Validation of *in vitro* Tests for Skin Corrosivity

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Summary
There have been several highly significant achievements in the area of alternatives for skin corrosivity testing in the last three years, most notably: (a) the validation, and subsequent endorsement, of two replacement alternative tests for skin corrosivity (the rat skin transcutaneous electrical resistance [TER] and EPISKIN™ human skin model assays) by the European Centre for the Validation of Alternative Methods (ECVAM); (b) an evaluation of the proposed OECD testing strategy for skin corrosion/irritation as it relates to the classification of corrosives; (c) completion of a successful prevalidation study on the use of the EpiDerm™ human skin model for corrosivity testing; (d) a review of CORROSITEX™ by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in the US; and (e) the submission of draft new test guidelines on skin corrosion to the OECD Secretariat and EU National Coordinators (Annex V test methods). It is now hoped that regulatory acceptance of the validated in vitro tests for skin corrosivity, at both EU and OECD levels, will be secured as quickly as possible.

Zusammenfassung: Validierung von in vitro Tests für die Ätzwirkung
In den letzten drei Jahren wurden mehrere bedeutende Erfolge bei der Entwicklung von Alternativmethoden zur Bestimmung der Ätzwirkung auf der Haut erzielt. Dies sind vor allem: (a) die Validierung und die darauffolgende Anerkennung von zwei Ersatzmethoden für die Ätzwirkung (Messung des elektrischen Widerstands an der Rattenhaut [TER] und der Testsatz mit humaner Haut EPISKIN™) durch das Europäische Zentrum für die Validierung von Alternativmethoden (ECVAM); (b) die Evaluierung der vorgeschlagenen OECD-Prüfstrategie für die Hautätzung und Hautreizung im Hinblick auf die Bewertung von ätzenden Stoffen; (c) die Fertigstellung einer erfolgreichen Prävalidierungsstudie mit dem humanen Hautmodell zu Ätzwirkung EpiDerm™; (d) die Nachprüfung von CORROSITEX™ durch das Interagency Coordinating Committee zur Validierung von Alternativmethoden in den USA (ICCVAM); und (e) die Vorlage von Entwürfen für neue Prüfrichtlinien für die Ätzwirkung auf der Haut beim OECD-Sekretariat und den National Coordinators der EU (Anhang V, Testmethoden). Es ist nun zu hoffen, dass die behördliche Anerkennung validierter in vitro Methoden für die Ätzwirkung auf der Haut sowohl auf EU- als auch auf OECD-Ebene so schnell wie möglich sichergestellt wird.

Keywords: 3R, replace, CORROSITEX™, EpiDerm™, EPISKIN™, human skin models, in vitro, rat skin transcutaneous electrical resistance assay, skin corrosion, Skin²™

1 Introduction

Dermal "corrosion" principally refers to the production of irreversible tissue damage in the skin. The assessment of acute skin corrosion/irritation potential is included in international regulatory requirements for the testing of chemicals. The standard approach used involves applying the test material to the shaved skin of albino rabbits (OECD, 1992). Testing for skin corrosion in laboratory animals has the potential to cause them considerable discomfort or pain, and it is recognised that the response in the rabbit is not always predictive of that found in humans. For these reasons, considerable effort has been directed toward the development and evaluation of alternative test methods for predicting chemical-induced acute dermal corrosion and irritation in recent years (Botham et al., 1998; Fentem et al., 1998).

A prevalidation study on *in vitro* skin corrosivity testing was conducted during 1993 and 1994 (Botham et al., 1995), as a first step towards defining those alternative tests which could be used within the context of OECD testing guideline 404 (OECD, 1992). Three tests were included in the prevalidation study: (a) the rat skin transcutaneous electrical resistance (TER) assay; (b) CORROSITEX™ (In Vitro International, Irvine, USA); and (c) the Skin²™ ZK1350 corrosivity test (Advanced Tissue Sciences, La Jolla, USA). Fifty coded chemicals (25 corrosives [C], 25 non-corrosives [NC]) were tested. The report on the outcome of the prevalidation study recommended that a formal validation study on alternative methods for skin corrosivity testing should be conducted (Botham et al., 1995).

2 ECVAM validation study

An international validation study on *in vitro* tests for replacing the *in vivo* rabbit test for skin corrosivity was conducted during 1996 and 1997 under the auspices of ECVAM (Fentem et al., 1998). The main objectives of the study were to: (a) identify tests capable of discriminating corrosives from non-corrosives for selected types of chemicals and/or all chemicals; and (b) determine whether these tests could identify correctly known R35 (UN packing group I) and R34 (UN packing groups II and III) chemicals. The tests evaluated were the rat skin TER assay, CORROSITEX, the Skin² ZK1350 corrosivity test, and EPISKIN™ (EPISKIN, Chaponost, France). Each test was conducted in three independent laboratories. Sixty coded chemicals were tested (Barratt et al., 1998).

Two of the tests evaluated, the TER and EPISKIN assays, met the criteria agreed by the Management Team concerning acceptable reproducibility and predictive ability (Fentem et al., 1998), for them to be considered scientifically validated for use as replacements for the rabbit test for distinguishing between C and NC chemicals for all of the chemical types studied (objective [a]). EPISKIN was also able to distinguish between known R35 and R34 chemicals, for all of the chemical types included in the study, on an acceptable number of occasions (objective [b]) (Fentem et al., 1998). The overall predictive ability of the TER and EPISKIN tests compared with the corrosivity classifications derived from the animal data are shown in table 1.

Table 1: Comparison of corrosivity classifications obtained from the TER and EPISKIN tests with those based on rabbit data - key statistical parameters (Fentem et al., 1998)

	TER assay	EPISKIN assay
Sensitivity (%)		
C	88	83
R34	18	75
R35	88	39
Specifity (%)	72	80
Predictivity (%)		
C	72	77
R34	40	64
R35	22	53
Accuracy (%)		
C/NC	79	81
R35/R34/NC	55	74

C=corrosive, NC=non-corrosive, R34=causes burns (EU risk phrase), R35=causes severe burns (EU risk phrase)

The scientific validity of the rat skin TER and EPISKIN tests was endorsed by the ECVAM Scientific Advisory Committee (ESAC) in March 1998 (ECVAM, 1998), as follows:

„The results obtained with the rat skin TER test 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 rat skin TER 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 C and NC chemicals for all of the chemical types studied. The Committee therefore agrees with the conclusion from this formal validation study that the rat skin TER test is scientifically validated for use as a replacement for the animal test for distinguishing between C and NC chemicals, and that the test is ready to be considered for regulatory acceptance."

„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 C and NC chemicals for all of the chemical types studied; it was also able to distinguish between known R35 (UN packing group I) and R34 (UN packing groups II and 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."

3 OECD testing strategy

It is becoming increasingly apparent that the development and implementation of stepwise (hierarchical) testing strategies, combining experimental data derived from a range of alternative methods (physicochemical techniques, structure-activity relationships [SAR], and *in vitro* tests), and which use animals only as a last resort when absolutely necessary, provides the most effective way forward for trying to predict toxicity while at the same time reducing the number of laboratory animals used for testing purposes (Botham et al., 1998). Flexible testing strategies provide a means to: (a) improve the scientific basis of toxicity testing; (b) implement the Three Rs, in terms of minimising the use and suffering of laboratory animals; (c) maximise the use of existing knowledge; and (d) optimise the use of resources.

Widespread concern over the use of the Draize rabbit test for assessing skin corrosion and irritation led to the proposal of a stepwise testing strategy at an OECD workshop in January 1996. Subsequently, the proposed testing strategy was adopted, with minor modifications, by the OECD Advisory Group on Harmonization of Classification and Labelling (OECD, 1998; Worth et al., 1998). An evaluation of the proposed OECD testing strategy as it relates to the classification of skin corrosives has been undertaken under the auspices of ECVAM (Worth et al., 1998). Using data on 60 chemicals generated during the ECVAM skin corrosivity validation study (Fentem et al., 1998), an assessment was made of the effect of applying three steps in the strategy, taken both individually and in sequence. The results indicated that chemicals can be classified as C or NC with sufficient reliability by the sequential application of three alternative methods - SAR (where available), pH measurement, and a single *in vitro* method - either the rat skin TER test or the EPISKIN assay (Worth et al., 1998).

4 Prevalidation study on the EpiDerm™ human skin model

An ECVAM-funded prevalidation study on the EpiDerm skin corrosivity test was coordinated by ZEBET during 1997/98, involving three phases: (a) protocol refinement; (b) protocol transfer; and (c) an

overall assessment of protocol performance (i.e. the reproducibility and predictive ability of the *in vitro* test). The objective of the study was to determine whether a test protocol developed for another human skin model (i.e. in addition to that for EPISKIN) could similarly discriminate C from NC for various chemical types. The EpiDerm test protocol developed by ZEBET incorporates the following prediction model, based on assessment of cell viability using the MTT assay after exposure to test chemical for 3 minutes and 1 hour: if the mean relative tissue viability after a 3-minute treatment is less than 50%, then classify as C; additionally, if the viability = 50% after 3 minutes, but is less than 15% after treatment for 1 hour, then also classify as C.

The test was conducted in three, independent, laboratories (ZEBET, Huntingdon Life Sciences and BASF), according to the ECVAM prevalidation scheme (Curren et al., 1995). In phase III, 24 coded chemicals (12 C, 12 NC) were tested; these were independently selected to be representative of the set of 60 chemicals tested in the ECVAM validation study (Barratt et al., 1998; Fentem et al., 1998). The results obtained were reproducible, both within and between the three laboratories. The EpiDerm test proved applicable to testing a diverse group of chemicals (both liquids and solids), including organic acids and bases, neutral organics, inorganic acids and bases, electrophiles and phenols. The concordances between the skin corrosivity classifications derived from the *in vitro* data and from the *in vivo* data were very good (Table 2); the test was able to distinguish between C and NC chemicals for all of the chemical types studied.

Table 2: Comparison of corrosivity classifications obtained from the EpiDerm test with those based on rabbit data – key statistical parameters (M. Liebsch, in preparation)

	EpiDerm assay
Sensitivity (%)	88
Specificity (%)	86
Predictivity (%)	
C	86
NC	87
Accuracy (%)	87

C=corrosive, NC=non-corrosive

5 Review of CORROSITEX™ by ICCVAM

ICCVAM, which has representation from 14 US federal agencies and programmes, conducted an independent scientific peer review of CORROSITEX in January 1999. The CORROSITEX assay involves measurement of the time required ("breakthrough time") for a chemical to pass through a hydrated collagen matrix (biobarrier) and supporting filter membrane. This is observed as a colour change in the chemical detection system (an aqueous solution of two pH indicator dyes). The peer review panel evaluated a submission prepared by In Vitro International, the manufacturers of CORROSITEX, according to the ICCVAM criteria for validation and acceptance of new toxicological test methods. The database used in the evaluation comprised results for 163 chemicals and chemical mixtures for which there were both CORROSITEX and *in vivo* rabbit corrosivity data. Published data from the ECVAM prevalidation (Botham et al., 1995) and validation studies (Fentem et al., 1998) were also considered during the review.

The final report on the outcome of this review should be available shortly, and it is expected that the findings will be similar to those from the ECVAM validation study; that is, that CORROSITEX may be valid for use, as an optional screen or as part of a tiered testing strategy, with restricted classes of chemicals (primarily acids and bases). However, in this respect, the advantages of CORROSITEX over simple pH determination remain to be demonstrated. In addition, many chemicals are incompatible with the chemical detection system and therefore cannot be tested in the CORROSITEX assay (Botham et al., 1995; Fentem et al., 1998).

6 New draft OECD and EU Annex V test guidelines

Further to the endorsement of the scientific validity of the rat skin TER and EPISKIN assays by the ESAC, several European Commission services (DGXI/E/2 and DGIII/E/3), and the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCC-NFP) which advises the Commission, reviewed all relevant documentation and subsequently added their endorsements to the ESAC statements.

A draft guideline on the use of the TER and EPISKIN tests for skin corrosion testing was prepared by the Management Team of the validation study, which was jointly submitted to the OECD Secretariat in December 1998 by DGXI/E/2 (on behalf of the Commission) and the UK government authorities. A draft Annex V test method on skin corrosion has also been prepared, for discussion by the EU National Coordinators for Test Methods. It is now hoped that regulatory acceptance of these validated replacement alternative tests for skin corrosion, at both EU and OECD levels, will be secured as quickly as possible, and that discussion of the draft guideline is made a priority in the work programmes for 1999/2000 of both the Commission (DGXI) and the OECD.

7 Conclusions and further activities

Skin corrosivity testing is a relatively simple procedure in biological terms. The endpoint is severe tissue destruction, not a subtle biological change, and the application route is topical, with no problems of dilution or distribution. These two factors made the development of non-animal methods for the prediction of skin corrosion easier than for other toxic effects exerted by subtle, multifactorial, mechanisms. Nevertheless, the validation of *in vitro* tests for skin corrosivity represents a significant achievement in relation to the replacement of toxicity tests known to cause considerable animal pain due to the nature of the endpoint under evaluation.

Whereas the replacement of animal tests for skin corrosion is a relatively simple target, the challenges involved in finding replacement alternative tests for skin irritation are greater given our limited understanding of the mechanistic basis of skin irritation *in vivo*, the complex series of reactions involved, and our inability at present to define the key relevant endpoints which could be evaluated *in vitro* in human skin models or other suitable test systems. Currently, most *in vitro* tests for skin irritation use cytotoxicity (e.g. MTT reduction) as the main endpoint; to varying extents they model dermal penetration of the chemical and its subsequent cytotoxi-

city. This may be sufficient in terms of enabling a simple discrimination between irritants and non-irritants following acute exposure, a hypothesis which is currently being evaluated in an ECVAM-supported prevalidation study on *in vitro* tests for acute skin irritation (ECVAM, 1999).

References

Barratt, M. D., Brantom, P. G., Fentem, J. H., Gerner, I., Walker, A. P. and Worth, A. P. (1998). The ECVAM international validation study on in vitro tests for skin corrosivity. 1. Selection and distribution of the test chemicals. *Toxicology in Vitro 12*, 471-482.

Botham, P. A., Chamberlain, M., Barratt, M. D., Curren, R. D., Esdaile, D. J., Gardiner, J. R., Gordon, V. C., Hildebrand, B., Lewis, R. W., Liebsch, M., Logemann, P., Osborne, R., Ponec, M., Régnier, J. F., Steiling, W., Walker, A. P. and Balls, M. (1995). A prevalidation study on in vitro skin corrosivity testing. The report and recommendations of ECVAM workshop 6. *ATLA 23*, 219-255.

Botham, P. A., Earl, L. K., Fentem, J. H., Roguet, R. and van de Sandt, J. J. M. (1998). Alternative methods for skin irritation testing: the current status. ECVAM Skin Irritation Task Force report 1. *ATLA 26*, 195-211.

Curren, R. D., Southee, J. A., Spielmann, H., Liebsch, M., Fentem, J. H. and Balls, M. (1995). The role of prevalidation in the development, validation and acceptance of alternative methods. *ATLA 23*, 211-217.

ECVAM (1998). ECVAM News & Views. *ATLA 26*, 275-280.

ECVAM (1999). ECVAM News & Views. *ATLA 27*, 221-223.

Fentem, J. H., Archer, G. E. B., Balls, M., Botham, P. A., Curren, R. D., Earl, L. K., Esdaile, D. J., Holzhütter, H. G. and 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 12*, 483-524.

OECD (1992). *OECD Guideline for the Testing of Chemicals, No. 404: Acute Dermal Irritation/Corrosion*, 6 pp. Paris: OECD.

OECD (1998). *Revised Proposal for the Harmonization of Hazard Classification Based on Skin Irritation/Corrosion*, ENV/MC/CHEM/HCL(98)4, 12 pp. Paris: OECD.

Worth, A. P., Fentem, J. H., Balls, M., Botham, P. A., Curren, R. D., Earl, L. K., Esdaile, D. J. and Liebsch, M. (1998). An evaluation of the proposed OECD testing strategy for skin corrosion. *ATLA 26*, 709-720.

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Erratum

In der Arbeit von Gysler et al., „Dreidimensionale Hautmodelle zur Erfassung der perkutanen Resorption", *ALTEX 16*, 67-72, sind bei den Abbildungen 2 und 3 (Seite 70) die Ordinatenwerte um eine Zehnerstelle zu gering ausgefallen. Richtig müssen diese beiden Abbildungen wie folgt aussehen:

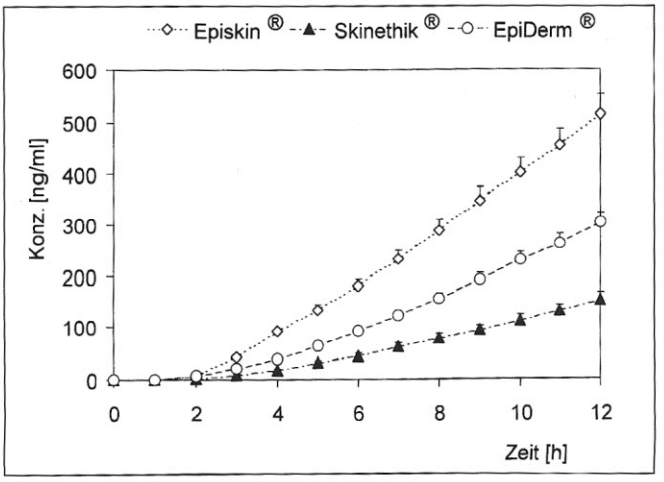

Abbildung 2: Penetration von Prednisolon durch humane Hautäquivalente. Konzentrationen im Akzeptormedium (kumulative Darstellung, n = 6).

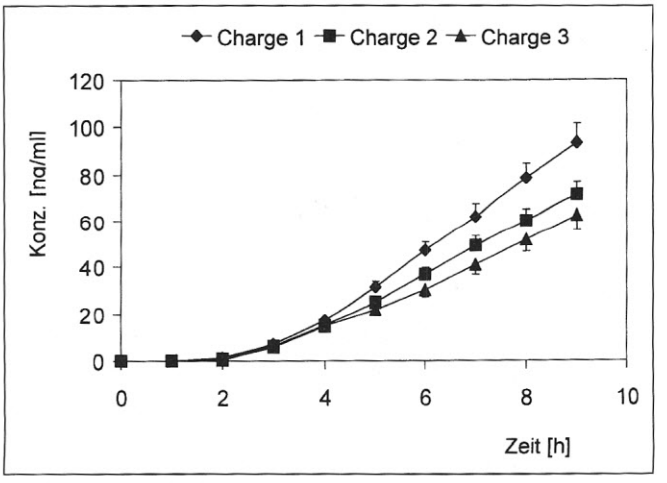

Abbildung 3: Variabilität der Barrierefunktion von Skinethic®. Vergleich der Prednisolon-Penetration (kumulative Darstellung) bei 3 unterschiedlichen Chargen (n = 6).