

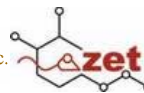
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Follow-up Validation of the EpiDerm Skin Irritation test

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Headlines

1. Background: history and outcomes of the ECVAM SIVS
2. EpiDerm protocol re-development and follow-up validation
3. Conclusion



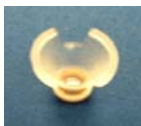
1. Background – ECVAM SIVS

- **ECVAM Skin irritation pre-validation (PV) study to replace the vivo rabbit test for hazard identification of chemicals (Fentem et al., 2000):**
5 methods (EPIKIN, EpiDerm SI tests included)
none successful.
- **Retrospective statistical analysis (Zuang et al., 2001):**
Although EpiDerm and EPIKIN tests used different test protocols the models provided **similar data**, when compared at **similar exposure times**



IDEA:

"...all well developed skin models have similar barrier properties and thus should provide similar results if the same protocol is used".



Skin Irritation Task Force therefore suggested, that **L'Oreal** (EPIKIN lead laboratory in the PV study) and **ZEBET** (EpiDerm lead laboratory in the PV study) should collaborate to develop "**common skin model protocol**".

- **Optimisation studies (Cotovio et al., 2005; Kandarova et al., 2005)**

EPIKIN protocol re-development (2001 – 2004):

exposure time 15 min, dose applied of 26,3 μl (mg)/ cm^2

post-incubation 42hours, endpoint MTT viability assay

(IL1, AK promising);

final EPIKIN protocol applied to EpiDerm (2003-2004)

(+ improvement of the technical procedures and increase of dose for EpiDerm model)

dose of 38,7 μl (mg)/ cm^2 : (other endpoints not investigated – due to lack of time)

Performance of optimised EpiDerm and EPIKIN tests:

45 samples (20 chemicals from pre-validation study + 25 chemicals from ECETOC database)

	<u>EpiDerm</u>	<u>EPIKIN</u>
Sensitivity:	70	85
Specificity:	88	80
Accuracy:	80	82

• **ECVAM Skin irritation validation study: 2004-2007 (Spielmann et al., 2007)**

Phase I: 20 Chemicals from New chemical database (NCD)

Difference between EpiDerm and EPISKIN: **1 chemical**

Phase II: 58 Chemicals (NCD, TSCA, ECETOC)

	EpiDerm		EPISKIN (MTT)	
	Specificity	Sensitivity	Specificity	Sensitivity
“Old” chemicals (ECETOC, TSCA)	87.5%	33.3%	79.2%	74.1%
“New” chemicals (NCD)	80.4%	70.8%	82.4%	75.0%
Overall	84.7%	56.3%	80.8%	74.7%

EPISKIN assay: validated, stand alone method (only MTT endpoint is fully validated)

EpiDerm assay: validated component of OECD 404 tiered strategy for identification of irritants.

NI result to be confirmed by another endpoint/assay,
until sensitivity will not be increased to match better the rabbit data

2. Increasing the sensitivity of the EpiDerm test protocol

Test protocol modification (Modified EpiDerm SIT):

- exposure extended from **15 min to 60 min**.
- part of the exposure at **37°C**
- application dose increased from **25 to 30 µl** (liquids)
- prediction model and post-exposure time not modified.

52 Chemicals tested at MatTek Corporation (2006-2008):

Chemicals from:

- **optimizations studies** (2001-2004) and **ECVAM SIVS validation study**
- **4h human patch tests** (Basketter et al., 2004)

Performance of the Modified EpiDerm SIT

(52 chemicals, 3 runs)

Sensitivity: 84% (53/63)

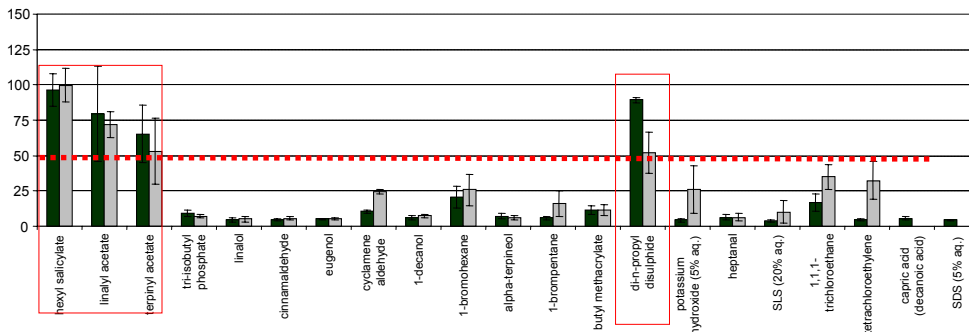
Specificity: 76% (71/93)

Results are similar to EPISKIN SIT (MTT assay)

■ EpiDerm - 60 min, 37°C

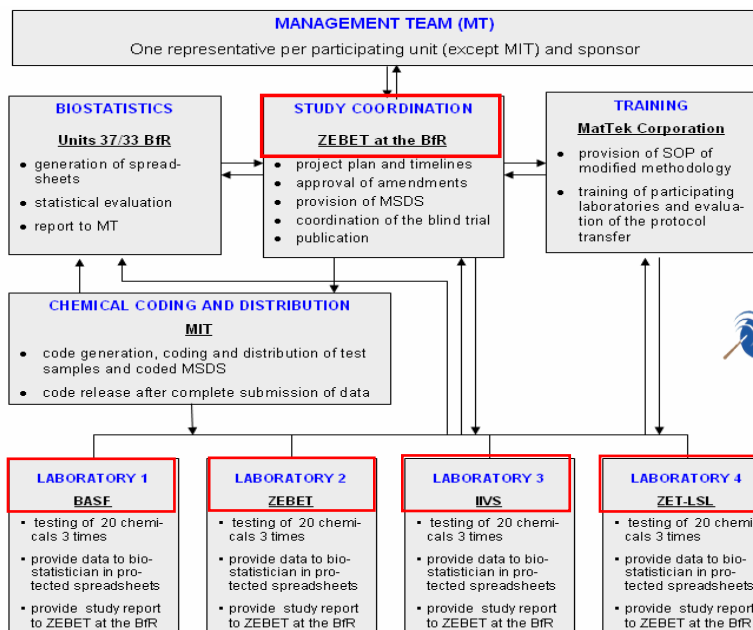
□ EPISKIN - 15 min, RT

21 In Vivo irritating chemicals (3 independent runs)



NI to human skin

3. Follow-up validation study: demonstration of inter-laboratory reproducibility



20 reference test chemicals - endorsed by ECVAM and PRP of the ECVAM SIVS for future validation studies

No	Chemical Name	CAS Number	EU label	GHS label	In vivo DIS
1	1-bromo-4-chlorobutane	6940-78-9	no	no	0
2	diethyl phthalate	84-66-2	no	no	0
3	di-propylene glycol	25265-71-	no	no	0
4	naphthalene acetic acid	86-87-3	no	no	0
5	allyl phenoxy-acetate	7493-74-5	no	no	0.3
6	isopropanol	67-63-0	no	no	0.3
7	4-methyl-thio-	3446-89-7	no	no	1
8	methyl stearate	112-61-8	no	no	1
9	allyl heptanoate	142-19-8	no	SLI	1.7
10	heptyl butyrate	5870-93-9	no	SLI	1.7
11	* hexyl salicylate	6259-76-3	R38	SLI	2
12	* terpinyl acetate	80-26-2	R38	SLI	2
13	tri-isobutyl phosphate	126-71-6	R38	SLI	2
14	* 1-decanol	112-30-1	R38	1	2.3
15	cyclamen aldehyde	103-95-7	R38	1	2.3
16	1-bromohexane	111-25-1	R38	1	2.7
17	* a-terpineol	98-55-5	R38	1	2.7
18	* di-n-propyl disulphide	629-19-6	R38	1	3
19	* butyl methacrylate	97-88-1	R38	1	3
20	heptanal*	111-71-7	R38	1	4 (3,3,5)

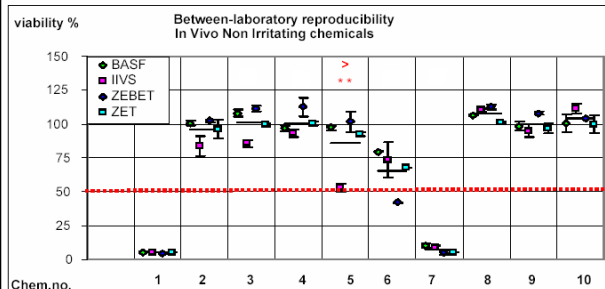
Rabbit Non Irritants (EU)

Rabbit Irritants (EU)

DIS – dominating median irritation score according to Hoffman et al., 2005 (44).

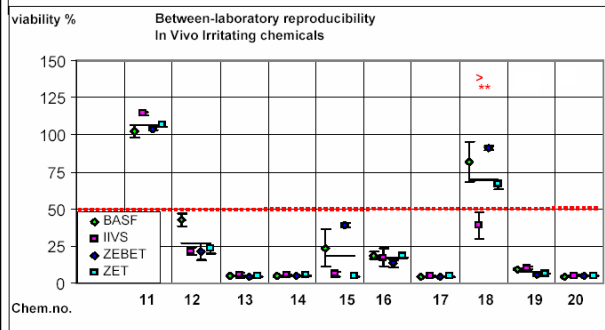
* Known to be NI to human skin (Basketter et al., 2004; Jirova et al., 2007)

Between-laboratory reproducibility of the 20 chemicals – MTT data



Laboratory	Sensitivity [%]	Specificity
BASF	73.3	80.0
ZEBET	76.7	73.3
IIVS	90.0	76.7
ZET	80.0	80.0
Overall	80.0	77.5

- reproducible
- high levels of sensitivity and specificity



EPISKIN (as benchmark test)
provides for this dataset
Sensitivity: **70 %**
Specificity: **80 %**

Comparison between EpiDerm and EPISKIN SIT performance (single laboratories, 3 runs)

Table 11: Comparison of the predictive performance of the Modified EpiDerm SIT and EPISKIN SIT (MTT).

No	Chemical Name	EU class	EpiDerm					EPISKIN			
			BASF	ZEBET	IIVS	ZET	Over all	Lab A	Lab B	Lab C	Over all
1	1-bromo-4-chlorobutane	no	R38	R38	R38	R38	R38	R38	R38	R38	R38
2	diethyl phthalate	no	no	no	no	no	no	no	no	no	no
3	di-propylene glycol	no	no	no	no	no	no	no	no	no	no
4	naphthalene acetic acid	no	no	no	no	no	no	no	no	no	no
5	allyl phenoxy-acetate	no	no	no	no	no	no	no	no	no	no
6	isopropanol	no	no	R38	no	no	no	no	no	no	no
7	4-methyl-thio-benzaldehyde	no	R38	R38	R38	R38	R38	no	R38	R38	R38
8	methyl stearate	no	no	no	no	no	no	no	no	no	no
9	allyl heptanoate	no	no	no	no	no	no	no	no	no	no
10	heptyl butyrate	no	no	no	no	no	no	no	no	no	no
11	hexyl salicylate	R38	no	no	no	no	no	no	no	no	no
12	terpinyl acetate	R38	R38	R38	R38	R38	R38	no	R38	no	no
13	tri-isobutyl phosphate	R38	R38	R38	R38	R38	R38	R38	R38	R38	R38
14	1-decanol	R38	R38	R38	R38	R38	R38	R38	R38	R38	R38
15	cyclamen aldehyde	R38	R38	R38	R38	R38	R38	R38	R38	R38	R38
16	1-bromohexane	R38	R38	R38	R38	R38	R38	R38	R38	R38	R38
17	a-terpineol	R38	R38	R38	R38	R38	R38	R38	R38	R38	R38
18	di-n-propyl disulphide	R38	no	no	R38	no	no	no	R38	no	no
19	butyl methacrylate	R38	R38	R38	R38	R38	R38	R38	R38	R38	R38
20	heptanal*	R38	R38	R38	R38	R38	R38	R38	--	--	R38

* tested only in the optimization studies in one laboratory (1,8); bold and blue: classified false negative or false positive with the MTT endpoint)

• Conclusion

- **Modified 60 min/42 h EpiDerm SIT provides comparable classifications as seen in the rabbit test and validated EPISKIN assay,**
- The test was **transferable** to “naïve” laboratory (ZET-LSL) as well as to experienced laboratories (BASF, IIVS, ZEBET),
- Modified 60 min/42h EpiDerm SIT proved to be **reproducible amongst laboratories and over time**,
- modified EpiDerm SIT provides **high level of sensitivity**. None of known human irritant was missed by the modified test,
- **The study was submitted on 23. 04. 2008 to ECVAM/Correlate as full replacement of the in vivo rabbit test (EU classification system)**

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Thank you for your attention !!!

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