EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

STATEMENT[1] 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

At its 30th meeting, held on 9 and 10 March 2009, the non-Commission members of the ECVAM Scientific Advisory Committee (ESAC) unanimously endorsed the following statement, subject to editorial finalisation by the ESAC secretariat and final ESAC consensus established by written procedure as of **9th April 2009**:

1. Performance of the ECVAM-validated skin irritation in vitro tests under UN GHS

Previously, three reconstructed human epidermis models (the EpiSkin, the modified EpiDerm SIT and the SkinEthic RHE test methods) have been validated by ECVAM primarily according to the previous EU classification system. This system is being replaced over the next few years by the new classification system laid out in the CLP regulation (see below) which is based on the United Nations' *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS; Ref. 1). For classification according to the new CLP rules the following deadlines apply: 1 December 2010 for the classification of substances and 1 June 2015 for the classification of mixtures (i.e. preparations). Importantly, the selection of test substances used for the ECVAM skin irritation validation study (SIVS; Ref. 2), performed from 2003 to 2007, already took account of the upcoming UN GHS classification system. Upon completion of the ECVAM SIVS, the EpiSkin test method was found to be a reliable stand-alone method for distinguishing between skin irritants and non-irritants (ESAC statement from April 2007, Ref. 3) and, hence, its performance as reference method with regard to the agreed predictive values was used to determine required standards of accuracy and reliability of the ECVAM skin irritation Performance Standards in May 2007. The modified EpiDerm SIT and the SkinEthic RHE test methods were subsequently validated on the basis of these Performance Standards using the 20 defined Reference Chemicals (ESAC statement from November 2008, Ref. 4).

In December 2008, the EU adopted the UN Globally Harmonised System (UN GHS) for Classification and Labelling and will implement this by means of the Regulation on the Classification, Labelling and Packaging of Substances and Mixtures (CLP Regulation EC 1272/2008; Ref. 5) which came into force on 20 January 2009 and will, after a transitional period, replace the previous EU legislations for the classification of substances and mixtures (i.e. preparations). In agreement with the provisions of the UN GHS system, the new CLP

[1] This statement has been updated during the 31st ESAC meeting, 7-8 July 2009 with respect to minor additions (e.g. providing the test results in table one behind the percentage figures) and, more importantly, one of the reference chemicals: As a result of an OECD Expert Consultation Meeting 15-17 June 2009, Washington D.C., 1,1,1,-trichloroethane, a substance listed in the Montreal Protocol, has been replaced by Tetrachloroethylene. Agreement on the update was established by written procedure as of 22.9.2009.

skin irritation classification system will use a single irritant category (category 2) and hence continues to use a total of two classification categories to distinguish irritant (category 2) from non-irritant (no-category) substances. However, according to the GHS rules for skin irritation classification and labelling, the cut-off score to distinguish between no-category and category 2 substances was shifted to an *in vivo* score of greater or equal 2.3 from a value of 2.0 (as used for the previous EU classification system). Consequently substances with an *in vivo* score between 2.0 and 2.3 that are considered irritant under the previous EU classification system will be considered non-irritants under the future CLP classification system, which does not implement the optional additional UN GHS category 3 ("mild irritants": substances with scores greater or equal to 1.5 and smaller than 2.3), which is available for those authorities (e.g. pesticides) that want to have more than one skin irritant category (Ref. 1).

The performance of all three tests under CLP (i.e. UN GHS using one single irritant category) has now been re-evaluated to take account of this shift of cut-off value into consideration and has been found satisfactory (Table 1; Ref. 6 for extensive background regarding the re-calculation of these values). While the specificity of the EpiSkin method is decreased from 81.8%* (previous EU system) to 71.1 %* (CLP), the test sensitivity has increased from 72%* (previous EU system) to 84.6%* (CLP). The two other methods show similar values for the specificity (both tests 69.2%*), and higher sensitivity values than the reference method under CLP.

The original ESAC statements relating to the scientific validity of these test methods therefore remains valid and, with regard to their use in the context of classification decisions, can now be extended to the CLP system. Updated accuracy values under CLP are provided in this statement.

Moreover, on the basis of the documentation available confirming the overall satisfactory performance of the three methods, the ESAC is of the opinion that no further work is required at this stage and that the existing information on the validation studies and additionally available background information is sufficient to explain and justify the changes in performance of the tests and key aspects of the performance standards (i.e. reference chemicals and defined accuracy values) necessitated by the threshold shift upon adaptation of the GHS system in the EU. As is common practice, adaptations to technical progress should be performed as appropriate and necessary. It should be noted, that any conclusions on the applicability domain are based, at this stage, mainly on the testing set used during the ECVAM SIVS.

*) All values are based on the final predictive decisions of the study calculated on the basis of the median of the individual laboratory predictions. Since the predictions are essentially categories (i.e. positive or negative) and take values of either 1 or 0, the final decision can be derived by using either the median or the mode.

Table 1. Accuracy values for the three ECVAM-validated skin irritation in vitro test methods under CLP (UN GHS)

	EpiSkin test method (58 chemicals[1])	**EpiSkin test method** (20 reference chemicals[3])	**Modified EpiDerm test method** (20 reference chemicals[3])	**SkinEthic test method** (20 reference chemicals[3])
Specificity (%) [2]	**71.1** (32/45)	**76.9** (10/13)	**69.2** (9/13)	**69.2** (9/13)
Sensitivity (%) [2]	**84.6** (11/13)	**85.7** (6/7)	**85.7** (6/7)	**100** (7/7)
Overall Accuracy (%) [2]	**74.1** (43/58)	**80** (16/20)	**75** (15/20)	**80** (16/20)

[1]) The test substances from the ECVAM Skin Irritation Validation Study (SIVS) conducted from 2003 to 2007.
[2]) Based on the median (or mode) of the individual laboratory predictions. The values in parentheses provide the correct predictions per total number of substances either per categorical group (for sensitivity and specificity values) or per total number of substances tested (for accuracy value).
[3]) Original 20 RC from the ECVAM Performance Standards May 2007

2. Adaptation of the Reference Chemicals and Defined Accuracy Values of the ECVAM Performance Standards

2.1 Updated list of Reference Chemicals

Due to the threshold shift resulting from the adoption of the UN GHS system in the EU, the reference chemicals set listed in the original ECVAM Performance Standards were no longer properly balanced with regard to an equal representation of Irritant versus Non-irritant substances.

To address this and other issues (i.e. global commercial availability, evidence that some substances are non-irritant in human, handling qualities) the reference chemical set was updated. The updated reference chemical set retains the false negative and false positive rates obtained with the EpiSkin method under UN GHS on the basis of the full set of 58 test substances from the ECVAM skin irritation validation study allowing for the appropriate future validation of modified or similar ("me-too") test methods.

Deletions

The following six substances were deleted (in vivo scores in parentheses):

1) d-propylene glycol (0)
2) allyl heptanoate (1.7)
3) terpinyl acetate (2.0)
4) tri-isobutyl phosphate (2.0)
5) alpha-terpineol (2.7)

6) butyl methacrylate (3.0)

Additions

The following six substances were added (in vivo scores in parentheses):

1) cinnamaldehyde (2.0)
2) 2-chloromethyl-3,5-dimethyl-4-methoxypyridine HCl (2.7)
3) 5% potassium hydroxide (3.0)
4) benzenethiol, 5-(1,1-dimethyl)-2 methyl (3.3)
5) 1-methyl-3-phenyl-1-piperazine (3.3)
6) Tetrachloroethylene (4.0)

Moreover, the updated reference chemicals (table 2) meet the following criteria:

1. the chemicals are commercially available
2. they are representative of the full range of Draize skin irritancy scores (from non-irritant to strong irritant)
3. they have a well-defined chemical structure
4. they are representative of the chemical functionalities used in the validation process
5. they are not associated with an extremely toxic profile (e.g. carcinogenic or toxic to the reproductive system) and they are not associated with environmental concerns or prohibitive disposal costs.

Table 2: Updated reference chemicals

Nr.	Reference Chemical	*In vivo* Score	EU *in vivo* category	GHS-EU *in vivo* category	EPISKIN classifi-cation
1	1-bromo-4-chlorobutane	0	no	no category	**I**
2	diethyl phthalate	0	no	no category	NI
3	naphthalene acetic acid	0	no	no category	NI
4	allyl phenoxy-acetate	0.3	no	no category	NI
5	isopropanol	0.3	no	no category	NI
6	4-methyl-thio-benzaldehyde	1	no	no category	**I**
7	methyl stearate	1	no	no category	NI
8	heptyl butyrate	1.7	no	optional cat. 3	NI
9	hexyl salicylate	2	R38	optional cat. 3	NI
10	cinnamaldehyde	2	R38	optional cat. 3	**I**
11	1-decanol *	2.3	R38	category 2	I
12	cyclamen aldehyde	2.3	R38	category 2	I
13	1-bromohexane	2.7	R38	category 2	I
14	2-chloromethyl-3,5-dimethyl-4-methoxypyridine HCl	2.7	R38	category 2	I
15	5% potassium hydroxide	3	R38	category 2	I
16	di-n-propyl disulphide *	3	R38	category 2	**NI**
17	benzenethiol, 5-(1,1-dimethylethyl)-2-methyl	3.3	R38	category 2	I
18	1-methyl-3-phenyl-1-piperazine	3.3	R38	category 2	I
19	heptanal	3.4	R38	category 2	I
20	Tetrachloroethylene	4	R38	category 2	I

**) Substances which are irritant in the rabbit but for which there is reliable evidence that they are non-irritant in humans.*

2.2 Updated defined accuracy values as specified in the ECVAM skin irritation Performance Standards

The defined accuracy values (to be included in the ECVAM skin irritation Performance Standards) are derived from the performance of the validated reference method EpiSkin with the updated reference chemicals and under GHS-EU and on the basis of additional considerations relating to relevance in the species of interest. The values are given in table 3.

Table 3: Defined Accuracy Values

	Defined Accuracy Values
Specificity (%)	**70**
Sensitivity (%)	**80**
Overall Accuracy (%)	**75**

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Ispra, 8th July 2009

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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 Maggy Jennings (Eurogroup for Animals)
Ms Dagmar Jírová (Czech Republic)
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Mr Hasso Seibert (ESF, acting as co-moderator at the meeting)
Ms Annalaura Stammati (Italy)
Mr Jan van der Valk (The Netherlands)
Mr Carl Westmoreland (COLIPA, acting as moderator at the meeting)

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 Eimear Kelleher (DG JRC)
Ms Karin Kilian (DG SANCO)
Mr Juan Riego Sintes (DG JRC)

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