EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
In-vitro Toxicology Unit
European Centre for the Validation of Alternative Methods (ECVAM)

STATEMENT ON THE SCIENTIFIC VALIDITY OF IN-VITRO TESTS FOR SKIN IRRITATION TESTING

At its 29th meeting, held on 4-5th November, 2008 at the European Commission in Brussels, the non-Commission members of the ECVAM Scientific Advisory Committee (ESAC) unanimously endorsed the following statement:

Two *in-vitro* skin irritation tests have been evaluated according to the principles outlined in the ECVAM document 'Performance Standards for Applying Human Skin Models to in-vitro skin irritation testing' (1). These performance standards were used to evaluate the reliability and accuracy of two test methods which are both based on reconstructed human epidermis and which measure or predict the same biological or toxic effect as the fully validated and accepted reference method (see ESAC statement, 2007 and validation study report) (2, 3).

A review of the data submitted on the following studies was conducted by an ESAC peer review panel:

1. EpiDerm SIT – update validation study: modification of the validated EpiDerm Test (MTT endpoint)
2. SkinEthic RHE assay –external catch up validation study (MTT endpoint)

It is concluded that the performance of these assays in these studies met the criteria outlined to be considered to have sufficient accuracy and reliability for prediction of R38 skin irritating and no-label (non-skin irritating) test substances compared to the validated and accepted method. Limitations associated with the previously validated and accepted in-vitro method for skin irritation (e.g. applicability domain) also apply to the two tests reviewed here (Ref. 4).

This endorsement takes account of the dossiers prepared for peer review; the views of independent experts who evaluated the dossiers against defined validation criteria; supplementary material made available to the Peer Review Panel by ECVAM; and the considered view of the Peer Review Panel appointed to oversee the process.

Joachim Kreysa

Head of Unit

In-Vitro Toxicology Unit

European Centre for the Validation of Alternative Methods

5th November 2008

References

1. ECVAM (2007) *Performance Standards for Applying Human Skin Models to In Vitro Skin Irritation Testing.* Online: http://ecvam.jrc.it/
2. ECVAM (2007) *Statement of the ECVAM Scientific Advisory Committee (ESAC) on the Validity of In Vitro Tests for Skin Irritation.* Online: http://ecvam.jrc.it/
3. ECVAM (2007) *Skin Irritation Validation Study Phase II: Analysis of the primary endpoint MTT and the secondary endpoint IL-1α.* Online: http://ecvam.jrc.it/
4. Spielmann, H., Hoffmann, S., Liebsch, M., Botham, P., Fentem, J., Eskes, C., Roguet, R., Cotović, J., Cole, T., Worth, A., Heylings, J., Jones, P., Robles, C., Kandárová, H., Gamer, A., Remmele, M., Curren, R., Raabe, H., Cockshott, A., Gerner, I. and Zuang, V. (2007) *The ECVAM International Validation Study on In Vitro Tests for Acute Skin Irritation: Report on the Validity of the EPISKIN and EpiDerm Assays and on the Skin Integrity Function Test.* ATLA **35,** 559-601.

The ESAC was established by the European Commission, and is composed of nominees from the EU Member States, industry, academia and animal welfare organisations, together with representatives of the relevant Commission services.

This statement was endorsed by the following members of the ESAC:

Ms Sonja Beken (Belgium)
Mr Albert Breier (Slovakia)
Ms Maija Dambrova (Latvia)
Ms Katalin Horvath (Hungary)
Ms Dagmar Jírová (Czech Republic)
Mr Roman Kolar (Eurogroup for Animals)
Ms Elisabeth Knudsen (Denmark)
Mr Manfred Liebsch (Germany)
Mr Lionel Larue (France)
Mr Gianni Dal Negro (EFPIA)
Mr Efstathios Nikolaidis (Greece)
Mr Constantin Mircioiu (Romania)
Mr. Walter Pfaller (Austria; moderator)
Mr Jon Richmond (UK)
Ms Vera Rogiers (ECOPA)
Mr Hasso Seibert (ESF)
Mr Dariusz Sladowski (Poland)
Mr Jan van der Valk (The Netherlands)
Mr Carl Westmoreland (COLIPA)
Mr Timo Ylikomi (Finland)

The following Commission Services and Observer Organisations were involved in the consultation process, but not in the endorsement process itself:

Ms Elke Anklam (IHCP; chairman)
Mr Joachim Kreysa (ECVAM)
Mr Jürgen Büsing (DG RTD)
Ms Silvia Casati (ECVAM, DG JRC)
Mr Thomas Cole (ECVAM, DG JRC, ESAC secretary)
Ms Laura Gribaldo (ECVAM, DG JRC)
Mr Claudius Griesinger (ECVAM, DG JRC)
Ms Eimear Kelleher (IHCP)
Ms Karin Kilian (DG SANCO)
Ms Barbara Mentré (DG ENTR)
Ms Pilar Prieto (ECVAM, DG JRC)
Mr Juan Riego Sintes (CPSQ, DG JRC)
Ms Sigrid Weiland
Ms Valérie Zuang (ECVAM, DG JRC)
Mr Patric Amcoff (OECD)
Mr Hajime Kojima (JaCVAM)
Mr William Stokes (NICEATM)
Mr Raymond Tice (NICEATM)
Ms Marilyn Wind (ICCVAM)

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Informative Annex

ECVAM Background Information on the Validation of two *in vitro* Test Methods for Skin Irritation Testing performed on the Basis of Performance Standards

Claudius Griesinger, Ispra, Italy, 11 November 2008

Contents

1. Background to Validation Studies based on Performance Standards

The ECVAM Performance Standards for applying human skin models to in vitro skin irritation testing (1) are based on the specifications of the two skin models that were validated during the ECVAM skin irritation validation study (SIVS), the commercially available EPISKIN and the EpiDerm test methods (2-4).

The Performance Standards describe guidance and minimum performance criteria that novel 'me-too' or modified test methods should fulfil so that they may be considered scientifically valid. The performance criteria include *inter alia* (a) a description of general and functional model conditions including acceptance criteria regarding the quality of individual tissues used as test system, (b) test acceptance criteria (e.g. guidance values for positive and negative control), (c) guidance regarding the test procedure and data interpretation (prediction model), (d) 20 reference chemicals that constitute a representative set of chemicals used during the full prospective validation study (3) as well as (e) performance criteria for test method reliability and predictivity.

The Performance Standards are intended as a tool to aid the evaluation, assessment and validation of novel methods on the basis of an experimental testing set of chemicals (the PS reference chemicals) that is markedly reduced in comparison with that of a full prospective validation study. According to OECD guidance document Nr. 34 on the validation and international acceptance of new or updated test methods for hazard assessment (5), two types of test methods can be evaluated on the basis of performance standards. These are

a) Test methods that are sufficiently similar with regard to structural and functional parameters in comparison with the validated methodology ("similar methods" or "me-too methods"). The corresponding validation process is referred to as "catch-up validation".

b) Modifications of validated methods ("modified methods") which are minor enough to warrant the limited experimental assessment as outlined in the Performance Standards. The corresponding validation process is referred to as "update validation".

2. Validation of two *in vitro* skin irritation methods in reference to the ECVAM in vitro Skin Irritation Performance Standards

2.1 Test methods endorsed

The two test methods endorsed by the 29th ESAC are

a) The SkinEthic RHE model, a similar/me-too method, submitted to ECVAM as a non-ECVAM coordinated catch-up study. The test was confirmed by ECVAM as sufficiently similar with regard to its structural and functional characteristics in reference to the Performance Standards and the test method was therefore admitted as a *non-ECVAM coordinated catch-up validation study*.

b) The EpiDerm SIT model, a modification of the previously validated EpiDerm method (2), submitted to ECVAM as a non-ECAVM coordinated update validation study. The main modification performed is the prolongation of the exposure time to the test substances from 15 ('common protocol', ECAVM SIVS) to 60 minutes, while all other essential model parameters remained unchanged. The test method was therefore admitted by ECVAM as a *Non-ECVAM coordinated update validation study*.

It is important to note that all human reconstructed tissue models that have been validated so far for the assessment of skin irritancy potential of xenobiotics, use a postincubation time of 42 hours. However, the assays differ with regard to the exposure time employed, i.e. the period that the epidermal surface is acutely treated with the xenobiotic. In contrast to the relatively short exposure time of 15 minutes outlined in the so-called "common protocol" of the ECVAM SIVS (3), the assays validated in the current context use extended exposure times: the modified EpiDerm SIT assay features, as stated above, an exposure time of 60 minutes while the SkinEthic RHE uses an exposure time of 42 minutes. The exposure times are understood to reflect the different barrier properties of the test systems and are adjusted for each test system in order to guarantee a dynamic response: the exposure time needs to be long enough to allow the development of measurable effects while being short enough to ensure that the system is not driven into saturation.

2.2 Submission, evaluation and peer review process

The SkinEthic RHE test method had been submitted by SkinEthic Laboratories, Nice, France on 7 April 2008. The EpiDerm SIT test method had been submitted on 23 April 2008 by the Federal Institute for Risk Assessment (BfR), Berlin, Germany.

Both test method submissions were evaluated by ECVAM on the basis of the criteria laid out in the ECVAM performance standards document (1). In addition to the external assessment of transferability provided in both test method submissions, the transferability of the SkinEthic RHE method as well as its standard operating procedure (SOP) were independently assessed and confirmed in-house at ECVAM from March to May 2008. Such independent assessment by ECVAM was deemed not necessary in the case of the EpiDerm SIT method since the EpiDerm model had undergone extensive assessment during the full skin irritation validation study (2-4) and since the modification of the test method was considered minor.

After ECVAM evaluation, the test method submissions and additional auxiliary material made available by ECAVM were reviewed by an ESAC Peer Review Panel and independently evaluated by this panel with regard to the ECAVM Performance Standards (1). The Peer Review Process was finalised on September 8, 2008.

3. Endpoints assessed by the two test methods

Both tests use the MTT test as primary endpoint. This colorimetric assay for cell viability is based on the mitochondrial reduction of the vital dye MTT [3-(4,5-Dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide] to a purple-coloured formazan. Cell viability has been demonstrated to be a suitable parameter to extrapolate on the irritancy potential of chemicals in human reconstructed epidermis models (3,4).

In addition, both the SkinEthic RHE and EpiDerm submission provided information on the secondary endpoint IL-1α (Interleukin 1 alpha). The data on IL-1α submitted in both dossiers did not demonstrate an improvement of the predictive capacity of the test methods. Therefore, for both methods, only the data for the MTT endpoint were considered with regard to the predictive capacity.

Background to the IL1 α endpoint:

As a result of the ECVAM SIVS, the IL-1α endpoint had been suggested as a potentially useful adjunct (2). IL-1α is an inflammatory mediator secreted by the non-classical pathway (6,7). The ECVAM SIVS had concluded that IL 1α may be capable, under certain conditions, to increase the sensitivity of human reconstructed epidermis assays (2-4), e.g. when used in a tiered testing approach to identify false negatives of the MTT endpoint.

4. Predictive values of the two test methods

Considering the MTT endpoint, the two validated method have predictive values as shown in Table 1, calculated on the basis of the *median (or mode) of the individual laboratory predictions* for each of the 20 reference chemicals. For comparison, the corresponding values for the reference method EPISKIN are provided. Both submitted test methods meet the values of predictivity indicated in the performance standards (specificity = 80% and sensitivity = 70%).

Table 1: *Predictive values (in %) for the MTT endpoint of the two novel validated in vitro tests for skin irritation testing (SkinEthic RHE and modified EpiDerm SIT) in comparison to the fully validated reference method (EPISKIN) of the ECVAM skin irritation validation study.*

	EPISKIN (reference method)	**Modified EpiDerm SIT**	**SkinEthic RHE**
Specificity	80	**80**	**80**
Sensitivity	70	**80**	**90**
False positive rate	*20*	***20***	***20***
False negative rate	*30*	***20***	***10***
Accuracy	75	**80**	**85**

5. References

1. ECVAM (2007) *Performance Standards for Applying Human Skin Models to In Vitro Skin Irritation Testing.* Online: http://ecvam.jrc.it/
2. ECVAM (2007) *Statement of the ECVAM Scientific Advisory Committee (ESAC) on the Validity of In Vitro Tests for Skin Irritation.* Online: http://ecvam.jrc.it/
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5. OECD (2005) *OECD guidance document on the validation and international acceptance of new or updated test methods for hazard assessment. OECD series on testing and assessment Nr. 34.*

6. Prudovsky I, Tarantini F, Landriscina M, Neivandt D, Soldi R, Kirov A, Small D, Kathir KM, Rajalingam D, Kumar TK. (2008) *Secretion without Golgi.* J Cell Biochem. **103(5):**1327-43.

7. Prudovsky I, Mandinova A, Soldi R, Bagala C, Graziani I, Landriscina M, Tarantini F, Duarte M, Bellum S, Doherty H, Maciag T. (2003) *The non-classical export routes: FGF1 and IL-1alpha point the way.* J Cell Sci. **116(Pt 24):**4871-81.