EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

ESAC STATEMENT ON THE PERFORMANCE STANDARDS (PS) FOR IN VITRO SKIN IRRITATION TESTING USING RECONSTRUCTED HUMAN EPIDERMIS

Contents

I. ESAC STATEMENT

At its 31st meeting, held on 7 and 8 July 2009, the non-Commission members of the ECVAM Scientific Advisory Committee (ESAC) unanimously endorsed the following statement, subject to final ESAC consensus established by written procedure as of 22.9.2009:

Upon completion of the ECVAM Skin Irritation Validation Study (SIVS) in 2007, ECVAM had defined Performance Standards for *in vitro* Skin Irritation Testing using Reconstructed Human Epidermis (RhE) on the basis of the EpiSkin as well as the original EpiDerm test methods (Ref. 1, 2). In 2008 ECVAM validated two test methods on the basis of these Performance Standards: one similar test method (the SkinEthic RHE assay) and one modified test method (the modified EpiDerm SIT) (Ref. 3).

The SIVS was designed and conducted prior to the adoption of the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (Ref. 4). Consequently, the SIVS evaluated the test methods under scrutiny primarily with respect to the EU classification system as described in the Dangerous Substances Directive (the "EU DSD" system) (Ref. 5) albeit considering the GHS classification system with respect to the selection of test substances used for validation.

In December 2008 the EU adopted the UN GHS (Ref. 4) and implements this by means of the Classification, Labelling and Packaging (CLP) Regulation (Ref. 6). This regulation came into force on 20 January 2009 and will gradually replace the EU DSD system.

The UN GHS system uses a slightly different cut-off value for distinguishing between substances considered irritant and those considered non-irritant: while the cut-off under EU DSD was an in vivo score of 2.0, the cut-off under UN GHS is 2.3.

With the adoption of the CLP regulation (Ref. 6), ECVAM carefully assessed the performance of the three validated in vitro skin irritation test methods. The ESAC reviewed this assessment confirmed that the performance of all three test methods was satisfactory also under UN GHS (Ref. 7).

As a second consequence of the adoption of the CLP regulation, the ECVAM two elements of the skin irritation Performance Standards required adaptation:

1) the set of Reference Chemicals listed in the PS

2) the defined accuracy and reliability values.

The original ECVAM Performance Standards for in vitro Skin Irritation have now undergone extensive update in view mainly of the EU's adoption of the UN globally harmonised classification and labelling system (UN GHS) (Ref. 4, 6) in 2008. The update was performed within the framework of a dedicated ECVAM / ESAC Task Force which gathered experts from the ESAC, from ECVAM, ESAC observers from ICCVAM and the OECD as well as invited experts from industry. Extensive documentation on both the technical details of the Performance Standards revision and the performance of all ECVAM-validated in vitro skin irritation methods has been prepared by ECVAM in collaboration with the BfR/Zebet (Ref. 8).

The ESAC concludes that the updated ECVAM Performance Standards (Ref. 9) can now be used to assess the scientific validity of proposed test methods for predicting acute skin irritation effects as classified and labelled under the provisions of CLP (Ref. 6).

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Head of Unit

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Ispra, 9 July 2009

II. REFERENCES TO THE STATEMENT

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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.ec.europa.eu/ "Publications / ESAC Statements"

3. ECVAM (2008) Statement of the ECVAM Scientific Advisory Committee (ESAC) on the scientific validity of in vitro tests for skin irritation testing. Online: http://ecvam.jrc.it/

4. United Nations Economic Commission for Europe (UNECE) (2008). UN Globally Harmonised System of Classification and Labelling of Chemicals, 2nd revised edition; Online: http://www.unece.org/trans/danger/publi/ghs/ghs_rev02/02files_e.html; (as of April 2009).ECVAM (2008) Statement of the ECVAM Scientific Advisory Committee (ESAC) on the scientific validity of in vitro tests for skin irritation testing. Online: http://ecvam.jrc.ec.europa.eu/ "Publications / ESAC Statements"

5. EC (2001) Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Union L225, 1-333.

6. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006)

7. ECVAM (2009) Statement on the performance under UN GHS of three in-vitro assays for skin irritation testing and the adaptation of the Reference Chemicals and defined Accuracy Values of the ECVAM Skin Irritation Performance Standards. Original statement March 2009, updated July 2009 to reflect further changes of the Reference Chemical set following the OECD acceptance process. Online: http://ecvam.jrc.ec.europa.eu/ under "Publications / ESAC Statements"

8. Griesinger, C., Barroso, J., Zuang, V., Cole, T., Genschow, E. and Liebsch M. (2009). Explanatory background document to the OECD draft test guideline on in vitro skin irritation testing. Available under “Validation Study Documents, section Skin Irritation” at: http://ecvam.jrc.ec.europa.eu

9. EC-ECVAM (2009). Performance Standards for in vitro skin irritation test methods based on Reconstructed human Epidermis (RhE). Available under “Validation Study Documents, section Skin Irritation”, at: http://ecvam.jrc.ec.europa.eu

III. THE ESAC

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 Argelia Castaño(Spain)
Ms Maija Dambrova (Latvia)
Ms Alison Gray (ESTIV)
Ms Katalin Horvath (Hungary)
Ms Dagmar Jírová (Czech Republic)
Mr Roman Kolar (Eurogroup for Animals)
Ms Elisabeth Knudsen (Denmark - acting as moderator at the meeting)
Mr Manfred Liebsch (Germany)
Mr Gianni Dal Negro (EFPIA)
Mr. Walter Pfaller (Austria)
Mr Tõnu Püssa (Estonia)
Mr Dariusz Sladowski (Poland)
Mr Jon Richmond (UK)
Ms Vera Rogiers (ECOPA)
Mr Michael Ryan (Ireland)
Ms Annalaura Stammati (Italy)
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:

Commission services
Mr Joachim Kreysa (DG JRC, Head of In vitro methods Unit/ECVAM, chairman)
Mr Claudius Griesinger (DG JRC, ESAC secretariat)
Ms Susanne Hoke (DG ENTR)
Ms Susanna Louhimies (DG ENV)
Mr Juan Riego Sintes (DG JRC)

The following observers were present
Mr Hajime Kojima (JaCVAM)
Mr William Stokes (NICEATM)
Ms Marilyn Wind (ICCVAM)

IV. INFORMATIVE ANNEXE TO THIS STATEMENT

1. General information on Performance Standards

Performance Standards are used to assess the reliability and relevance of new test methods in reference to a validated method / validated methods. To qualify for a validation based on Performance Standards new methods have to fulfil the specific condition of being sufficiently similar to the previously validated 'Reference Method(s)'. Two types of methods qualify for Performance Standards-based validations: Novel but sufficiently similar test methods (so-called "me-too's") and modifications of the validated method(s) that are minor enough to leave the modified method sufficiently similar with respect to the Reference Method.

Performance Standards are defined on the basis of the properties and performance of a validated test method / methods. Such validated methods used to define Performance Standards are referred to as 'Reference Method'.

Performance Standards are constituted by three essential elements:

1) **Essential test method components**, defining the essential structural, functional and procedural characteristics of the test method

2) A list of **Reference Chemicals**, defining a set of substances that at should be tested during validation of *Similar* or *Modified Test Methods* and that ideally appropriately reflect the properties (i.e. chemical classes) of testing set used for validation of the Reference Method as well as the Reference Method's predictive values.

3) Defined **accuracy and reliability values** which appropriately reflect the predictive capacity of the Reference Method. These values thus define the acceptance range with respect to the predictive capacity of new *Similar* or *Modified* Methods.

2. Detailed background on the Update of the ECVAM Performance Standards for in vitro Skin Irritation Testing

The original PS were defined in May 2007 after completion of the ECVAM Skin Irritation Validation Study (SIVS) conducted between December 2003 to August 2006 (Spielmann et al., 2007) and the ESAC peer review process finalised with the issuing of the ESAC statement on the reference methods in 2007 (ECVAM 2007). During the SIVS, the reliability, relevance and limitations (including chemical applicability domain) of two commercially available Reconstructed human Epidermis (RhE) models (EpiSkin™ and EpiDerm™) were analysed. The SIVS was designed and conducted prior to the adoption of the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, 2008). Consequently, the SIVS evaluated the test methods under scrutiny primarily with respect to the EU classification system as described in the Dangerous Substances Directive (the “EU DSD” system) (EC 2001) albeit considering the GHS classification system during selection of substances to be tested in the SIVS. Thus, the original PS, including both the list of *Reference Chemicals* and the *Accuracy Target Values,* were based on the EU DSD (EC 2001), which consists of two categories: *no label* (non-classified substances) and *R38* (irritant substances) with a cut-off *in vivo* score of 2.0.

In December 2008 the EU adopted the UN GHS (United Nations 2008) and implements this by means of the Classification, Labelling and Packaging (CLP) Regulation (EC 2008). This regulation came into force on 20 January 2009 and will replace, after a transitional period, the previous EU legislations (EC

2001) for the classification of substances and mixtures (i.e. preparations). The EU classification system based on GHS (the "CLP" system) (EC 2008) directly transposes the UN GHS system (United Nations 2008) which foresees one irritant category. The EU will not use an additional optional category for mild irritants ("Category 3") that will apply only to some authorities (e.g. pesticides) (UN GHS*). Therefore the CLP system continues to use two categories to distinguish *non-classified* (No Category) from irritant (Category 2) substances. However, according to the new rules for skin irritation classification and labelling (C&L) (United Nations 2008; EC 2008), the cut-off score to distinguish between No Category and Category 2 substances was shifted to 2.3 (UN GHS or CLP) from a value of 2.0 (EU DSD). Consequently substances with an *in vivo* score between 2.0 and 2.3 that were considered irritant under the EU DSD are now non-classified under UN GHS, which does not use the optional Category 3 ($1.7 \leq$ Cat 3 < 2.3).

This had practical consequences on the ECVAM PS:

(a) the set of Reference Chemicals (RC) was not balanced any more (three former R38 substances had become not classified under UN GHS) (Griesinger et al., 2008) and although this can be regarded as reflecting the real prevalence of irritants much better, it is good practice to have a balanced distribution of RC enabling assessment of both classified (irritant) and non-classified substances on the basis of equal numbers of test substances;
(b) the accuracy target values did not match the changed prevalence which results from the cut-off shift (Griesinger et al., 2008): with a higher cut-off, more substances will not be classified in the future and, inversely, the prevalence of skin irritant substances will decrease.

Therefore, the global adoption of GHS (in the EU through regulation EC 1272/2008 - CLP regulation) (EC 2008) made necessary an **update of the original ECVAM PS** in order to balance the set of RC and carefully adjust the accuracy target values (Griesinger et al., 2008). Minor adaptations include more precise specifications concerning:

1) Recommendations regarding the training set for developing similar or modified test methods that may qualify for PS-based equivalence validation studies, in particular limitations regarding the use of RC for test development/optimisation purposes.
2) The number of times that invalid runs may be retested.
3) The number of invalid run sequences (i.e. absence of 3 valid independent runs in a single laboratory) after retesting that are acceptable for the data set to be considered qualified for the purpose of an equivalence validation study.
4) The calculation of Reliability (Reproducibility) and Predictive Capacity (Accuracy)

V. REFERENCES TO THE INFORMATIVE ANNEXE

EC (2001) Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Union L225, 1-333.

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