EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection
In-vitro Methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

ECVAM RETROSPECTIVE VALIDATION STUDY ON CYTOTOXICITY/CELL-FUNCTION BASED *IN VITRO* ASSAYS FOR THE PREDICTION OF EYE IRRITATION

ESAC PEER REVIEW

Background Information

History, Study Management and Study Timelines

In the field of eye irritation, most of the previous evaluation and validation studies on alternative methods for eye irritation have succeeded in demonstrating the reproducibility and reliability of promising new methods, but have failed to identify a single assay for the full replacement of the *in vivo* Draize rabbit eye test (1-11). This was due to various factors, among which are the limited quality of the existing *in vivo* data (as reflected in the limited consistency of the Draize test), methodological limitations of the *in vitro* systems and of the statistical analyses used at the time. In addition, it is generally recognised that the range of criteria for injury and inflammation covered by the Draize rabbit eye test is unlikely to be replaced by a single *in vitro* test. In 2004, an expert group assisted ECVAM in identifying the most promising alternative methods and the prospects for their validation in the framework of the scientific working process to establish timetables for the implementation of the marketing and testing bans, as required by EU Directive 2003/15/EC, the 7th amendment to the Cosmetics Directive (12, 13). The major recommendations made by the expert group in the area of eye irritation were the following:

1. To make use of testing strategies utilizing the strengths of particular *in vitro* assays to address required ranges of irritation potential and/or chemical classes.

2. To assess retrospectively in a weight of evidence approach the existing data of the most promising *in vitro* test methods.

3. To have a high quality and reliable large *in vivo* data set and a corresponding analysis of these for the validation studies.

4. To consider differences in regulatory classification schemes in various countries and regions (e.g. Japan, US, Europe) and to compare existing data with the recently established UN GHS for eye irritation (14).

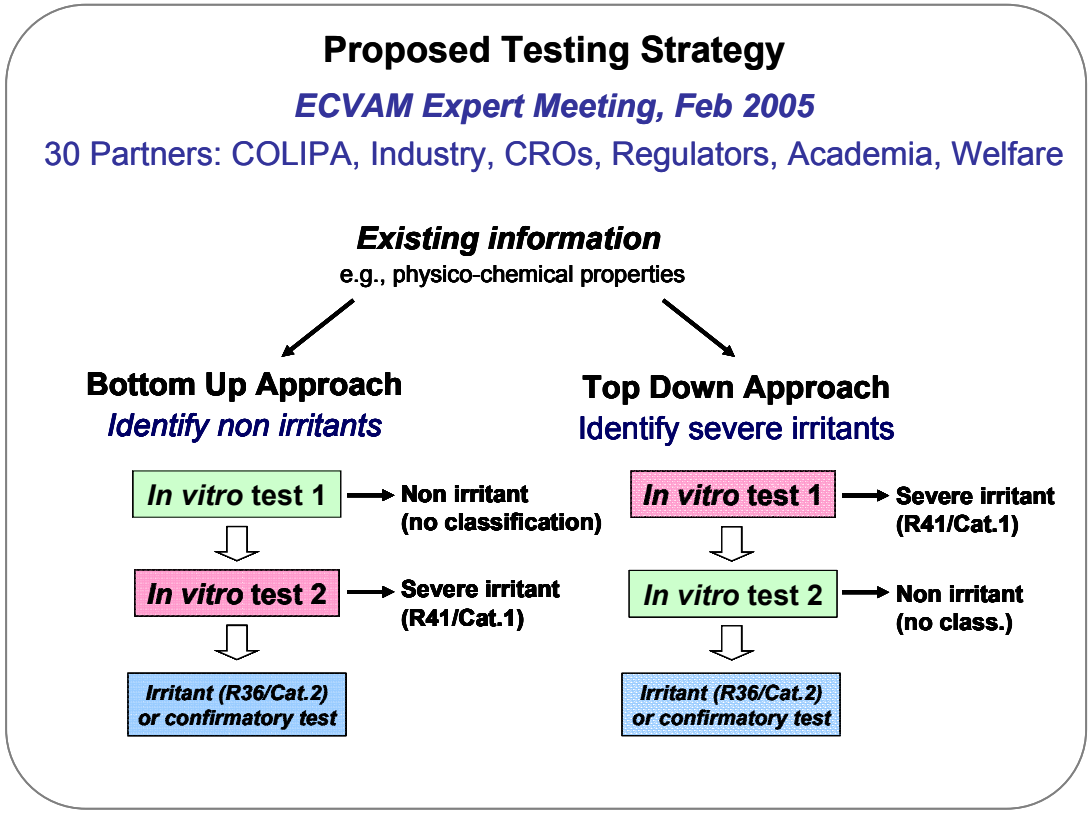
5. To support further development of mechanistically-based alternative methods including the assessment of depth of injury and recovery as potential indicators for irreversibility and reversibility of effects.

To address these recommendations and to progress the most advanced *in vitro* test methods towards validation in a timely manner, ECVAM established a Task Force on eye irritation in June 2004, and reinforced its collaboration with ICCVAM, COLIPA and

industry in general. In addition, a joint ECVAM/ICCVAM workplan with defined shared leaderships was developed to favour harmonisation and avoid duplication of work. It was agreed that ICCVAM would take the lead in the evaluation of the organotypic assays (BCOP, ICE, IRE, HET-CAM), whereas ECVAM would take the lead in the evaluation of the cytotoxicity/cell-function based assays (CM, FL, NRR, RBC) and Reconstructed human Tissue (RhT) models (EpiOcular™, SkinEthic HCE™).

Furthermore, ECVAM organised an expert meeting in February 2005, which involved more than 30 participants coming from industry, regulatory bodies, contract laboratories, academia, and welfare groups. Participants were requested, to nominate test methods for eye irritation for a specific applicability domain, to provide supportive *in vivo* and *in vitro* data, and to identify potential test strategies to assess eye irritation based on their experiences and uses of the test methods. Two testing strategies were suggested, and a number of gaps and recommendations were identified (15). The testing scheme proposes using a Bottom-Up (begin with using test methods that can accurately identify non-irritants) or Top-Down (begin with using test methods that can accurately identify severe irritants) progression of *in vitro* tests (based on expected irritancy). Irrespective of the starting point, the approach would identify non-irritants and severe irritants, leaving all others to the irritant EU R36 / GHS 2A/2B categories (Figure 1).

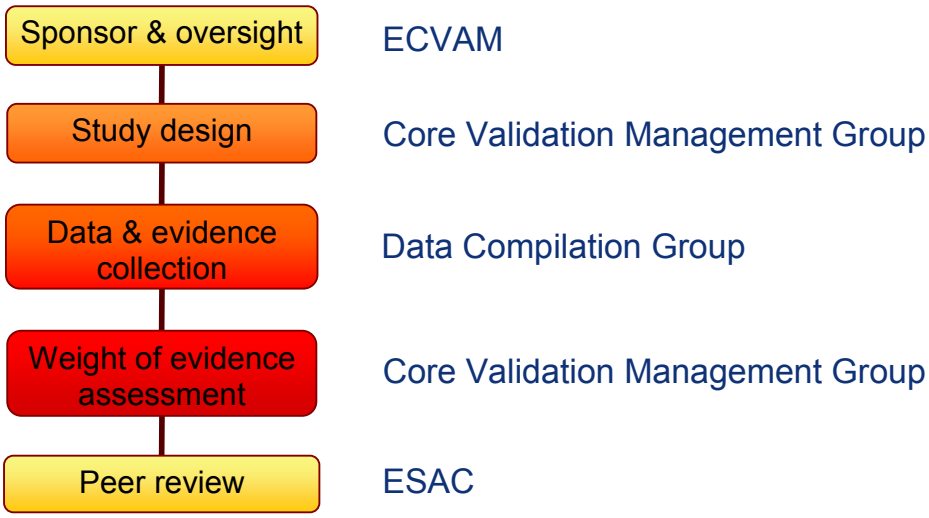
Figure 1. Bottom-Up and Top-Down *In Vitro* Testing Strategy Approach for Eye Irritation.



Subsequent to the reviews and outcome of the activities described above, ECVAM together with its Task Force on eye irritation recommended in 2004, that a retrospective validation study on the most promising cytotoxicity based assays, such as the Neutral Red Release and the Red Blood Cell assays; and cell-function based assays, such as the Cytosensor Microphysiometer and the Fluorescein Leakage assays, should be initiated. The retrospective validation of the Neutral Red Release, the Red Blood Cell test, the Fluorescein Leakage assay and the Cytosensor Microphysiometer was initiated in October 2005. The study was based on the retrospective collection of existing data compiled according to the ECVAM modular approach to validation and weight of evidence principles (16, 17).

The validation study was managed as shown in Figure 2.

Figure 2. Management and responsibilities in the retrospective validation study on cytotoxicity/cell-function based assays for eye irritation.



ECVAM acted as sponsor and was responsible for overseeing the study. A Core Validation Management Group managed the study, whereas a data Compilation Group composed of experts in the methods evaluated, were responsible for the collection of data and supporting evidence on the assays, by compiling comprehensive Background Review Documents (BRDs). With regard to the compilation of BRDs, the core Validation Management Group was responsible for approving: a) their structure, b) the search strategies used, c) the study and data selection procedures, d) the acceptance and exclusion criteria of studies and data, e) the protocol components considered most crucial, including PM, f) the data management procedures and, g) the completeness of the BRDs and their quality. The core VMG was also responsible for the respect of timelines and study progression, for the evidence-based evaluation, and finally, for

drafting conclusions and recommendations on the usefulness and limitations of the assays.

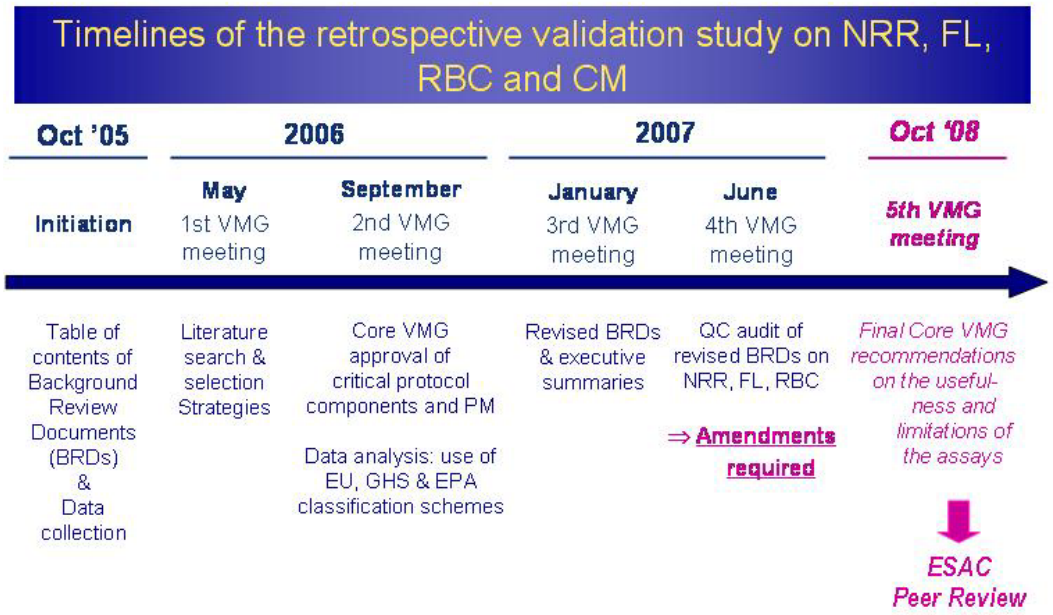
The Data Compilation Group was selected through a Joint Research Centre (JRC) Open Call for Tender.

In order to ensure that the BRDs were of a certain quality, Quality Control Audits were carried out.

The Quality Control Audits were carried out between May 2007 and July 2008 upon availability of the BRDs.

An overview on the timelines for the completion of the validation study is given in Figure 3.

Figure 3. Overview of timelines of the retrospective validation study on cytotoxicity/cell-function based assays for eye irritation.



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Documents

	Documents for the CM Assay	Doc No
Summary Report	Summary Report drafted by ECVAM – CM Test Method Evaluation Report doc-01_CM_Summary_Report.pdf	01
Background Review Document	Background Review Document (BRD) prepared by Institute for In Vitro Sciences - Final Revision 20/08/2008 doc-02_CM_BRD_Report_Aug08.pdf	02
Annexes	doc-03_CM_BRD_Annexes_Aug08.pdf	03
	Documents for the FL Assay	
Summary Report	Summary Report drafted by ECVAM – FL Test Method Evaluation Report doc-04_FL_Summary_Report.pdf	04
Background Review Document	Background Review Document (BRD) prepared by the University of Nottingham- Final Revision 01/2008 doc-05_ FL_BRD_Report_Jan08.pdf	05
Annexes	doc-06_ FL_BRD_Annexes_Jan08.pdf	06
Supporting Documents	doc-07_ FL_BRD_Supporting-Documents_Jan08.pdf	07
Additional Files	***Folder: FL_BRD_Additional-Files_Jan08*** ○ doc-08_ FL between laboratory Annex III.xls ○ doc-09_ FL BRD Predictive Capacity Annex Vai.xls ○ doc-10_ FL BRD Predictive Capacity Annex Vaii.xls ○ doc-11_ FL BRD Predictive Capacity Annex Vc.xls ○ doc-12_ FL intralaboratory Annex IIa.xls ○ doc-13_ FL Predicitive Capacity Annex Vb.xls ○ ***Folder: Annex VII*** ▪ doc-14_ COLIPA-BRDv6 2.xls ▪ doc-15_ CTFA PIIIv6 -FROM IIVS.xls	 08 09 10 11 12 13 14 15

	▪ doc-16_ EC_HOstudysummaryclassificationv6(aug06)(2).xls	16
	Documents for the NRR Assay	
Summary Report	Summary Report drafted by ECVAM – NRR Test Method Evaluation Report doc-17_NRR_Summary_Report.pdf	17
Background Review Document	Background Review Document (BRD) prepared by the University of Nottingham- Final Revision 12/2007 doc-18_NRR_BRD_Report_Dec07.pdf	18
Annexes	doc-19_NRR_BRD_Annexes_Dec07.pdf	19
Supporting Documents	doc-20_NRR_BRD_Supporting-Documents_Dec07.pdf	20
Additional Files	***Folder: NRR_BRD_Additional-Files_Dec07*** o doc-21_Between Laboratory Reproducibility Annex III.xls o doc-22_Predictive Capacity Annex Vb.xls o doc-23_Predictive Capacity Raw Data Annex Vai.xls o doc-24_Predictive Capacity Summarised Data Annex Vaii.xls o doc-25_Within Laboratory Reproducibility Annex II.xls o doc-26_Within Laboratory Reproducibility Annex IIa.xls o ***Folder: Raw in vivo data Annex Vaiii*** ▪ doc-27_COLIPA-BRDv6 2.xls ▪ doc-28_ECVAM V6 CTFA PI.xls ▪ doc-29_ECVAM V6 CTFA PII.xls ▪ doc-30_ECVAM V6 CTFA PIII -FROM IIVS.xls ▪ ***Folder: CTFA in vivo data from AVON*** • doc-31_Draize_Data_4-7-93_Phase_I.pdf • doc-32_EyeIrritationStudyInRabbits_LVP_PhaseIII_Page1-151_4-1.pdf • doc-33_PrimaryEyeIrritationStudyInRabbits_(RandomBlockDesign).pdf • doc-34_PrimaryEyeIrritationStudyInRabbits_Volume1of2_-_Haz.pdf • doc-35_PrimaryEyeIrritationStudyInRabbits_Volume2of2_-_Ha.pdf	21 22 23 24 25 26 27 28 29 30 31 32 33 34 35

	○ ***Folder: Relevant unpublished data Annex VII*** ▪ doc-36_Data Taken From Various Publications Reports.xls ▪ ***Folder: CTFA Data*** • doc-37_CTFA PI NRR.pdf • doc-38_CTFA PII NRR.pdf • doc-39_CTFA PIII NRR.pdf ▪ ***Folder: Wella Data*** • doc-40_NRRdataforECVAM-BRDwithoutRecipeNo(1)(1).xls • doc-41_SupplementaryinformationtoECVAMNRR-BRDdatasheet[2].pdf	36 37 38 39 40 41
	Documents for the RBC Assay	
Summary Report	Summary Report drafted by ECVAM – RBC Test Method Evaluation Report doc-42_RBC_Summary_Report.pdf	 42
Background Review Document	Background Review Document (BRD) prepared by the University of Barcelona - Final Revision 09/2008 doc-43_RBC_BRD_Report_Sep08.pdf	 43
Annexes	doc-44_RBC_BRD_Annexes_Sep08.pdf	44
	Minutes of the 5th VMG Meeting (14th – 15th October 2008)	
Minutes	Minutes of the 5th VMG meeting including: Meeting Objectives, Observations, Recommendations and Follow-up Actions doc-45_Minutes_5th_VMG_Meeting_(Oct08).pdf	 45

Criteria for Evaluation (ECVAM Modular Approach)

1. Data collection
Are the data collection procedures and selection clearly defined?

2. Goal of the study
Clearly understandable?
Scientific rationale given?
Regulatory rationale?

3. Test definition (Module 1)
Are the test and its purpose well defined?
Are the proposed standardised protocol and prediction model adequate?

4. Data quality
Quality of the evaluated data.
Are they sufficient to assess the study goal?
Quality of the reference data?

5. Test materials
Is the number of evaluated substances sufficient?
Are they representative of proposed applicability domain?

6. Within-laboratory variability (Module 2)
- assessment of reproducibility of the data in the same laboratory

7. Transferability (Module 3)
- how easy is it to transfer the test to a second laboratory?

8. Between-laboratory variability (Module 4)
- assessment of reproducibility of the data in different laboratories

9. Predictive capacity (Module 5)
Have the predictive capacity of the methods been properly assessed?
Is the assay relevant for their stated purpose?

10. Applicability domain (Module 6)
Is the proposed applicability domain well defined?

11. Performance standards (Module 7)
Have appropriate performance standards been defined for the test?

12. Readiness for regulatory purposes

General considerations
Please address any other consideration you might have in relation to the proposed approach under this section.

Conclusions and recommendation
Please summarise your conclusions on the validity of the approach under this section.

Time for evaluation
The panel members should report back to the chair of the ESAC peer review panel, by **26 February 2009.**

Please see actions/timelines on page 15.

Evaluation report
The panel should provide a consensus report addressing the above topics and any other important issues. The report should include a draft statement on the validity of the approach.

Validation guidelines

- Balls et al. (1995) Practical aspects of the validation of toxicity test procedures. ECVAM Workshop Report 5. *ATLA* **23**, 129-147.

- Hartung et al (2004) A modular approach to the ECVAM principles on test validity. *ATLA* **32**, 467-472.

- OECD (2005) Guidance document on the Validation and International Acceptance of new or updated test methods for hazard assessment, adopted by OECD joint Meeting on 10 June 2005, Paris, France.

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EXPERTS on the ESAC Peer Review Panel of the ECVAM Eye Irritation Validation Study

External experts	
Internal ESAC members	

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ESAC PEER REVIEW

Actions / Timelines

WHAT	WHEN	WHO
(1) Launch ESAC Peer Review and provide password to Peer Review Panel (PRP) to access documents on the ECVAM website	27 January 2009	ECVAM
(2) Submission of individual peer review reports to chair of ESAC peer review panel (JvV) with copy to ECVAM (VZ)	26 February 2009	PRP members
(3) Peer Review Panel meeting at ECVAM, JRC, Ispra (Italy), to discuss and finalize review	2-4 March 2009	PRP members
(4) Prepare and agree final PRP consensus report and draft statement on scientific validity of *in vitro* methods for the prediction of eye irritation during meeting at ECVAM	2-4 March 2009	PRP members
(5) Circulate agreed PRP report and draft of ESAC statement to ESAC members	5 March 2009	ECVAM
(6) Present outcome of PRP report and finalise ESAC statement on scientific validity at the ESAC meeting	**9-10 March 2009**	PRP chair