



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

3
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15 **I. ESAC STATEMENT**

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

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

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

31 In December 2008 the EU adopted the UN GHS (Ref. 4) and implements this by means of the
32 Classification, Labelling and Packaging (CLP) Regulation (Ref. 6). This regulation came into force on
33 20 January 2009 and will gradually replace the EU DSD system.

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

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



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

42 1) the set of Reference Chemicals listed in the PS

43 2) the defined accuracy and reliability values.

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

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

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60 Joachim Kreysa

61 Head of Unit

62 In-Vitro Methods Unit

63 European Centre for the Validation of Alternative Methods

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69 Ispra, 9 July 2009

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92 regulations and administrative provisions relating to the classification, packaging and labelling
93 of dangerous substances. Official Journal of the European Union L225, 1-333.

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

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99 skin irritation testing and the adaptation of the Reference Chemicals and defined Accuracy
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102 the OECD acceptance process. Online: <http://ecvam.jrc.ec.europa.eu/> under "Publications /
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112 **III. THE ESAC**

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

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

118

- 119 Ms Argelia Castaño (Spain)
120 Ms Maija Dambrova (Latvia)
121 Ms Alison Gray (ESTIV)
122 Ms Katalin Horvath (Hungary)
123 Ms Dagmar Jírová (Czech Republic)
124 Mr Roman Kolar (Eurogroup for Animals)
125 Ms Elisabeth Knudsen (Denmark - acting as moderator at the meeting)
126 Mr Manfred Liebsch (Germany)
127 Mr Gianni Dal Negro (EFPIA)
128 Mr. Walter Pfaller (Austria)
129 Mr Tõnu Püssa (Estonia)
130 Mr Dariusz Sladowski (Poland)
131 Mr Jon Richmond (UK)
132 Ms Vera Rogiers (ECOPA)
133 Mr Michael Ryan (Ireland)
134 Ms Annalaura Stamatì (Italy)
135 Mr Jan van der Valk (The Netherlands)
136 Mr Carl Westmoreland (COLIPA)
137 Mr Timo Ylikomi (Finland)

138

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

141 **Commission services**

- 142 Mr Joachim Kreysa (DG JRC, Head of In vitro methods Unit/ECVAM, chairman)
143 Mr Claudius Griesinger (DG JRC, ESAC secretariat)
144 Ms Susanne Hoke (DG ENTR)
145 Ms Susanna Louhimies (DG ENV)
146 Mr Juan Riego Sintès (DG JRC)

147

148 **The following observers were present**

- 149 Mr Hajime Kojima (JaCVAM)
150 Mr William Stokes (NICEATM)
151 Ms Marilyn Wind (ICCVAM)



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153 IV. INFORMATIVE ANNEXE TO THIS STATEMENT

154 1. General information on Performance Standards

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

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

165 Performance Standards are constituted by three essential elements:

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

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

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

175

176 2. Detailed background on the Update of the ECVAM Performance Standards for in vitro Skin 177 Irritation Testing

178 The original PS were defined in May 2007 after completion of the ECVAM Skin Irritation Validation
179 Study (SIVS) conducted between December 2003 to August 2006 (Spielmann et al., 2007) and the
180 ESAC peer review process finalised with the issuing of the ESAC statement on the reference methods
181 in 2007 (ECVAM 2007). During the SIVS, the reliability, relevance and limitations (including
182 chemical applicability domain) of two commercially available Reconstructed human Epidermis (RhE)
183 models (EpiSkinTM and EpiDermTM) were analysed. The SIVS was designed and conducted prior to
184 the adoption of the United Nations (UN) Globally Harmonized System of Classification and Labelling
185 of Chemicals (GHS) (United Nations, 2008). Consequently, the SIVS evaluated the test methods under
186 scrutiny primarily with respect to the EU classification system as described in the Dangerous
187 Substances Directive (the "EU DSD" system) (EC 2001) albeit considering the GHS classification
188 system during selection of substances to be tested in the SIVS. Thus, the original PS, including both
189 the list of *Reference Chemicals* and the *Accuracy Target Values*, were based on the EU DSD (EC
190 2001), which consists of two categories: *no label* (non-classified substances) and *R38* (irritant
191 substances) with a cut-off *in vivo* score of 2.0.

192

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



196 2001) for the classification of substances and mixtures (i.e. preparations). The EU classification
197 system based on GHS (the "CLP" system) (EC 2008) directly transposes the UN GHS system (United
198 Nations 2008) which foresees one irritant category. The EU will not use an additional optional
199 category for mild irritants ("Category 3") that will apply only to some authorities (e.g. pesticides) (UN
200 GHS*). Therefore the CLP system continues to use two categories to distinguish *non-classified* (No
201 Category) from irritant (Category 2) substances. However, according to the new rules for skin
202 irritation classification and labelling (C&L) (United Nations 2008; EC 2008), the cut-off score to
203 distinguish between No Category and Category 2 substances was shifted to 2.3 (UN GHS or CLP)
204 from a value of 2.0 (EU DSD). Consequently substances with an *in vivo* score between 2.0 and 2.3 that
205 were considered irritant under the EU DSD are now non-classified under UN GHS, which does not use
206 the optional Category 3 ($1.7 \leq \text{Cat } 3 < 2.3$).

207 This had practical consequences on the ECVAM PS:

- 208
- 209 (a) the set of Reference Chemicals (RC) was not balanced any more (three former R38 substances had
210 become not classified under UN GHS) (Griesinger et al., 2008) and although this can be regarded as
211 reflecting the real prevalence of irritants much better, it is good practice to have a balanced distribution
212 of RC enabling assessment of both classified (irritant) and non-classified substances on the basis of
213 equal numbers of test substances;
 - 214 (b) the accuracy target values did not match the changed prevalence which results from the cut-off
215 shift (Griesinger et al., 2008): with a higher cut-off, more substances will not be classified in the future
216 and, inversely, the prevalence of skin irritant substances will decrease.

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

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

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231 **V. REFERENCES TO THE INFORMATIVE ANNEXE**

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233 28th time Council Directive 67/548/EEC on the approximation of laws, regulations and
234 administrative provisions relating to the classification, packaging and labelling of dangerous
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