

Section 6.1.4

Acute Eye Irritation

Annex Point IIA6.1.4

Chicken enucleated eye test

Official
use only

	1 REFERENCE
1.1 Reference	██████████ 1996. Chicken Enucleated Eye Test with three samples of lactic acid; an alternative to the Draize eye irritation test with albino rabbits. TNO Report nr. V96.157
1.2 Data protection	Yes
1.2.1 Data owner	Purac Biochem BV
1.2.2 Companies with letter of access	No
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its entry into Annex I
	2 GUIDELINES AND QUALITY ASSURANCE
2.1 Guideline study	CEET = Chicken Enucleated Eye Test is an alternative test system: in their latest updates of the guidelines on eye irritancy testing, both the EEC and the OECD allow for the use of alternative ex vivo/ in vitro test systems
2.2 GLP	Yes
2.3 Deviations	No
	3 MATERIALS AND METHODS
3.1 Test material	Three different forms of lactic acid are tested: 1. Purac H60: powder 2. Purac HS88: liquid: an aqueous solution, as given in Section 2 3. Purac BF S36 Lactic acid B : liquid: a buffered solution
3.1.1 Lot/Batch number	1. Purac H60: RB 374 EA 2. Purac HS88: AP 5074L 3. Purac BF S36 Lactic acid B : HM 960207
3.1.2 Specification	1. Purac H60: L+ lactic acid solid adduct with Ca-lactate 2. Purac HS88: L+ lactic acid aqueous solution ; CAS 79-33-4, as given in Section 2 3. Purac BF S36 Lactic acid B : L+ lactic acid, sodium lactate: CAS 79-33-4 and CAS 867-56-1
3.1.2.1 Description	1. Purac H60: creamy, yellow powder 2. Purac HS88: clear colourless liquid , as given in Section 2 3. Purac BF S36 Lactic acid B : clear colourless liquid
3.1.2.2 Purity	1. Purac H60: 60% lactic acid and 40% Ca-lactate 2. Purac HS88: circa 88% lactic acid, as given in Section 2 3. Purac BF S36 Lactic acid B : 73 – 84 % lactic acid

Section 6.1.4

Acute Eye Irritation

Annex Point IIA6.1.4

Chicken enucleated eye test

3.1.2.3 Stability	1. Purac H60: no information 2. Purac HS88: no information, as given in Section 2 3. Purac BF S36 Lactic acid B: no information
3.2 Test Animals	
3.2.1 Species	Chickens were used as eye donors
3.2.2 Strain	ROSS spring chickens
3.2.3 Source	Poultry slaughterhouse v.d. Bor, Nijkerkerveen, The Netherlands
3.2.4 Sex	Male and female
3.2.5 Age/weight at study initiation	7 weeks old / 2.5-3.0 kg
3.2.6 Number of animals per group	Not applicable for this test: the Chicken Enucleated Eye Test (CEET) is an 'ex vivo bioassay' and the eyes of the chicken were used
3.2.7 Control animals	Not applicable; however, control eyes were used.
3.3 Administration/ Exposure	
3.3.1 Preparation of test substance	Test substances were used undiluted
3.3.2 Amount of active substance instilled	The two liquid samples were each applied in an amount of 0.03 mL in such a way that the entire surface of the cornea was bathed with the test material. For the solid sample 0.03 gram powder was applied.
3.3.3 Exposure period	10 seconds
3.3.4 Postexposure period	After the 10 seconds exposure the corneal surface was rinsed with 20 ml of isotonic saline
3.4 Examinations	
3.4.1 Ophthalmoscopic examination	Not applicable
3.4.1.1 Scoring system	The scoring system for the CEET is included in the Annexes to the study report. At 0, 30, 75, 120, 180 and 240 minutes after treatment
3.4.1.2 Examination time points	
3.4.2 Other investigations	Not applicable
3.5 Further remarks	
	RESULTS AND DISCUSSION
3.6 Clinical signs	Not applicable
3.7 Average score	
3.7.1 Cornea	Not applicable
3.7.2 Iris	Not applicable

Section 6.1.4

Acute Eye Irritation

Annex Point IIA6.1.4

Chicken enucleated eye test

3.7.3	Conjunctiva	See under 3.9	
		Not applicable	
3.7.3.1	Redness		
		Not applicable	
3.7.3.2	Chemosis		
3.8	Reversibility	Not applicable	
3.9	Other	CEET:	
	Swelling %	Maximum score and category for: 1. Purac H60: 17 %; category II 2. Purac HS88: 28 %; category III 3. Purac BF S36 Lactic acid B : 6 %; category II	
	Opacity	Maximum score and category for: 1. Purac H60: 2.0; category III 2. Purac HS88: 4.0; category IV 3. Purac BF S36 Lactic acid B : 0.5; category I	
	Fluorescein retention	Maximum score and category for: 1. Purac H60: 2.0; category III 2. Purac HS88: 3.0; category IV 3. Purac BF S36 Lactic acid B : 1.0; category II	
3.10	Overall result	The three lactic acid samples cause different corneal effects in the CEET: 1. Purac H60: moderate corneal effects 2. Purac HS88: severe corneal effects 3. Purac BF S36 Lactic acid B : slight corneal effects	X
		4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1	Materials and methods	Three different forms of lactic acid were tested for eye irritating/corrosive potential in an <i>ex vivo</i> bioassay, namely the Enucleated Eye Test with chicken eyes (CEET). The eyes were collected from a slaughter-house for chickens (which were killed for human consumption).	
4.2	Results and discussion	On the basis of the results obtained with this <i>in vitro</i> (<i>ex vivo</i>) assay and according to the EU classification, the following was concluded: 1. Purac H60: irritating to the eyes (R36) 2. Purac HS88: severely irritating to the eyes (R41) 3. Purac BF S36 Lactic acid B : not irritating to the eyes	
4.3	Conclusion		X
4.3.1	Reliability	1	
4.3.2	Deficiencies	N	

Section 6.1.4

Acute Eye Irritation

Annex Point IIA6.1.4

Chicken enucleated eye test

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2008/05/08
Materials and Methods	Applicant's version is acceptable.
Results and discussion	3.9 See CA-Table 1
Conclusion	Purac HS88 (circa 88% lactic acid, pH 2): severely irritating to the eyes (R41)
Reliability	1
Acceptability	Acceptable without restrictions
Remarks	None
COMMENTS FROM ...	
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

Appendix

Table A6_1_4E-1. Results of eye irritation study

Use this table, if relevant effects occur.

	Cornea	Iris	Conjunctiva	
			redness	chemosis
score (average of animals investigated)	0 to 4	0 to 2	0 to 3	0 to 4
60 min	Not applicable for CEET			
24 h				
48 h				
72 h				
Average 24h, 48h, 72h				
Area effected				
Maximum average score (including area affected, max 110)				

Section 6.1.4**Acute Eye Irritation****Annex Point IIA6.1.4**

Chicken enucleated eye test

Reversibility*
average time for reversion
<i>Give method of calculation maximum average score.</i>
* <i>c : completely reversible</i> <i>n c : not completely reversible</i> <i>n : not reversible</i>

CA-Table 1:

Summary of the maximum mean scores for corneal swelling, opacity and fluorescein, the irritation categories assigned, and final (EC-)classification¹ of the three **lactic acid samples**

Test material ²	Maximum mean score for:			Categories	Classification
	Swelling	Opacity	Fluorescein		
H60 (powder)	17	2.0	2.0	II/III/III	R36
HS 88 (liquid)	28	4.0	3.0	III/IV/IV	R41
BF 536 (liquid)	6	0.5	1.0	II/I/II	NI

¹ Classification in non labelling (i.e. not irritant, NI), irritant to eyes (R36), and risk of serious eye damage (R41) as employed by the European Communities (EC standards, Official Journal of the European Communities, L 110 A, Volume 36, 4 May 1993).