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ECVAM European Centre for the Validation of Alternative Methods

STATEMENT ON THE SCIENTIFIC VALIDITY OF THE EPISKIN™ TEST (AN *IN VITRO* TEST FOR SKIN CORROSIVITY)

At its 10th meeting, held on 31 March 1998 at the European Centre for the Validation of Alternative Methods (ECVAM), Ispra, Italy, the ECVAM Scientific Advisory Committee (ESAC)[1] unanimously endorsed the following statement:

> The results obtained with the EPISKIN™ test (involving the use of a reconstructed human skin model) in the ECVAM international validation study on *in vitro* tests for skin corrosivity were reproducible, both within and between the three laboratories that performed the test. The EPISKIN test proved applicable to testing a diverse group of chemicals of different physical forms, including organic acids, organic bases, neutral organics, inorganic acids, inorganic bases, inorganic salts, electrophiles, phenols and soaps/ surfactants. The concordances between the skin corrosivity classifications derived from the *in vitro* data and from the *in vivo* data were very good. The test was able to distinguish between corrosive and non-corrosive chemicals for all of the chemical types studied; it was also able to distinguish between known R35 (UN[2] packing group I) and R34 (UN packing groups II & III) chemicals. The Committee therefore agrees with the conclusion from this formal validation study that the EPISKIN test is scientifically validated for use as a replacement for the animal test, and that it is ready to be considered for regulatory acceptance.

The ESAC has been regularly kept informed of the progress of the study, and this endorsement was based on an assessment of various documents, including, in particular, the report on the results and evaluation of the validation study by the Management Team, which is to be published in *Toxicology in Vitro*.[3]

This validation study was conducted in accordance with the general principles laid down in the report of the CAAT[2]/ERGATT[2] workshop held in 1990,[4] guidelines contained in the report of an ECVAM/ERGATT workshop held in 1995,[5] criteria laid down by ECVAM and the ECB,[2,6] criteria recommended at an OECD[2] workshop held in 1996,[7] and the US ICCVAM[2] report on validation and regulatory acceptance.[8] The outcome of a prevalidation study on *in vitro* tests for skin corrosivity was published in 1995, as ECVAM workshop report 6.[9] A separate report on the selection of the test chemicals for the validation study is to be published alongside the Management Team's report in *Toxicology in Vitro*.[10]

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1. The ESAC was established by the European Commission, and is composed of representatives of the EU Member States, industry, academia and animal welfare, together with representatives of the relevant Commission services. The following members of the ESAC were present at the meeting on 31 March 1998:

Dr B Blaauboer (ERGATT)	Dr P Botham (ECETOC)
Professor J Castell (Spain)	Dr D Clark (UK)
Dr B Garthoff (EFPIA)	Professor A Guillouzo (France)
Dr C Hendriksen (The Netherlands)	Dr R Lorenzini (Italy)
Professor G Papadopoulos (Greece)	Professor V Rogiers (Belgium)
Dr B Rusche (Eurogroup for Animal Welfare)	Dr O de Silva (COLIPA)
Professor H Spielmann (Germany)	Dr O Svendsen (Denmark)
Professor H. Tritthart (Austria)	Dr M Viluksela (Finland)
Professor E Walum (Sweden)	
Professor M Balls (ECVAM)	Mr G Corcelle (DGXI)
Dr J Fentem (ECVAM)	Dr G Fracchia (DGXII)
Ms S Louhimies (DGXI)	Dr M Robert (DGIII)
Mr A Van Elst (DGXXIV)	

2. CAAT: Center for Alternatives to Animal Testing, Baltimore, USA; ECB: European Chemicals Bureau, Ispra, Italy; ERGATT: European Research Group for Alternatives in Toxicity Testing, Utrecht, The Netherlands; ICCVAM: *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods, Research Triangle Park, USA; OECD: Organization for Economic Cooperation and Development, Paris, France; UN: United Nations.

3. Fentem JH, Archer GEB, Balls M, Botham PA, Curren RD, Earl LK, Esdaile DJ, Holzhütter H-G & Liebsch M (1998) The ECVAM international validation study on *in vitro* tests for skin corrosivity. 2. Results and evaluation by the Management Team. *Toxicology in Vitro*, in press.

4. Balls M, Blaauboer B, Brusick D, Frazier J, Lamb D, Pemberton M, Reinhardt C, Roberfroid M, Rosenkranz H, Schmid B, Spielmann H, Stammati AL & Walum E (1990) Report and recommendations of the CAAT/ERGATT workshop on the validation of toxicity test procedures. *ATLA* 18: 303-337.

5. Balls M, Blaauboer BJ, Fentem JH, Bruner L, Combes RD, Ekwall B, Fielder RJ, Guillouzo A, Lewis RW, Lovell DP, Reinhardt CA, Repetto G, Sladowski D, Spielmann H & Zucco F (1995) Practical aspects of the validation of toxicity test procedures. The report and recommendations of ECVAM workshop 5. *ATLA* 23: 129-147.

6. Balls M & Karcher W (1995) The validation of alternative test methods. *ATLA* 23: 884-886.

7. Anon. (1996) *Final Report of the OECD Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Toxicological Test Methods.* 60pp. Paris: OECD.

8. Anon. (1997) *Validation and Regulatory Acceptance of Toxicological Test Methods. A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods.* 105 pp. Research Triangle Park, NC: NIEHS.

9. Botham PA, Chamberlain M, Barratt MD, Curren RD, Esdaile DJ, Gardiner JR, Gordon VC, Hildebrand B, Lewis RW, Liebsch M, Logemann P, Osborne R, Ponec M, Régnier J-F, Steiling W, Walker AP & Balls M (1995) A prevalidation study on *in vitro* skin corrosivity testing. The report and recommendations of ECVAM workshop 6. *ATLA* 23: 219-255.

10. Barratt MD, Brantom PG, Fentem JH, Gerner I, Walker AP & Worth AP (1998) The ECVAM international validation study on *in vitro* tests for skin corrosivity. 1. Selection and distribution of the test chemicals. *Toxicology in Vitro,* in press.

General information about the ECVAM skin corrosivity validation study:

A. The study was coordinated from ECVAM, and the Management Team (MT) was chaired by Dr Julia Fentem (ECVAM). The other four MT members acted as representatives of the "lead laboratories" and each took responsibility for one of the four tests included in the validation study: Dr Rodger Curren (Microbiological Associates Inc., USA; CORROSITEX™), Dr Lesley Earl (Unilever, UK; rat skin TER assay), Mr David Esdaile (Rhône-Poulenc Agro, France; EPISKIN™), and Dr Manfred Liebsch (ZEBET, Germany; Skin^{2TM} assay). The study was principally funded by ECVAM, under the terms of 14 separate contracts with the participating organisations. Professor Michael Balls (ECVAM) and Dr Philip Botham (ESAC; ZENECA CTL, UK) represented the sponsors in any contacts with the MT. In addition to ECVAM, the participating organisations were: Agence du Medicament (France), BASF Aktiengesellschaft (Germany), BIBRA International (UK), COVANCE (UK), Humboldt University (Germany), Huntingdon Life Sciences (UK), INRS (France), Microbiological Associates Inc. (USA), Microbiological Associates Ltd (UK), Rhône-Poulenc Agro (France), Sanofi Recherche (France), Unilever Research (UK), ZEBET, BgVV (Germany) and ZENECA CTL (UK).

B. This study began in 1996, as a follow-up to a prevalidation study on *in vitro* tests for replacing the *in vivo* Draize rabbit test for skin corrosivity. The main objectives were to: (a) identify tests capable of discriminating corrosives (C) from non-corrosives (NC) for selected groups of chemicals (e.g. organic acids, phenols) and/or all chemicals (single chemical entities only); and (b) determine whether the tests could identify correctly known R35 (UN packing group I) and R34 (UN packing groups II & III) chemicals. The tests selected for inclusion in the validation study were: (a) the rat skin TER assay; (b) CORROSITEX™; (c) the Skin^{2TM} ZK1350 corrosivity test; and (d) EPISKIN^{TM}. Each test was conducted in three independent laboratories, according to the principles, criteria and procedures for undertaking validation studies outlined previously by ECVAM in conjunction with international experts in this area. Prediction models for the four tests were clearly defined in the test protocols.

C. A test set of 60 chemicals was selected by an independent Chemicals Selection Sub-Committee, including organic acids (6C/5NC), organic bases (7C/3NC), neutral organics (9NC), phenols (2C/3NC), inorganic acids (6C/1NC), inorganic bases (2C/2NC), inorganic salts (1C/2NC), electrophiles (3C/5NC) and soaps/surfactants (3NC). The first set of ten coded chemicals was distributed independently of the MT and participating laboratories in

June 1996. Further to the satisfactory completion of the first phase of the study, the remaining 50 coded chemicals were distributed in September 1996. The results obtained were submitted to ECVAM's statistician, Dr Graeme Archer, for independent analysis in consultation with Dr Hermann-Georg Holzhütter (Humboldt University, Berlin, Germany). Data analysis and preparation of the final reports took place between May and October 1997.

D. EPISKIN™ is a three-dimensional human skin model comprising a reconstructed epidermis with a functional stratum corneum. Its use for skin corrosivity testing involves topical application of test materials to the surface of the skin for 3, 60 and 240 min, and the subsequent assessment of their effects on cell viability by using the MTT assay. An in-house evaluation and prevalidation of the test was conducted during 1994-96. On the basis of these studies, the test protocol was refined prior to its inclusion in this validation study.

EPISKIN Prediction Model:

Treatment time (mins)	**Viability (%)**	**C/NC**	**EU risk phrase**	**UN packing group**
3	< 35	C	R35	I
3 /60	≥ 35 / < 35	C	R34	II
60 /240	≥ 35 / < 35	C	R34	III
240	≥ 35	NC	no label	-

E. The prediction model for the EPISKIN test was used to classify the corrosivity potentials of the 60 test chemicals on the basis of the *in vitro* data obtained in the three laboratories conducting the test. Comparing these *in vitro* classifications with the *in vivo* classifications independently assigned to the chemicals before the blind trial began gave the following key statistical parameters:

Sensitivity:	C	83%
	R34/II & III	75%
	R35/I	39%
Specificity:		80%
Predictivity:	C	77%
	R34/II & III	64%
	R35/I	53%
Accuracy:	C/NC	81%
	R35/R34/NC	74%

The underprediction and overprediction rates for the EPISKIN test relative to the study objectives were:

Objective (a): C *v* NC	underprediction rate	17%
	overprediction rate	20%

Objective (b): R35/I *v* R34/II & III *v* NC

underprediction rate	
R35/I → NC	17%
R34/II & III → NC	18%
overprediction rate	
NC → R35/I	1%
NC → R34/II & III	19%
R34/II & III → R35/I	8%

F. In order for the EPISKIN test to be considered for use for legislative and other purposes, measures will be taken to press for the updating of OECD Testing Guideline 404 and Annex V method B.4 of *Directive 67/548/EEC*.

G. A statement on the scientific validity of the rat skin transcutaneous electrical resistance (TER) assay for skin corrosivity testing was also endorsed by the ESAC on 31 March 1998. The two other methods included in the validation study, CORROSITEX and Skin^2, did not meet all of the criteria for them to be considered acceptable as replacement tests. The corrosivity potentials of about 40% of the test chemicals could not be assessed with CORROSITEX, although it may be valid for testing specific classes of chemicals (such as organic bases and inorganic acids). The Skin^2 assay, as conducted in this validation study, had an unacceptably high underprediction rate (57%), although it had a specificity of 100%. It is recognised that both of these methods could be useful if they were incorporated into a tiered testing strategy for skin corrosivity.