EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)

ESAC Request 2016-02

EURL ECVAM Scientific Advisory Committee

(ESAC)

EURL ECVAM REQUEST FOR ESAC ADVICE

on the

SkinEthic™ Human Cornea Epithelium (HCE) Eye Irritation Test (EIT)

Title page information	
Abbreviated title of ESAC request	SkinEthic™ HCE EIT
ESAC REQUEST Nr.	2016-02
Template used for preparing request	EP 3.02
Date of finalising request	27/05/2016
Date of submitting request to ESAC	27/05/2016
Request discussed through	ESAC42
Opinion expected at (date)	Written procedure following ESAC42
File name of this request	**ER2016-02_ESAC_REQUEST_SKINETHIC_HCE_EIT.pdf**

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1. TYPE OF REQUEST

Request Type	Identify request ("YES")
R1 ESAC Peer Review **of a Prevalidation Study or Validation Study**	**YES, external validation study** **(i.e. not coordinated by EURL ECVAM)**
If R1)applies please specify further:	
►Prevalidation Study	**NO**
►Prospective Validation Study	**YES** Background In December 2008, two reconstructed human eye tissue models for *in vitro* assay of eye irritation potential, EpiOcular™ Eye Irritation Test (EIT) and SkinEthic™ Human Cornea Epithelium (HCE), were sponsored for validation as alternatives to the traditional *in vivo* standard practice with rabbits (Draize test). The eye irritation validation study (EIVS) was conceived as a ring trial of comparative performance among six participant laboratories (three for each test method), testing selected chemicals to evaluate reliability (reproducibility within and between laboratories of results obtained *in vitro*) and relevance (predictive capacity of effects documented *in vivo*). Neither test method was able to comply fully with the acceptance criteria set by the validation management group (VMG). Therefore, further optimisation was recommended. With minor refinement to the EpiOcular™ EIT protocol, the method was successfully validated in 2013. The SkinEthic™ HCE protocol was subject to more comprehensive revision, followed by another validation ring trial (three laboratories) completed in 2015. In November 2015 the revised SkinEthic™ HCE test method was submitted for assessment by EURL ECVAM and formal peer-review by ESAC.
►Retrospective Validation Study	**NO**
►Validation Study based on Performance Standards	**NO**
R2 Scientific Advice on a test method submitted to EURL ECVAM for validation **(e.g. the test method's biological relevance etc.)**	**NO**
R3 Other Scientific Advice **(e.g. on test methods, their use; on technical issues such as cell culturing, stem cells, definition of performance standards etc.)**	**NO**

2. TITLE OF STUDY OR PROJECT FOR WHICH SCIENTIFIC ADVICE OF THE ESAC IS REQUESTED

L'Oréal-coordinated validation of the *in vitro* SkinEthic™ Human Cornea Epithelium (HCE) Eye Irritation Test (EIT)

3. BRIEF DESCRIPTION OF THE STUDY OR PROJECT

1) Serious eye damage/eye irritation and regulatory tests

Eye irritation is the result of reversible anterior surface tissue trauma, causing degeneration of vision. Serious eye damage is not fully reversible within 21 days of exposure (UN, 2013). Traditionally, eye irritation has been determined by the *in vivo* rabbit Draize eye test (OECD Test Guideline 405, 2012).

Validated *in vitro* alternative methods include:

- organotypic assays: Bovine Corneal Opacity and Permeability test (OECD updated Test Guideline 437, 2013) and Isolated Chicken Eye test (OECD updated Test Guideline 438, 2013).
- cell-based methods: Fluorescein Leakage assay (OECD Test Guideline 460, 2012), Cytosensor Microphysiometer assay (OECD draft Test Guideline, 2012) and Short Time Exposure assay (OECD Test Guideline 491, 2015).
- reconstructed human cornea-like epithelium (RhCE) test: EpiOcular™ EIT (OECD Test Guideline 492, 2015).

2) SkinEthic™ HCE EIT purpose

At present, no single *in vitro* method can fully replace the *in vivo* Draize eye test for assessment of serious eye damage/eye irritation. However, tiered combination of alternatives (so-called Top-Down/Bottom-Up approach) can reduce/replace reliance on *in vivo* procedures (Scott *et al*., 2010; OECD draft Guidance, 2015). Top-Down differentiates chemicals inducing serious eye damage (GHS category 1) as priority, while Bottom-Up first discriminates 'non-irritants' (GHS no category).

The SkinEthic™ HCE EIT method is intended for inclusion in a Top-Down/Bottom-Up assessment strategy, particularly relevant for industrial chemicals or chemicals used in human exposure products, such as cosmetics ingredients which are banned from animal testing. The method is therefore required as an alternative, also effectively reducing the need for animal studies by their partial replacement. The SkinEthic™ HCE is the second RhCE test method that is validated following EpiOcular™ EIT. It is however important to have at least two of these methods validated and accepted by regulatory authorities in order to guarantee the widespread availability of this technology and avoid potential market monopolies.

In a tiered assessment strategy, the SkinEthic™ HCE EIT method is applicable as a first step in Bottom-Up discrimination of 'non-irritants' or as a confirmatory last step in a Top Down approach. However, the method is not intended to differentiate category 1 from 2 on its own.

3) SkinEthic™ HCE EIT principle

The test method addresses eye irritation caused by topical exposure to chemicals, manifested *in vivo* as local inflammation and/or opacity, resulting mechanistically from cell damage (cytotoxicity).

The *in vitro* test system uses immortalized human cornea epithelial cells, cultured to form a Reconstructed human Cornea-like Epithelium (RhCE), i.e. a three-dimensional tissue similar to the human corneal epithelium. The test method was developed to model *in vivo* topical exposure, with

prediction of positive or negative irritation response from cell viability assay. Tissue viability is determined quantitatively as a percentage, relative to a negative control (100% viable) by standardised MTT assay (photometric measurement of purple formazan production from enzymatic reduction of the vital dye MTT). Tissues treated with eye irritants show a decrease in viability relative to the negative control, with discrimination of positive or negative GHS classification defined by an optimised viability threshold percentage (prediction model).

The revised SkinEthic™ HCE test submission is complete with comprehensive protocols (SOPs) for eye irritation testing of liquids (EITL) and solids (EITS) as used in the validation study (Attachments 1a & 1b, respectively) and as intended for test method users (Attachments 1c & 1d, respectively).
Critical elements of the SOPs include:

- test system description (Human Cornea Epithelium tissue model, with quality control).
- TT: test treatment (application, exposure, incubation, MTT-formazan extraction).
- viability determination (MTT formazan assay: OD measurement, HPLC).
- prediction model: EITL (60% threshold) and EITS (50% threshold).

Acceptance criteria (for qualified test, qualified run, and complete test):

- NgC: negative control (PBS): $1.4 \leq OD \leq 2.5$ (mean of 2 replicate tissues).
- PC: positive control (methyl acetate): viability $\leq$ 30% (mean of 2 tissue replicates).
- viability difference between run replicates ≤ 20 (NgC, PC, TT).

4) SkinEthic™ HCE EIT optimisation

The test method was developed by L'Oréal, with prediction model optimization using 125 chemicals, including 71 liquids and 54 solids (Attachment 2).

A principal criterion for selection of test chemicals was availability of supporting complete and quality assured *in vivo* Draize eye irritation data. The selection was limited to commercially available chemicals.

The chemicals, incorporating 44/125 (35%) previously selected for the original ring trial eye irritation validation study (EIVS) provided a range of properties, including:

- Chemical class (functional group): soap/surfactant, organics (neutral, acid and base) and inorganic base.
- Several colour interfering chemicals, MTT reducers and MTT reducing coloured chemicals.
- GHS classification: 49% not classified (NC) and 51% classified (C) (divided as 53% Category 1 and 47% Category 2.

As distribution of physical state and GHS classification category, the 125 chemicals covered: 34 Category 1 (19 liquids and 15 solids), 21 Category 2A (16 liquids and 5 solids), 9 Category 2B (4 liquids and 5 solids) and 61 No Category (32 liquids and 29 solids).

The complement of chemicals used for development and optimization represents a significant and balanced set.

5) SkinEthic™ HCE EIT training and transfer

Transferability of the method was demonstrated using 18 chemicals (9 solids / 9 liquids) including strong colorants and MTT reducers known to cause interference, aiming to cover all experimental eventualities.

Two training days are required for a naïve laboratory, including practical application and data evaluation. Actual transfer of the method was arranged over two weeks, testing the 18 chemicals in replicate independent series to allow evaluation of:

- adherence to acceptance criteria.

- single and dual operator comparison.
- predictive concordance.

Results demonstrated accurate and reproducible implementation.

The training exercise has been described in full, with detailed method SOPs (Attachments 4a and 4b) and assessment reports (Attachments 5a, 5b, 5c, and 5d).
SOP implementation (transfer) by the naïve laboratories has also been reported in full (Attachments 6a and 6b) indicating the method is both robust and transferable.

6) SkinEthic™ HCE EIT validation

The ring trial validation study (EITL and EITS) for evaluation of within/between laboratory reproducibility (WLR/BLR) and predictive capacity (PC) included 120 chemicals (60 liquids, 60 solids) tested in three laboratories (L'Oréal, Charles River, Vito) with an additional 80 chemicals (45 liquids, 35 solids) tested by L'Oréal (lead laboratory).

The chemical selection (Attachment 2) again covered a range of properties:

- the full range of *in vivo* eye irritation GHS Categories (1, 2A, 2B, or No Category).
- the *in vivo* determinants of classification (cornea opacity, iritis, conjunctiva redness, chemosis, reversibility/persistence).
- wide representation of organic functional groups.
- known chemical structures.
- coloured and/or direct MTT reducers.
- availability through laboratory retail supply, at reasonable cost.

The processing and analysis of all data from the three laboratories in the ring trial was contracted to an independent consultant statistician who has compiled 2 comprehensive reports, respective of the liquid and solid protocols (Attachments 8a and 8b). The reports are clear and concise, uniformly applying the acceptance criteria and prediction model to determine within laboratory reproducibility (WLR) between laboratory reproducibility (BLR) and predictive capacity (PC).

The test submitter (L'Oréal) has also compiled all data used for method evaluation, provided as attachments in *pdf* and *xls* formats:

- Attachment 3a: Data used for relevance and reliability assessment (EITL and EITS).
- Attachment 3b: EITL: WLR assessment: 60 ring trial chemicals, 3 labs (L'Oreal, VITO, CRL).
- Attachment 3c: EITL: WLR assessment: 45 additional chemicals, 1 lab (L'Oreal).
- Attachment 3d: EITS: WLR assessment: 60 ring trial chemicals, 3 labs (L'Oreal, VITO, CRL).
- Attachment 3e: EITS: WLR assessment: 35 additional chemicals, 1 lab (L'Oreal).
- Attachment 7a: EITL: BLR assessment: 60 ring trial chemicals, 3 labs (L'Oreal, VITO, CRL).
- Attachment 7b: EITS: BLR assessment: 60 ring trial chemicals, 3 labs (L'Oreal, VITO, CRL).
- Attachment 9: EIT: Predictive capacity (PC) assessment.

7) SkinEthic™ HCE EIT results

Within Laboratory Reproducibility (WLR)

WLR (concordance of predicted classification) based on the set of 120 chemicals, was reported as follows:

- CRL: 91.7% (EITL 88.3% and EITS 95.0%).
- VITO: 94.2% (EITL 93.3% and EITS 95.0%).
- L'Oreal: 95.8% (EITL 95.0% and EITS 96.7%).

WLR for the extended set of 200 chemicals (tested by L'Oreal only) was:

- 95.0% (EITL 93.3% and EITS 96.8%).

The test submission report concluded that the SkinEthic™ HCE EIT method (liquids/solids) has been shown to exceed the minimum requirement for WLR of 85% set by the validation management group (VMG) of EIVS. The WLR is also comparable to that obtained previously for a similar method, EpiOcular™ EIT.

Between Laboratory Reproducibility (BLR)

Fifty six of the 60 liquid chemicals were consistently classified (NC/C) by the three laboratories resulting in a BLR (concordance of predicted classification) of 93.3% (95% CI: 84.1% - 97.4%). BLR based on pair-wise comparison, was reported as follows:

- L'Oreal versus CRL: 93.3% (56/60 chemicals).
- L'Oreal versus VITO: 95.0% (57/60 chemicals).
- CRL versus VITO: 98.3% (59/60 chemicals).

Fifty eight of the 60 solid chemicals were consistently classified (NC/C) by the three laboratories resulting in a BLR (concordance of predicted classification) of 96.7% (95% CI: 88.6% - 99.1%). BLR based on pair-wise comparison, was reported as follows:

- L'Oreal versus CRL and L'Oreal versus VITO: 96.7% (58/60 chemicals).
- CRL versus VITO: 100%.

The test submission report concluded overall BLR for the SkinEthic™ HCE EIT method, based on the set of 120 chemicals, was 95.0% (EITL 93.3% and EITS 96.7%) exceeding the defined minimum requirement of 80% set by the VMG of EIVS.

For comparison, the test submission reported BLR from the previous ring trial validation of the similar test method EpiOcular™ EIT was 94.4% for liquids and 92.0% for solids.

Predictive Capacity (PC)

PC (ring trial) was evaluated by comparing *in vitro* viability with respect to prediction model (all runs, per laboratory and cumulatively) with documented *in vivo* classifications according to GHS.

The statistics report summarises the frequency distribution of true versus false predictions, respective of irritant classification (C) and non-irritant classification (NC). From these frequencies are calculated the sensitivity (rate of correct prediction for C, with false negatives), the specificity (rate of correct prediction for NC, with false positives) and overall accuracy (rate of correct prediction, C or NC) expressed as percentages:

Liquids protocol (EITL) predictive capacity (ring trial):

in vivo	**Cumulative**		**L'Oréal**		**CRL**		**VITO**	
	C	**NC**	**C**	**NC**	**C**	**NC**	**C**	**NC**
Classified	283	5	96	0	94	2	93	3
No Category	77	175	29	55	23	61	25	59
Total	**540**		**180**		**180**		**180**	
Sensitivity (%)	98.3		100		97.9		96.9	
False Negatives (%)	1.7		0		2.1		3.1	
Specificity (%)	69.4		65.5		72.6		70.2	
False Positives (%)	30.6		34.5		27.4		29.8	
Accuracy (%)	84.8		83.9		86.1		84.4	

From statistical bootstrap resampling (which estimates uncertainty in predictive capacity, as 95% CI) (10,000 re-samples at n=1 for the 60 chemicals) the statistics report indicates overall predictive capacity for the liquids protocol (EITL):

Parameter	Estimate	95% CI
Sensitivity (%)	98.2	93.8; 100
Specificity (%)	69.4	60.7; 75.0
Accuracy (%)	84.8	80.0; 88.3

Solids protocol (EITS) predictive capacity (ring trial):

in vivo	**Cumulative**		**L'Oréal**		**CRL**		**VITO**	
	C	**NC**	**C**	**NC**	**C**	**NC**	**C**	**NC**
Classified	249	21	83	7	83	7	83	7
No Category	63	206	22	68	19	71	22	67
Total	**539**		**180**		**180**		**179**	
Sensitivity (%)	92.2		92.2		92.2		92.2	
False Negatives (%)	7.8		7.8		7.8		7.8	
Specificity (%)	76.6		75.6		78.9		75.3	
False Positives (%)	23.4		24.4		21.1		24.7	
Accuracy (%)	84.4		83.9		85.6		83.3	

From statistical bootstrap resampling (which estimates uncertainty in predictive capacity, as 95% CI) (10,000 re-samples at n=1 for the 60 chemicals) the statistics report indicates predictive capacity for the solids protocol (EITS):

Parameter	Estimate	95% CI
Sensitivity (%)	91.9	90.0; 93.3
Specificity (%)	76.6	73.3; 80.0
Accuracy (%)	84.3	81.7; 86.7

The test submission also reports sensitivity, specificity and accuracy for the extended set of chemicals (including 45 additional liquids and 35 additional solids tested by the lead laboratory only) quoting similar figures.

8) HPLC spectrophotometry

The MTT-reduction assay for tissue viability, relevant to all *in vitro* test methods based on Reconstructed human Tissues (RhT) is limited by interference with coloured chemicals.

The test method R&D has overcome this limitation using High/Ultra High Performance Liquid Chromatography Performance (HPLC-UPLC)-spectrophotometry for endpoint detection of formazan.

The HPLC-UPLC method has been shown to be highly reproducible (BLR) between different laboratories.

Based on this, the test submission report concludes that HPLC/UPLC is relevant to all *in vitro* RhT test methods irrespective of the test system and test method and can be applied to any of the other RhT test systems within the relevant OECD Test Guidelines. Indeed, the HPLC/UPLC-spectrophotometry technique has already been implemented in OECD TGs 431 (in vitro skin corrosion based on RhE), 439 (in vitro skin irritation based on RhE) and 492 (in vitro serious eye damage/eye irritation based on RhCE).

References

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Scott, L., Eskes, C., Hoffmann, S., Adriaens, E., Alepée, N., Bufo, M., Clothier, R., Facchini, D., Faller, C., Guest, R., Harbell, J., Hartung, T., Kamp, H., Varlet, B.L., Meloni, M., McNamee, P., Osborne, R., Pape, W., Pfannenbecker, U., Prinsen, M., Seaman, C., Spielmann, H., Stokes, W., Trouba, K., Berghe, C.V., Goethem, F.V., Vassallo, M., Vinardell, P., Zuang, V. (2010): A proposed eye irritation testing strategy to reduce and replace *in vivo* studies using Bottom-Up and Top-Down approaches. Toxicol In Vitro 24, 1-9.

United Nations (UN) Globally Harmonized System (GHS) for Classification and Labelling of Chemicals (2013): 5th (revised) edition.

4. OBJECTIVES, QUESTIONS, TIMELINES

4.1 OBJECTIVE

Objective *Why does EURL ECVAM require advice on the current issue?*	EURL ECVAM requests an ESAC opinion on the reliability (reproducibility within and between laboratories of results obtained *in vitro*) and relevance (predictive capacity of effects documented *in vivo*) of the SkinEthic™ Human Cornea Epithelium (HCE) Eye Irritation Test (EIT) for prediction of eye irritation potential of chemicals. The opinion of ESAC should support EURL ECVAM with respect to the development of an EURL ECVAM recommendation on the Reconstructed human Cornea-like Epithelium (RhCE) assays for serious eye damage/eye irritation testing outlining (1) the scientific basis of the assays, (2) their overall performance (transferability, reproducibility and predictive capacity) as assessed during the validation studies and based on other (e.g. published) information, (3) their applicability and limitations, and 4) their proposed use. ESAC's advice should enable EURL ECVAM to conclude, within its EURL ECVAM Recommendation, on the potential adequacy of the SkinEthic™ HCE EIT for routine testing of serious eye damage/eye irritation for regulatory purposes.

4.2 QUESTION(S) TO BE ADDRESSED

Questions *What are the questions and issues that should be addressed in view of achieving the objective of the advice?*	The ESAC peer review of the SkinEthic™ HCE EIT should address the following aspects: (1) Scientific basis in relation to serious eye damage/eye irritation. (2) Clarity of the test definition, including: - purpose and need of the test method. - biological/mechanistic relevance in relation to the test system used and the endpoint measured. - protocol clarity and completeness. - clarity and adequacy of the prediction model and its development. (3) Clarity of the definition of the study objective(s). (4) Appropriateness of the study design and execution considering the study objective(s), including: - number and selection criteria for test chemicals (e.g., range of documented effects *in vivo*, etc.). - quality assurance of reference data (*in vivo*) for predictive capacity assessment. - number of participating laboratories. - number of replicates, number of repetitions, rules for retesting and handling of deviations. (5) Study management and conduct. (6) Results compilation and statistical analyses reporting. - appropriateness of calculation of WLR and BLR on the basis of the generated data.

	- appropriateness of calculation of Predictive Capacity on the basis of the generated data. - appropriateness of identification of limitations/applicability domain on the basis of the generated data. (7) Transferability and reproducibility (WLR/BLR). (8) Predictive capacity for distinguishing chemicals not requiring classification from chemicals requiring classification as Category 1 (serious eye damage) or Category 2 (eye irritation) and relevance to a tiered (Top-Down/Bottom-Up) testing strategy. (9) Applicability and any known limitations, assessed from the selection of the test chemicals (range of molecular class and physical properties) and analyses of possible reasons for misclassifications. (10) Possible gaps, if any, between study design and study conclusions. (11) Whether the information provided in the submission is sufficient to substantiate the proposed use of the test method within a Bottom-Up/Top-Down testing strategy. (12) Usefulness and applicability of HPLC/UPLC-spectrophotometry as an alternative endpoint detection system to standard photometry in SkinEthic™ HCE EIT. (13) What additional work, if necessary, should be undertaken in future to further characterise the test method and its proposed use. ESAC's advice should conclude on the regulatory applicability of the SkinEthic™ HCE EIT (i.e., for implementation as an EU test method and OECD Test Guideline).

4.3 TIMELINES

Timelines concerning this request *When does EURL ECVAM require the advice?*	**Timeline**	**Indication**
	Finalised ESAC Opinion required by:	**June 2016**
	Request to be presented to ESAC by written procedure (e.g. due to urgency) prior to the next ESAC	**YES**
	Request to be presented to ESAC at ESAC plenary meeting	**NO**

5. EURL ECVAM PROPOSALS ON HOW TO ADDRESS THE REQUEST WITHIN ESAC

5.1 EURL ECVAM PROPOSAL REGARDING REQUEST-RELATED STRUCTURES REQUIRED

Specific structures required within ESAC to address the request *Does the advice require an ESAC working group, an ESAC rapporteur etc.?*	**Structure(s) required**	**Required according to EURL ECVAM? (YES/NO)**
	S1 ESAC Rapporteur	**NO**
	S2 ESAC Working Group	**ESAC members** - José M. Navas (Chair) - Kristina Kejlová - Annete Kopp-Schneider - Renate Kraetke - Jon Richmond **ICATM nominations** - Dave Allen (NICEATM/ICCVAM) - Kyung-Min Lim (College of Pharmacy, Ewha Womans University; nominated by KoCVAM)
	S3 Invited Experts	**NO**
	Ad S3: If yes – list names and affiliations of suggested experts to be invited and specify whether these are member of the EEP	
	If other than above (S1-S3):	

5.2 DELIVERABLES AS PROPOSED BY EURL ECVAM

Deliverables *What deliverables (other than the ESAC opinion) are required for addressing the request?*	**Title of deliverable other than ESAC opinion**	**Required? (YES/NO)**
	D1 ESAC Rapporteur Report and draft opinion	**NO**
	D2 ESAC Peer Review Report and draft opinion	**YES**
	If other than above (D1-D2):	

6. LIST OF DOCUMENTS TO BE MADE AVAILABLE TO THE ESAC

Count	Description of document	Already available? (YES/NO)	File name
1	SkinEthic™ HCE test submission (TST)	YES	TST SkinEthic HCE EIT_Amended.pdf
2	EURL ECVAM Assessment Report on the SkinEthic™ HCE test submission	YES	SkinEthic_HCE_assessment_report_2016-05-09_final.pdf
3	Protocol of the SkinEthic™ HCE EITL (Liquid)	YES	Attachment 1a.pdf
4	Protocol of the SkinEthic™ HCE EITS (Solid)	YES	Attachment 1b.pdf
5	SkinEthic™ HCE EITL (Liquid) DB-ALM protocol	YES	Attachment 1c.pdf
6	SkinEthic™ HCE EITS (Solid) DB-ALM protocol	YES	Attachment 1d.pdf
7	SkinEthic™ HCE EIT - List of test items including their CAS number and basic physical/chemical properties for optimisation/transfer/WLR/BLR/Predictive capacity	YES	Attachment 2.pdf
8	SkinEthic™ HCE EIT - Data used for relevance and reliability assessment (EITL and EITS)	YES	Attachment 3a.pdf
9	SkinEthic™ HCE EITL - WLR assessment (60 chemicals – 3 labs)	YES	Attachment 3b.pdf
10	SkinEthic™ HCE EITL - WLR assessment (45 additional chemicals – 1 lab)	YES	Attachment 3c.pdf
11	SkinEthic™ HCE EITS - WLR assessment (60 chemicals – 3 labs)	YES	Attachment 3d.pdf
12	SkinEthic™ HCE EITS - WLR assessment (35 additional chemicals – 1 lab)	YES	Attachment 3e.pdf
13	Training protocol of the SkinEthic™ HCE EITL (Liquids)	YES	Attachment 4a.pdf
14	Training protocol of the SkinEthic™ HCE EITS (Solids)	YES	Attachment 4b.pdf
15	Training report of the SkinEthic™ HCE EITL (Liquids) - VITO	YES	Attachment 5a.pdf
16	Training report of the SkinEthic™ HCE EITL (Liquids) - CRL	YES	Attachment 5b.pdf
17	Training report of the SkinEthic™ HCE EITS (Solids) - VITO	YES	Attachment 5c.pdf
18	Training report of the SkinEthic™ HCE EITS (Solids) – CRL	YES	Attachment 5d.pdf
19	Transfer report of the SkinEthic™ HCE EITL (Liquids) – VITO & CRL	YES	Attachment 6a.pdf
20	Transfer report of the SkinEthic™ HCE EITS (Solids) – VITO & CRL	YES	Attachment 6b.pdf
21	SkinEthic™ HCE EITL - BLR assessment (60 chemicals – 3 labs)	YES	Attachment 7a.pdf
22	SkinEthic™ HCE EITS - BLR assessment (60 chemicals – 3 labs)	YES	Attachment 7b.pdf

23	Statistical analysis and reporting of the SkinEthic™ HCE EITL (Liquids)	YES	Attachment 8a_Revised.pdf
24	Statistical analysis and reporting of the SkinEthic™ HCE EITS (Solids)	YES	Attachment 8b.pdf
25	SkinEthic™ HCE EIT – Predictive capacity assessment	YES	Attachment 9.pdf
26	Project plan of the SkinEthic™ HCE EITL (Liquids)	YES	Attachment 10a.pdf
27	Project plan of the SkinEthic™ HCE EITS (Solids)	YES	Attachment 10b.pdf
28	SkinEthic™ HCE EIT - HPLC/UPLC-spectrophotometry (24 chemicals – 1 lab)	YES	Attachment 11.pdf
29	Publication on the validation of EITL	YES	Alépée et al. 2016 - SkinEthic HCE liquids.pdf
30	Publication on the validation of EITS	YES	Alépée et al. 2016 - SkinEthic HCE solids.pdf

7. TERMS OF REFERENCE OF THE ESAC WORKING GROUP

7.1 ESTABLISHMENT OF THE ESAC WORKING GROUP

The ESAC unanimously agreed by written procedure on the 18th of February 2016 on the composition of a new ESAC Working Group for the review of test methods in the area of serious eye damage/eye irritation.

7.2 TITLE OF THE ESAC WORKING GROUP

Full title:
ESAC Working Group on Eye Irritation Test Methods

Abbreviated title:
ESAC WG Eye Irritation

7.3 MANDATE OF THE ESAC WORKING GROUP

The ESAC WG is requested to conduct a scientific review of the l'Oréal-coordinated validation study concerning the SkinEthic™ HCE EIT. The review needs to address the questions put forward to ESAC by EURL ECVAM under section 4.2 of the current request.

The review should focus on the appropriateness of design and conduct of the study in view of the study objective and should provide an appraisal to which extent the conclusions of the test submitter are substantiated by the information generated during the study and how the information generated relates to the scientific background available.

7.4 DELIVERABLES OF THE ESAC WORKING GROUP

The ESAC WG is requested to deliver to the chair of the ESAC and the ESAC Coordinator a detailed **ESAC Working Group Report** outlining its analyses and conclusions and a **draft ESAC Opinion.** A template has been appended (Appendix 1) intended to facilitate the drafting of the WG report.

The conclusions drawn in the report should be based preferably on consensus. If no consensus can be achieved, the report should clearly outline the differences in the appraisals and provide appropriate scientific justifications.

7.5 PROPOSED TIMELINES OF THE ESAC WORKING GROUP

Item	Proposed date/time	Action	Deliverable
1	6 May 2016	Teleconference of the Working Group	Agree procedure
2	11-13 May 2016	Working Group meeting	Draft ESAC WG report and draft ESAC opinion
3	27 May 2016	Circulation of final WG report and draft ESAC opinion to ESAC	Final draft ESAC WG report and draft ESAC opinion
4	9-10 June 2016	Endorsement of WG report and ESAC opinion at ESAC42 meeting	Final ESAC WG report and ESAC opinion

7.6 QUESTIONS WHICH SHOULD BE ADDRESSED BY THE ESAC WORKING GROUP

The review should address the **questions put forward to ESAC by EURL ECVAM** (see section 4.2) and the information requirements of the ESAC Working Group Template, where applicable. The ESAC Coordinator will provide guidance if needed.

When preparing the final ESAC WG report to address these questions, the ESAC WG is requested to use a pre-defined reporting template. This template (see appendix 1) follows ECVAM's modular approach and addresses to which extent the standard information requirements have been addressed by the study. The template allows moreover for addressing the issues specific studies outlined in section 4.2. The Secretariat will provide guidance if necessary.

APPENDIX 1 REPORTING TEMPLATE

The appended ESAC WG template suggests a structure that is in close agreement with the EURL ECVAM information requirements ("modules") for scientific review following validation and allows at the same time for the description of the analysis and conclusions concerning more specific questions.

The template can be used for various types of validation studies (e.g. prospective full studies, retrospective studies, performance-based studies and prevalidation studies). Depending on the study type and the objective of the study, not all sections may be applicable.

However, for reasons of consistency and to clearly identify which information requirements have not been sufficiently addressed by a specific study, this template is uniformly used for the evaluation of validation studies.

The current template is

TEMPLATE_ESAC-WG_REPORT-v6.doc