered or diffuse areas; details of the iris are clearly visible
757	2	Easily discernible translucent area; details of the iris are slightly obscured
758		
759	3	Severe corneal opacity; no specific details of the iris are visible; size of the pupil is barely discernible
760		
761	4	Complete corneal opacity; iris invisible
762		The mean corneal opacity value for all test eyes is calculated for all observation time points.
763		

764 c. Fluorescein retention  
 765 The mean fluorescein retention value for all test eyes is calculated for the  
 766 30-minute observation time point only. When test substances have adhered  
 767 to the cornea, fluorescein retention can be determined whenever the test  
 768 substance has sufficiently loosened. The following scale is used for  
 769 scoring:

	<u>Score</u>	<u>Observation</u>
770		
771	0	No fluorescein retention
772	0.5	Very minor single cell staining
773	1	Single cell staining scattered throughout the treated area of the cornea
774		
775	2	Focal or confluent dense single cell staining
776	3	Confluent large areas of the cornea retaining fluorescein

777 d. Morphological effects include “pitting” of corneal epithelial cells,  
 778 “loosening” of epithelium, “roughening” of the corneal surface and  
 779 “sticking” of the test substance to the cornea. These findings can vary in  
 780 severity and may occur simultaneously. The classification of these  
 781 findings is subjective according to the interpretation of the investigator.  
 782 On the basis of severity of the observed findings, these effects are divided  
 783 into four categories: 1 = none; 2 = slight; 3 = moderate; 4 = severe.

784 e. A histopathological evaluation of the corneal tissue should be included  
 785 when the standard ICE endpoints (i.e., corneal opacity, swelling, and  
 786 fluorescein retention) produce borderline results. A standardized scoring  
 787 scheme using the formal language of pathology to describe any effects  
 788 should be included.

## 789 **7.0 EVALUATION OF TEST RESULTS**

790 Results from the three test method endpoints, corneal opacity, corneal swelling, and  
 791 fluorescein retention should be evaluated separately (as in **Section 9.0**), and also  
 792 combined to generate an Irritancy Classification for a test material (as in **Section 10.0**).

## 793 **8.0 CRITERIA FOR AN ACCEPTABLE TEST**

794 A test is considered acceptable if the concurrent negative or vehicle/solvent controls and  
 795 the concurrent positive controls give an Irritancy Classification that falls within  
 796 nonirritating and severely irritating/corrosive classes, respectively.

## 797 9.0 DATA INTERPRETATION

798 Interpretation of corneal thickness, corneal opacity, and fluorescein retention using four  
 799 irritancy categories is done according to the following scales:

### 800 9.1 Corneal Thickness

801

Mean Corneal Swelling (%)	Category
0 to 5	I
> 5 to 12	II
> 12 to 18 (>75 minutes after treatment)	II
> 12 to 18 (<75 minutes after treatment)	III
> 18 to 26	III
> 26 to 32 (>75 minutes after treatment)	III
> 26 to 32 (<75 minutes after treatment)	IV
> 32	IV

### 802 9.2 Corneal Opacity

803

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

### 804 9.3 Fluorescein Retention

805

Mean Fluorescein Retention Score at 30 minutes post-treatment	Category
0.0-0.5	I
0.6-1.5	II
1.6-2.5	III
2.6-3.0	IV

## 806 10.0 ASSESSMENT OF THE EYE IRRITANCY

807 The severe irritancy classification for a test substance is assessed by reading the irritancy  
 808 classification that corresponds to the combination of categories obtained for corneal  
 809 swelling, corneal opacity, and fluorescein retention, as presented in the scheme below.

810 Classification                      Combinations of the 3 Endpoints

811 Severely Irritating                      3 x IV

812	2 x IV, 1 x III
813	2 x IV, 1 x II*
814	2 x IV, 1 x I*
815	Corneal opacity $\geq 3$ at 30 min (in at least 2 eyes)
816	Corneal opacity = 4 at any time point (in at least 2 eyes)
817	Severe loosening of the epithelium (in at least 1 eye)
818	*Combinations less likely to occur.

819 Even if an ocular corrosive or severe irritant classification is not obtained for a test  
 820 substance, ICE data would be useful in conjunction with *in vivo* data or valid *in vitro* test  
 821 data to further evaluate the usefulness and limitations of the ICE test method for  
 822 identifying non-severe irritants and nonirritants. Therefore, it is recommended that the  
 823 complete classification scheme of the ICE test method (i.e., corrosive/severe irritants,  
 824 non-severe irritants, or nonirritants) be applied and that these data are reported in parallel  
 825 with any other data obtained (i.e., from the *in vivo* rabbit eye test or an adequately  
 826 validated *in vitro* test method). The remaining categories in the ICE test method  
 827 classification scheme include the following:

828	Moderate Irritant	At least two category scores of III, or at most one category
829		of IV
830	Mild Irritant	At least two category scores of II, or at most one category
831		III
832	Non-Irritant	At most one category score of II

### 833 **11.0 STUDY REPORT**

834 The study report should include the following information, if relevant to the conduct of  
 835 the study:

#### 836 Test and Control Substances

- 837 • Chemical name(s) such as the structural name used by the Chemical Abstracts  
 838 Service (CAS), followed by other names, if known
- 839 • The CAS Registry Number (RN), if known
- 840 • Purity and composition of the substance or preparation (in percentage(s) by weight),  
 841 to the extent this information is available
- 842 • Physicochemical properties such as physical state, volatility, pH, stability, chemical  
 843 class, water solubility relevant to the conduct of the study
- 844 • Treatment of the test/control substances prior to testing, if applicable (e.g., warming,  
 845 grinding)
- 846 • Stability, if known

#### 847 Information Concerning the Sponsor and the Test Facility

- 848 • Name and address of the Sponsor

- 849 • Name and address of the test facility
- 850 • Name and address of the Study Director
- 851 • Storage and transport conditions of eyes (e.g., date and time of eye collection, time  
852 interval prior to initiating testing)
- 853     Justification of the Test Method and Protocol Used
- 854     Test Method Integrity
- 855 • The procedure used to ensure the integrity (i.e., accuracy and reliability) of the test  
856 method over time (e.g., periodic testing of proficiency substances, use of historical  
857 negative and positive control data)
- 858     Criteria for an Acceptable Test
- 859 • Acceptable concurrent negative control ranges based on historical data
- 860 • Acceptable concurrent positive control ranges based on historical data
- 861 • If applicable, acceptable concurrent benchmark control ranges based on historical  
862 data
- 863     Test Conditions
- 864 • Description of test system used
- 865 • Slit-lamp microscope used (e.g., model)
- 866 • Calibration information and instrument settings for the slit-lamp used
- 867 • Information for the chicken eyes used, including statements regarding their quality
- 868 • Details of test procedure used
- 869 • Test substance concentration(s) used
- 870 • Description of any modifications of the test procedure
- 871 • Reference to historical data of the model (e.g., negative and positive controls,  
872 proficiency substances, benchmark substances)
- 873 • Description of evaluation criteria used
- 874     Results
- 875 • Description of other effects observed
- 876 • Digital photographs of the eye
- 877     Discussion of the Results
- 878     Conclusion
- 879     A Quality Assurance Statement for Good Laboratory Practice (GLP)-Compliant  
880 Studies

- 881 • This statement indicates all inspections made during the study, and the dates any  
882 results were reported to the Study Director. This statement also serves to confirm  
883 that the final report reflects the raw data.

884 If GLP-compliant studies are performed, then additional reporting requirements provided  
885 in the relevant guidelines (11)(12)(13)(14) should be followed.

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