

## Assessment

### VALIDATION OF THE HUMAN EPIDERMIS MODEL SKINETHIC FOR SKIN CORROSION TESTING ACCORDING TO NEW OECD TEST GUIDELINE 431

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#### INTRODUCTION

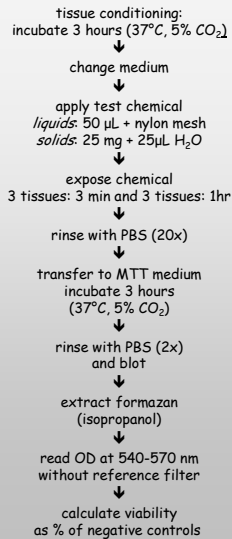
In the year 1998 the EPISKIN and TER *in vitro* corrosivity test were successfully validated and met the acceptance criteria previously defined by the Management Team of the ECVAM International Validation Study (1). Because between 1998 and 2001 the model EPISKIN (EPISKIN SNC, Lyon, F) was not commercially available, an ECVAM Catch up validation study was successfully performed with the human epidermal model EpiDerm (MatTek, Ashland, USA) (2).

In 2002 National Co-ordinators of OECD Test Guideline Programme (WNT) endorsed New Draft Test Guidelines TG430 (TER) and TG431 (Human Skin Model) for *In Vitro* Skin Corrosion Testing. In Guideline TG 431 general functional and performance criteria were defined if other (or new) skin or epidermis models are used in the context of this guideline (3).

To show that this concept works, in summer 2003 ZEBET tested several chemicals from the ECVAM validation trial with the the SkinEthic human reconstituted epidermal model (HRE) (SkinEthic, Nice, F), applying the validated EpiDerm skin corrosion test protocol and prediction model to SkinEthic. After a slight technical adaptation the SkinEthic results were identical with the EpiDerm results (4).

During December 2003 - February 2004 a blind trial employing ZEBET (D), Safepharma (UK) and BASF (D) was performed in which the 12 reference chemicals specified in OECD Test Guideline 431 were evaluated in each laboratory three times independently at different occasions. Data on reproducibility between laboratories, as well as predictions obtained are presented.

#### PROTOCOL



#### SKINETHIC PREDICTION (BLIND TRIAL)

Chemical	In vivo	Run	Laboratory		
			SAFEPHARM	ZEBET	BASF
1,2-Diaminopropane	R35	1	C	C	C
		2	C	C	C
		3	C	C	C
Acrylic acid	R35	1	C	C	C
		2	C	C	C
		3	C	C	C
2-tert. Butylphenol	R34	1	C	C	C
		2	C	C	C
		3	C	C	C
Potassium hydroxide (10% aq)	R34	1	C	C	C
		2	C	C	C
		3	C	C	C
Sulphuric acid (10 % wt)	R34	1	C	NC*	C
		2	C	C	C
		3	C	C	C
Octanoic acid	R34	1	C	C	C
		2	C	C	C
		3	C	C	C
4-Amino-1,2,4-triazole	R34	1	NC	NC	NC
		2	NC	NC	NC
		3	NC	NC	NC
Eugenol	NC	1	NC	NC	NC
		2	NC	NC	NC
		3	NC	NC	NC
Phenethyl bromide	NC	1	NC	NC	NC
		2	NC	NC	NC
		3	NC	NC	NC
Tetrachloroethylene	NC	1	C	NC	NC/C
		2	C	NC	NC/C
		3	NC	NC	NC
Isostearic acid	NC	1	NC	NC	NC
		2	NC	NC	NC
		3	NC	NC	NC
4-(Methylthio)-benzaldehyde	NC	1	NC	NC	NC
		2	NC	NC	NC
		3	NC	NC	NC

NC\* - problems with spreading of the chemical on the surface

#### MATERIAL AND METHOD

##### Human skin model

SkinEthic HRE - 0.63 cm<sup>2</sup> (SkinEthic, France)

##### Test materials : 12 OECD reference chemicals (3)

Nr.	Chemical name	In vivo classification
1	1,2-Diaminopropane	Severely Corrosive
2	Acrylic Acid	Severely Corrosive
3	2-tert. Butylphenol	Corrosive
4	Potassium hydroxide (10% aq)	Corrosive
5	Sulfuric acid (10% wt)	Corrosive
6	Octanoic acid (caprylic acid)	Corrosive
7	4-Amino-1,2,4-triazole	Not corrosive
8	Eugenol	Not corrosive
9	Phenethyl bromide	Not corrosive
10	Tetrachloroethylene	Not corrosive
11	Isostearic acid	Not corrosive
12	4-(Methylthio)-benzaldehyde	Not corrosive

8N KOH - used as positive control  
Deionized H<sub>2</sub>O - used as negative control

##### Prediction model

tissue viability (%)	prediction
3 min: < 50	corrosive (C)
3 min: ≥ 50 and 1 hr: < 15	corrosive (C)
3 min: ≥ 50 and 1 hr: ≥ 15	non-corrosive (NC)

Prediction model for low concentrated aqueous solutions of inorganic acids:\*

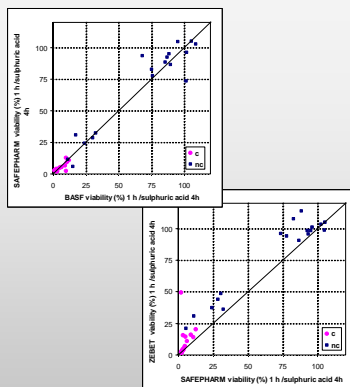
tissue viability (%)	prediction C / NC
3 min: < 50	corrosive (C)
3 min: ≥ 50 and 1 hour: < 15	corrosive (C)
3 min: ≥ 50 and 4 hours < 15	corrosive (C)
3 min: ≥ 50 and 4 hours: ≥ 15	non-corrosive (NC)

\* Since the surface of SkinEthic HRE is very hydrophobic, spreading and permeation of aqueous solutions of inorganic acids is difficult. Therefore prolongation of exposure to four hours is necessary.

#### TER, EpiDerm™, and EPISKIN™ PERFORMANCE in the ECVAM studies (1)(2) :

Chemical	TER (1)						EPISKIN (1)									EpiDerm - phase II and III (2)						
	LAB 1		LAB 2		LAB 3		LAB 1			LAB 2			LAB 3			LAB 1		LAB 2		LAB 3		
	I	II	I	II	I	II	I	II	III	I	II	III	I	II	III	I	II	I	II	I	II	
Acrylic acid																						
1,2-Diaminopropane	R35	R35	R35	R35	R35	R35	R34	R34	R34	R34	R34	R34	R34	R34	R34	C	C	C	C	C	C	C
2-tert-Butylphenol	NC	R35	NC	R35	R35	R35	R34	R34	R34	R34	R34	R34	R34	R34	R34	C	NC	C	C	C	C	C
Potassium hydroxide (10% aq.)	R35	R35	R35	R35	R35	R35	R34	R34	R34	R34	R34	R34	R34	R34	R34	R35	C	C	C	C	C	C
Sulphuric acid (10% wt.)	R34	R34	NC	R34	R34	R34	R34	R34	R34	R34	R34	R34	R34	R34	R34	C	C					
Octanoic acid (caprylic acid)	R35	R35	R35	R35	NC	NC	R34	R34	R34	R34	R34	R34	R34	R34	R34	C	C	C	C	C	C	C
4-Amino-1,2,4-triazole	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Eugenol	NC	R34	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Phenethyl bromide	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Tetrachloroethylene	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	R34	R34	R34	NC	NC	NC	NC	NC	NC
Isostearic acid	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
4-(Methylthio)-benzaldehyde	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC

#### INTERLABORATORY REPRODUCIBILITY



Example: mean tissue viability values obtained from testing the 12 reference chemicals in three independent tests with one hour exposure (4 hours for sulphuric acid 10 % wt.). Chemicals corrosive *in vivo* are shown as pink circles, non-corrosive chemicals are shown as blue squares.

#### CONCLUSION

⇒ Results obtained with SkinEthic HRE were reproducible, both within and between laboratories, and over time. The protocol was 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.

⇒ Concordance between the *in vitro* predictions of skin corrosivity potential obtained with SkinEthic HRE and *in vitro* predictions obtained with the accepted tests of OECD TG 430 and TG 431 were very good. The test was able to distinguish between corrosive and non-corrosive chemicals for all of the chemical types studied.

#### REFERENCES

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