Overview of Skin Irritation and Corrosion Test Methods and Strategies

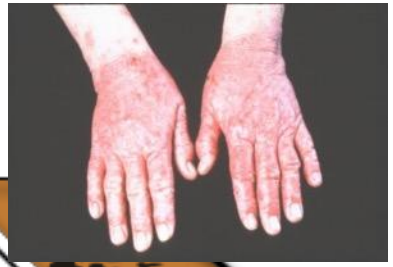
Hans Raabe, M.S.
Chief Operating Officer
Institute for In Vitro Sciences, Inc.

IIVS
Institute for In Vitro Sciences
Advancing Science & Animal Welfare Together

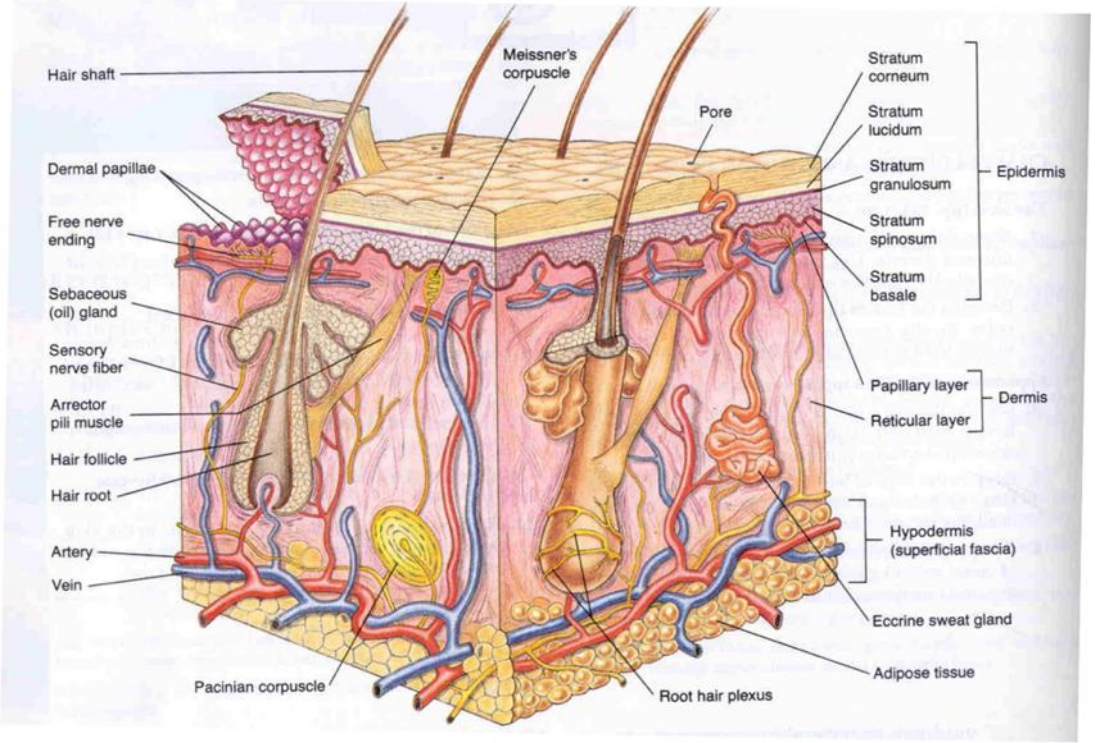
Overview

- What are the goals of a testing program?
- Modeling the physiology of human skin
- Reconstructed human epidermal models
  characteristics and limitations
- Test procedures and skin toxicology endpoints

Protecting our primary line of defense

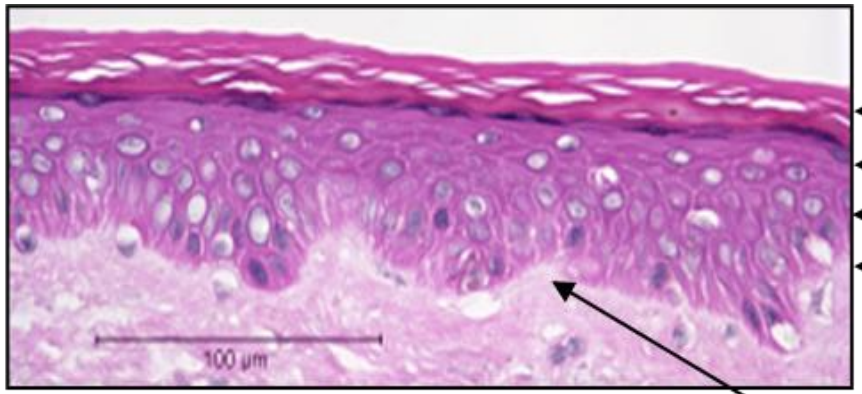


Human Skin Physiology



- Primary physical barrier against mechanical, chemical and microbial insults
- Control loss of systemic hydration
- Immune network
- Unique defense system against UV irradiation

Human Skin Physiology



Native human skin

Stratum corneum

Stratum granulosum

Stratum spinosum

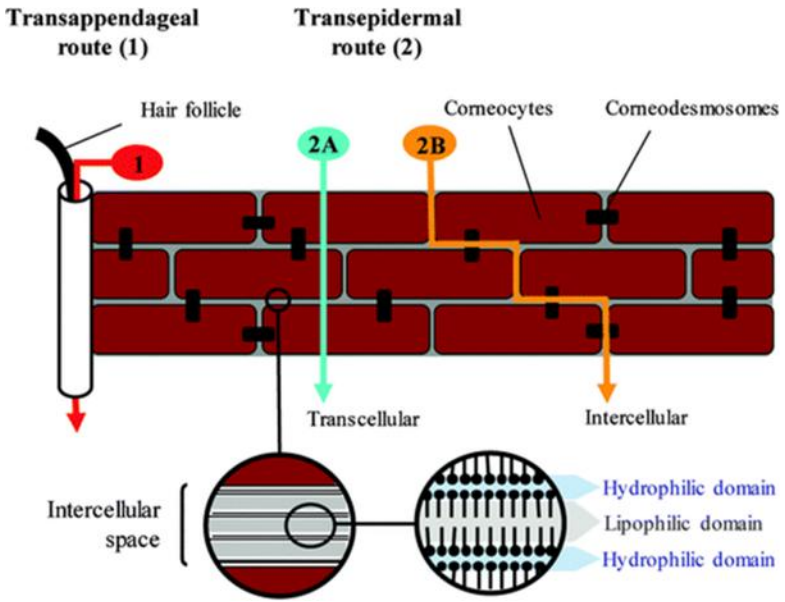
Stratum basale - layer of proliferative keratinocytes

Dermal papillae – high surface area interactions with epidermis

Image courtesy of Institute for In Vitro Sciences (IIVS)

Skin Physiology

"Bricks and mortar"



Hadgraft J, Lane ME. Skin: the ultimate interface. Phys Chem Chem Phys. 2011 Mar 28;13(12):5215-22. doi: 10.1039/c0cp02943b. Epub 2011 Feb 24. PMID: 21350740.

Intercellular lipid arrangement

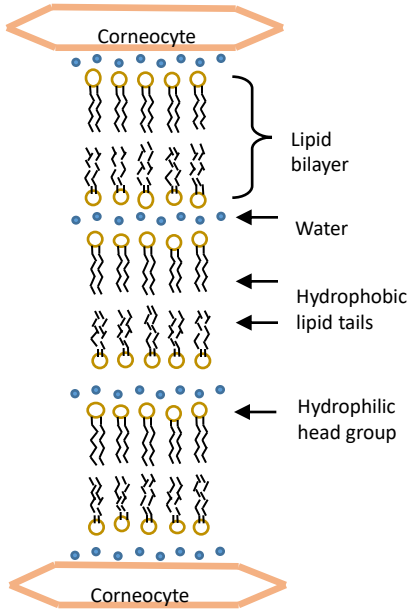
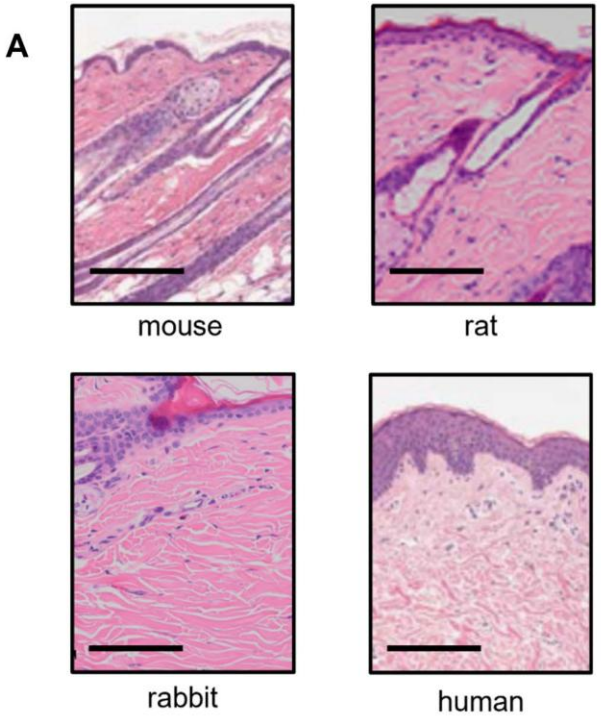


Image courtesy of Institute for In Vitro Sciences (IIVS)

Species and Model Comparisons

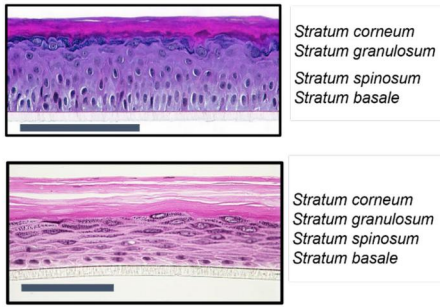
Mammalian / human skin sections



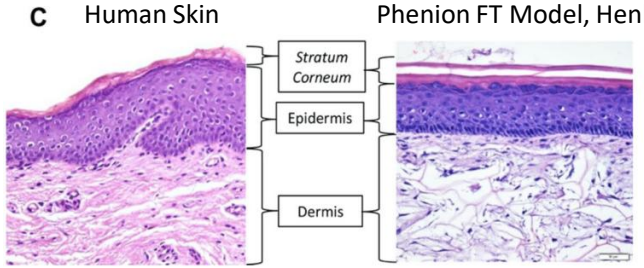
Human cell-based epidermal models

SkinEthic™ RHE, EPISKIN

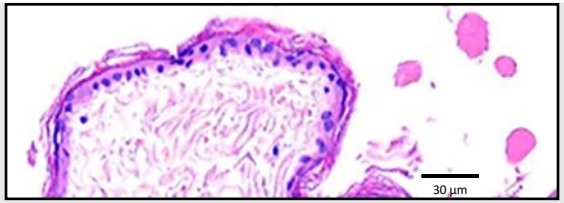
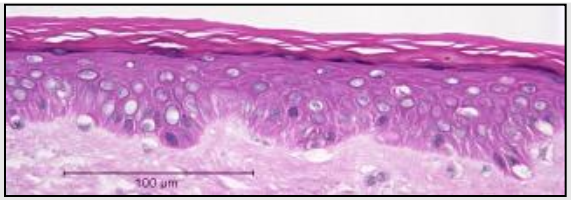
EpiDerm™, MatTek



Human cell-based full thickness model

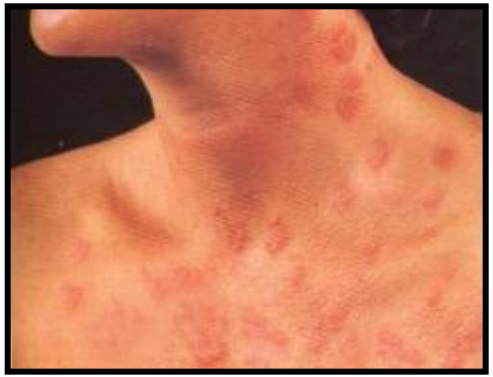


Species differences between human and rabbit skin

Characteristic	Rabbit Skin	Human Skin
Structure	Limited to three strata: *stratum basale,* *s. spinosum, and* *s. corneum*	Five distinct functional layers: *stratum basale,* *s. spinosum*, *s. granulosum*, *s. lucidum, and* *s. corneum*
Histology	 image modified from Uhm, Jeong, Lee, et al. (2023) Toxicol Res. 39, 477–484 (2023)	 image courtesy of Institute for In Vitro Sciences
Thickness	Total Skin: 1.21 ± 0.04 mm Epidermis: 0.03 mm Dermis: 1.18 mm	Total Skin: 3 mm Epidermis: 0.050 mm Dermis: 2 - 4 mm (~1 mm for eye lids)
Hair follicle density	4 to 5 follicles per mm^2	0.2 to 0.3 follicles per mm^2 (arms and legs)
Skin barrier functional composition	(–) filaggrin (FLG) (–) claudin (CLDN1) (–) E-cadherin (CDH1)	(+) filaggrin (FLG) (+) claudin (CLDN1) (+) E-cadherin (CDH1)

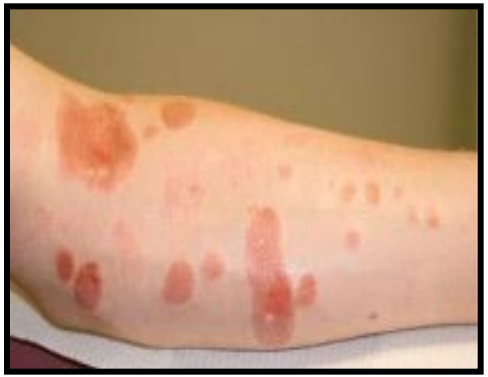
The impact of substances on human skin

Skin Irritation



- **Reversible damage** of the skin following exposure to a chemical or chemical mixture
- Characterized macroscopically by erythema (redness) and oedema
- Damage to keratinocytes and dermal cells leads to inflammation

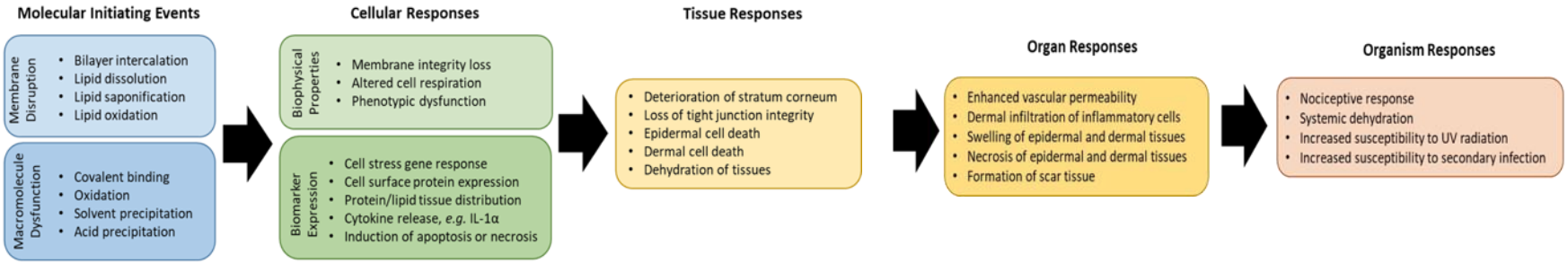
Skin Corrosion



- **Irreversible damage** of the skin following exposure to a chemical or chemical mixture
- Visible necrosis through the epidermis and into dermis - macroscopically typified by ulcers, bleeding, sloughing of epidermis, etc.
- Likely scar formation

http://www.survivingdisasters.info/emergency-first-aid/c/chemical-burn

Biological basis for skin irritation / corrosion



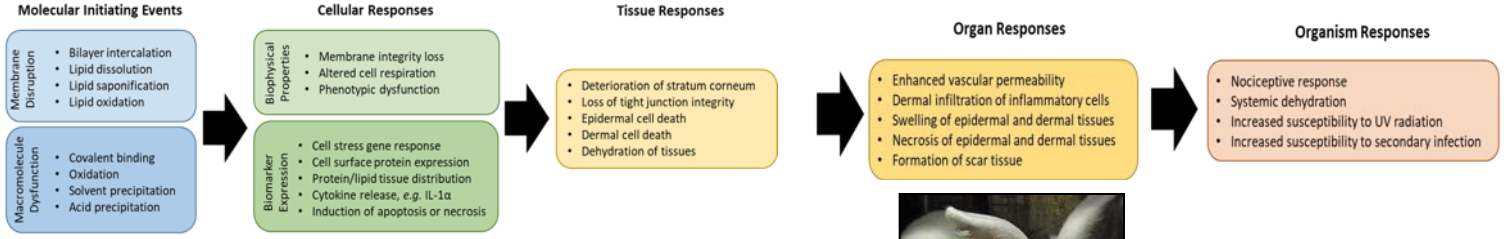
Generalized mechanisms of skin irritation and corrosion in mammals. Key events upstream of erythema and edema that can be measured quantitatively include cytotoxicity, tissue dehydration, and cytokine release

Dermal exposure to chemicals, mixtures, and formulations can lead to a wide range of adverse responses

To affect the underlying epidermal keratinocytes or fibroblasts (and other cells), chemicals must first be able to breach or damage the outermost epidermal barrier layer (*stratum corneum*)

- Corrosive chemicals are both highly cytotoxic and can rapidly penetrate deep into the dermis. Consequently, severe reactions result in irreversible epidermal and dermal necrosis and subsequent scar formation
- Irritant chemicals may be less cytotoxic and / or less likely to permeate beyond the epidermis. Mild to moderate inflammation reactions are observed such as transient erythema and edema
- Cellular stress and cell death throughout the epidermis and dermis are key to the adverse outcomes

Draize Test



Animals: 1-3 rabbits (sequential testing)

Test substance: 0.5 mL or 0.5 g of usually undiluted liquid or moistened solid test substance applied on 6 cm² skin surface

Exposure and Observations: 3 minutes, 1 hour or 4 hours (addresses skin corrosion)
4 hours and above (addresses skin irritation)

Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4

Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges or area well defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Draize J. H., Woodard G., Clavery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes, J. Pharmacol. Exp. Ther., 82, 377-390 (1944).

Registration of Substances using the rabbit Draize skin irritation test

UN GHS: Reaction Score

Average of erythema or oedema in at least
2 of 3 animals over 24, 48 and 72 hours

US EPA: Primary Dermal Irritation Index (PDII)

Sum of erythema (1 / 24 / 48 / 72 hours) + Sum of oedema (1 / 24 / 48 / 72 hours)

4 intervals (1 / 24 / 48 / 72 hours) x no. of animals

US EPA	Hazard Category			
	I	II	III	IV
PDII	Corrosive	>5.0	2.1-5.0	0-2
Irritation Potential		Severely Irritating	Moderately Irritating	Slightly Irritating
Signal Word	DANGER	WARNING	CAUTION	CAUTION

http://www2.epa.gov/sites/production/files/2014-07/documents/chapter7_revised_final_0714.pdf.

Evaluating the Reliability of the Draize Test

Regulatory Toxicology and Pharmacology 122 (2021) 104920

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Analysis of variability in the rabbit skin irritation assay

John P. Rooney [a,*], Neepa Y. Choksi [a], Patricia Ceger [a], Amber B. Daniel [a], James Truax [a], David Allen [a], Nicole Kleinstreuer [b]

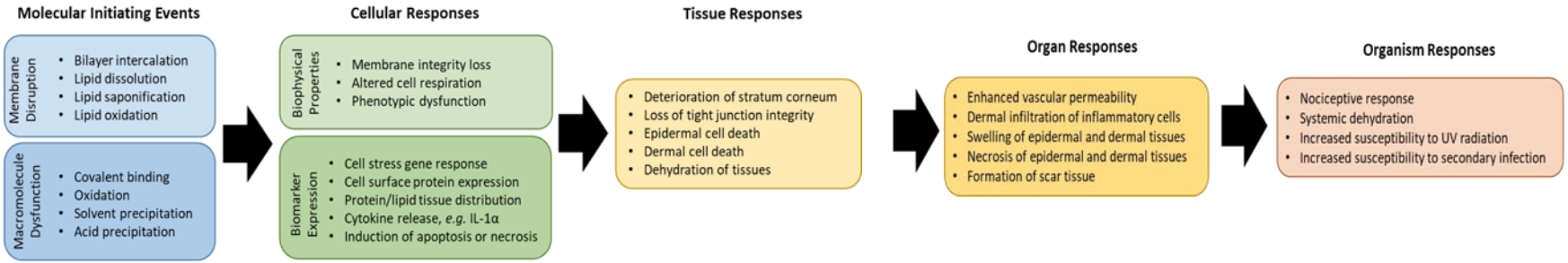
[a] Integrated Laboratory Systems, LLC, 601 Keystone Park Dr, Suite 800, Morrisville, NC, 27560, USA
[b] National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, National Institutes of Environmental Health Sciences, Research Triangle Park, NC, 27709, USA

- Cat. 1 substances had an 86.2% probability of receiving the same classification when tested multiple times.
- Cat. 2 substances had a 63.6% chance of retesting as Cat. 2 and a combined 23.5% chance of being classified as a Cat. 3 or NC upon repeat testing.
- Cat. 3 substances had a 45.2% chance of retesting as Cat. 3, a 28.6% chance of testing at Cat. 2, and a 23.8% chance of testing at NC.
- NC substances had a 92.1% probability of receiving the same classification when tested multiple times.

Table 1
Conditional probabilities for the 'Full EPA' (A), 'Clean EPA' (B), 'Curated EPA' (C), and 'GHS' (D) datasets.

A. Full EPA Data Set					
Prior Result	I	II	III	IV	Total
I	76.0%	8.0%	8.5%	7.5%	313
II	12.0%	28.1%	35.3%	24.6%	89
III	5.8%	5.0%	43.5%	45.7%	357
IV	2.2%	1.9%	11.6%	84.4%	1672
B. Clean EPA Data Set					
Prior Result	I	II	III	IV	Total
I	87.5%	1.9%	7.5%	3.1%	140
II	17.4%	47.8%	8.7%	26.1%	11
III	4.2%	0.8%	42.5%	52.5%	110
IV	0.8%	0.8%	9.7%	88.8%	576
C. Curated EPA Data Set					
Prior Result	I	II	III	IV	Total
I	86.3%	4.2%	7.1%	2.5%	207
II	14.1%	44.9%	20.5%	20.5%	35
III	6.9%	5.2%	53.6%	34.3%	133
IV	0.9%	2.0%	9.1%	88.0%	690
D. GHS Data Set					
Prior Result	Cat 1	Cat 2	Cat3	NC	Total
Cat 1	86.2%	7.7%	0.3%	5.7%	257
Cat 2	13.0%	63.6%	5.9%	17.6%	152
Cat3	2.4%	28.6%	45.2%	23.8%	19
NC	2.2%	4.2%	1.4%	92.1%	781

Biological basis for skin irritation / corrosion



Generalized mechanisms of skin irritation and corrosion in mammals. Key events upstream of erythema and edema that can be measured quantitatively include cytotoxicity, tissue dehydration, and cytokine release

Image courtesy of Institute for In Vitro Sciences (IIVS)

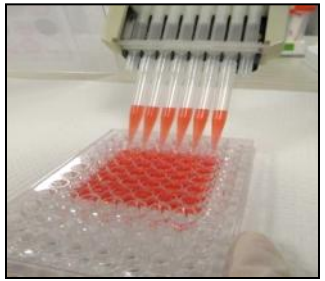


Image courtesy of Institute for In Vitro Sciences (IIVS)

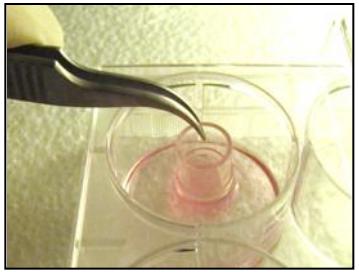


Image courtesy of Institute for In Vitro Sciences (IIVS)

Evaluation of skin corrosion and irritation potential using *in vitro* assays validated for regulatory purposes

Membrane Barrier Test Method (Corrositex®) (OECD TG 435)

Membrane Barrier Test Method (Corrositex®) (OECD 435)

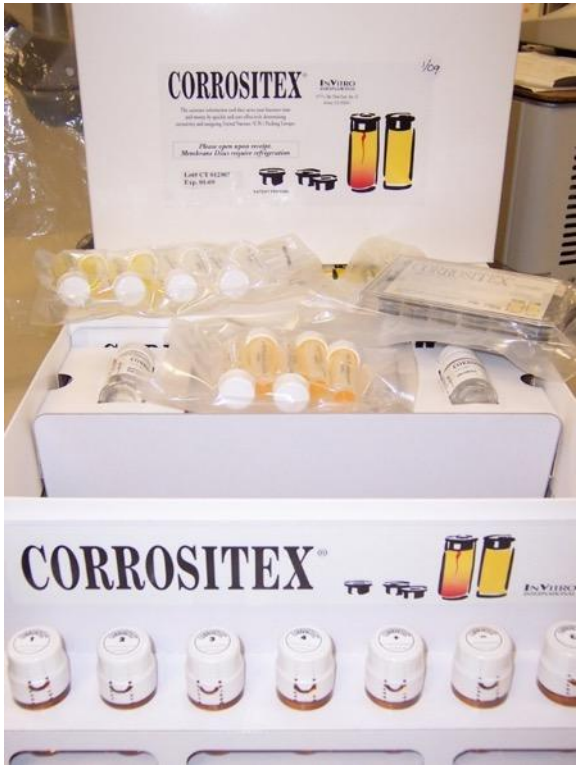
Brief overview and current regulatory status

- **Test system:** Artificial membrane designed to respond to corrosive substances in a manner similar to animal skin *in situ*
- **Assay endpoint:** The time (in minutes) required for a test substance to penetrate through the Corrositex Biobarrier Membrane and produce a color change in the Chemical Detection System (CDS)
- **Assay controls:** Negative (10% citric acid, 5% propionic acid); Positive (sodium hydroxide)

- **Applicability:** Assigns UN Packing Group to corrosives or verifies if a test substance is non-corrosive
- **Limitations:** Materials with a pH of ≥ 4.5 and ≤ 8.5 generally fail to qualify for testing based on the CDS used in the kit provided by In Vitro International

---

- **Regulatory status:** OECD Test Guideline 435 (TG 435, updated 2015)



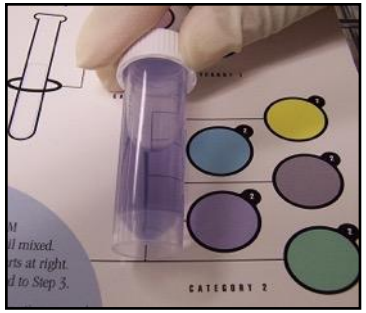
Technical Notes

Pre-assay considerations

Q: Does the test substance qualify for the assay?

- Test substance is added to a tube containing Chemical Detection System (CDS).
- Materials with a pH of ≥ 4.5 and ≤ 8.5 generally fail to qualify for testing.

Q: Which time-monitor protocol should be used?



- Test substance is added to two different buffer tubes to select the appropriate timetable and protocol
- “Category 1” test substances are tested for up to 4 hours (for strong acid / base)
- “Category 2” test substances are tested for up to 1 hour (for weak acid / base)

Assay conduct considerations

Assay setup: ensure bright white lighting and a white background to allow timely visualization of color change critical for data interpretation and classification

Red stream is evidence of chemical breaking through biobarrier

Images courtesy of Institute for In Vitro Sciences (IIVS)

Corrositex®: Typical Protocol

Matrix Powder Solution

Biobarrier Preparation

Biobarrier Placement

Break Through Observations

Prepare the biobarrier membrane: biobarrier matrix powder is completely solubilized with the biobarrier diluent at 64-68°C. The solubilized biomatrix is added to a membrane disc containing the porous cell membrane.

To initiate testing, place each biobarrier onto a vial containing CDS

Add the test substance onto four replicate biobarrier membranes and continuously monitor the CDS for the first 10 min.

Observe vials until a color change occurs

Once a color change occurs in all four vials, the mean break through times are calculated

Images courtesy of Institute for In Vitro Sciences (IIVS)

"Category" 1 Protocol

Mean Time to Produce a Change in Chemical Detection System	Packing Group
≤ 3 Minutes	I
> 3 Minutes - 1 Hour	II
> 1 - 4 Hours	III
> 4 Hours	Not Applicable

"Category 2" Protocol

Mean Time to Produce a Change in Chemical Detection System	Packing Group
≤ 3 Minutes	I
> 3 Minutes - 30 minutes	II
> 30 - 60 minutes	III
> 60 minutes	Not Applicable

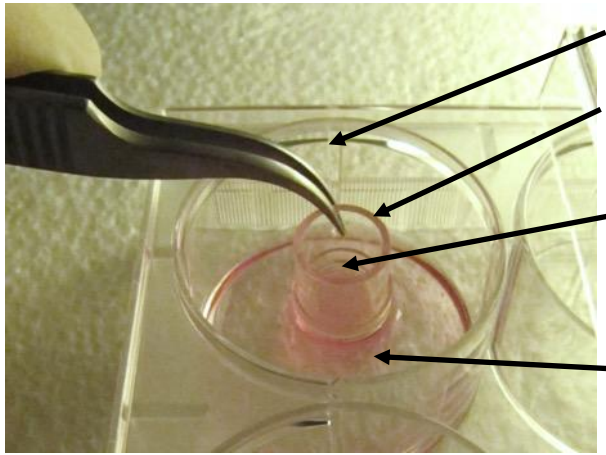
Sensitivity	Specificity	False negative rate	False positive rate	Packing Group Accuracy
89%	75%	11%	25%	96%

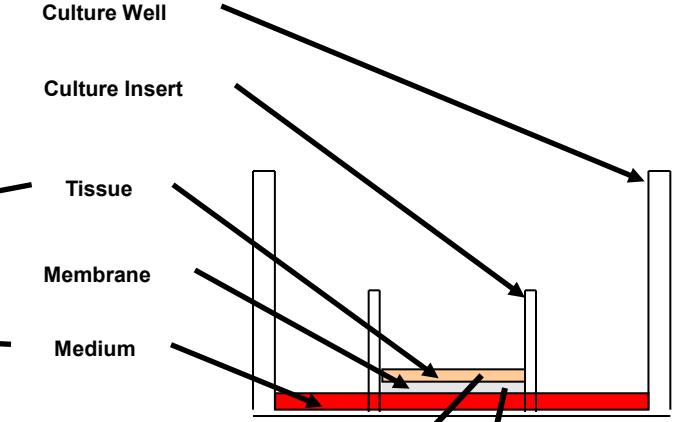
Evaluation of skin corrosion and irritation potential using *in vitro* assays validated for regulatory purposes

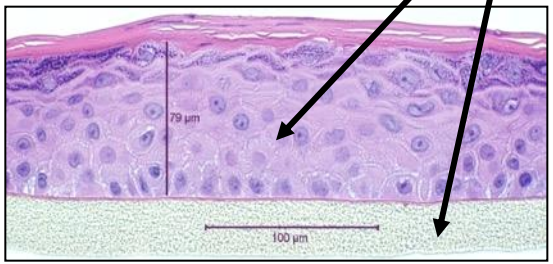
RhE Test Method – Skin Corrosion Assay (OECD TG 431)

Reconstructed Human Epidermal Model

cultured at Air / Liquid Interface





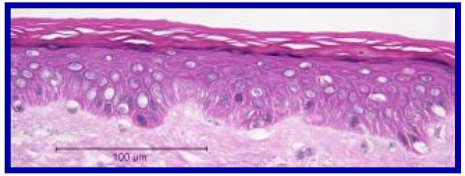


Images courtesy of Institute for In Vitro Sciences (IIVS)

In Vitro Reconstructed Human Epidermis (RhE) Models Validated for Regulatory Purposes

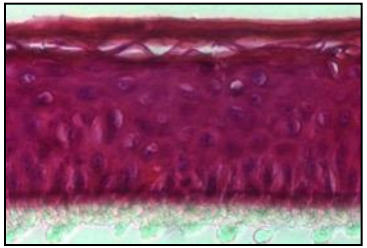
General model criteria

- Viable human keratinocytes
- Distinct differentiated layers
- *Stratum corneum* provides barrier function
- RhE model vendors ensure consistent batches

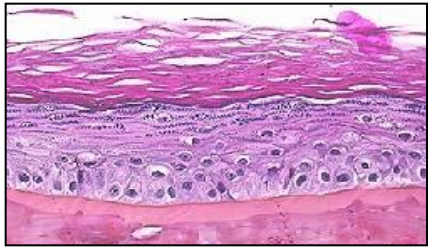


Native human skin

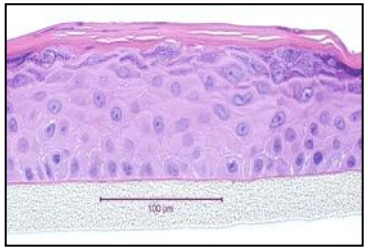
epiCS®



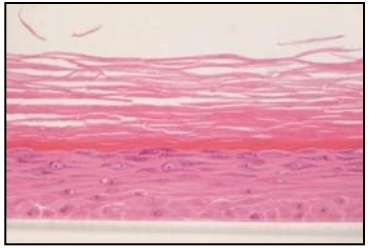
EpiSkin™ (SM)



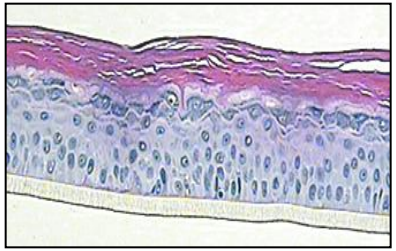
EpiDerm™ (EPI-200)



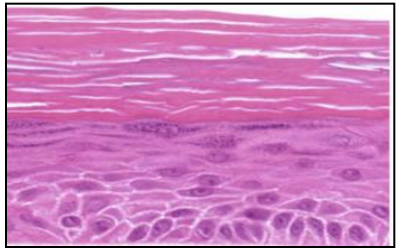
LabCyte EPI-MODEL



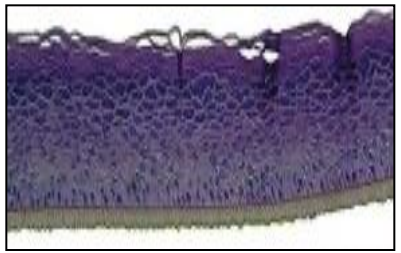
SkinEthic™ RHE



KeraSkin™



Skin+®

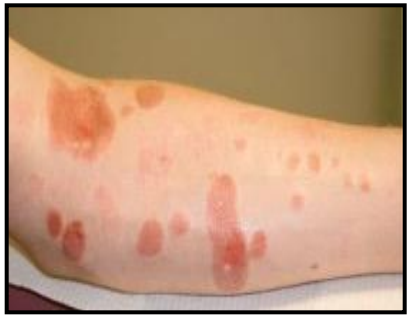


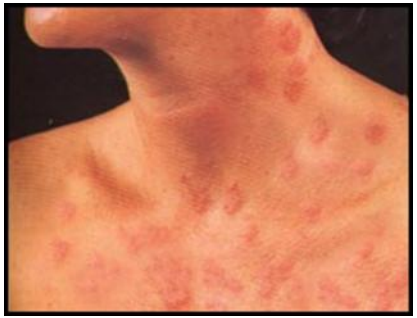
Time-to-Toxicity Concept

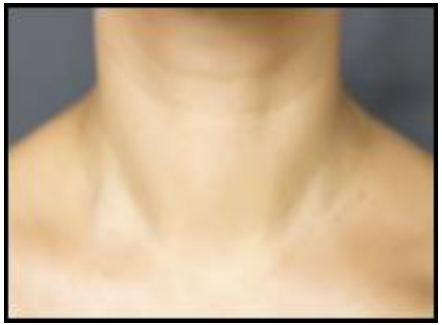
In Vivo Outcomes

Time-to-Onset of Clinical Observations
(approximated to Draize scale)

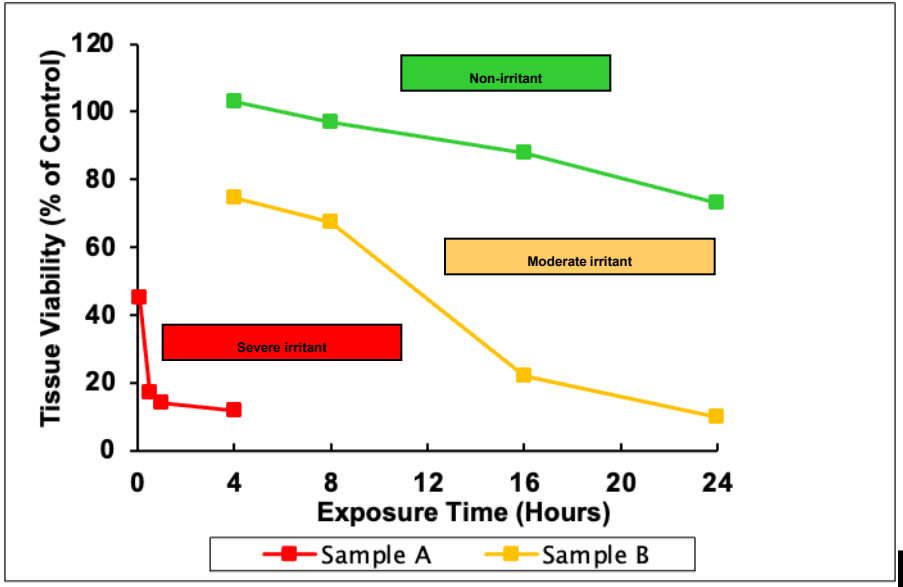
3 min	60 min	4 hrs	8 hrs	1 day	3 days	7+ days
Corrosive	Severe		Moderate		Mild to Non-irritant	
				Personal Care		Cosmetics
			Household Cleaning Products			
		Industrial Chemicals				







Time-to-Toxicity Concept Applied In Vitro



Costin, G.-E. et al. In Vitro Safety Testing Strategies for Skin Irritation Using 3D Reconstructed Human Epidermis Models. Rom. J. Biochem. 46 (2), 149-163 (2009).

MatTek Corporation MTT Effective Time-50 (ET-50) Protocol For Use with Epiderm™ Skin Model (EPI-200) (09/06/05).

MatTek suggests the following as guidance for assigning verbal descriptors for expected in vivo irritation based on the ET_{50} scores using the EpiDerm (EPI-200) model.

Chemical	Expected *in vivo* irritation	ET_{50} (hrs)
Concentrated nitric acid	Strong/severe, possibly corrosive	<0.5
1% Sodium Dodecyl Sulfate	Moderate	0.5-4
1% Triton X-100	Moderate to mild	4-12
Baby shampoo	Very mild	12-24
10% Tween 20	Non-irritating	24

RhE Test Method - Skin Corrosion Assay (OECD TG 431)

Brief overview and current regulatory status

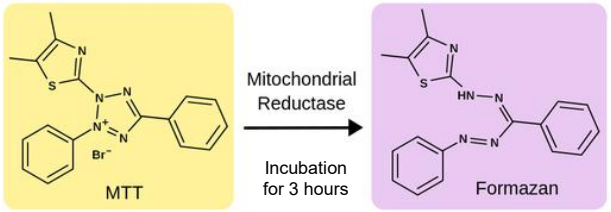
- **Test system:** RhE models: [EpiDerm™ (EPI-200); EpiSkin™ (SM) (discontinued); SkinEthic™ RHE; epiCS®; LabCyte EPI-MODEL 24 SCT]
- **Assay endpoint:** Tissue viability (%): assessed by reduction of the vital dye MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) by viable cells
- **Assay controls:** Negative: (sterile, deionized water or NaCl solution 9g/L)
  Positive: (8N KOH or glacial acetic acid – only for 4 hr exposure)

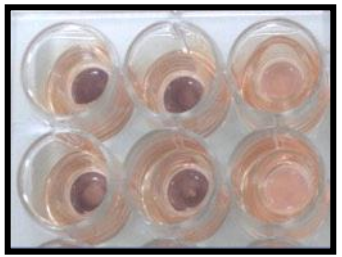
……

- **Applicability:** The results can be used for regulatory purposes for distinguishing corrosive from non-corrosive test substances.
  The method also allows for sub-categorization, *i.e.*, 1A vs. 1B-and-1C vs. non-corrosive test substances.
- **Limitations:** The method does not allow discriminating between skin corrosive sub-categories 1B and 1C according to the UN GHS due to a limited set of well-known *in vivo* corrosive Category 1C chemicals.

……

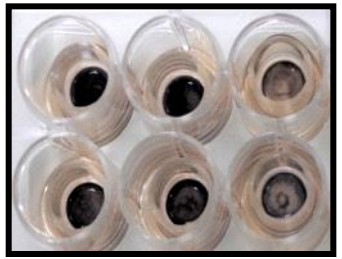
- **Regulatory status:** OECD Test Guideline 431 (updated 2025 to remove EpiSkin)





After 10-15 minutes

high viability low viability



After 3 hours

Images courtesy of Institute for In Vitro Sciences (IIVS)

Skin Corrosion Protocol – OECD Test Guideline 431

Tissue Receipt

Upon receipt, incubate tissues for at least 1 hour at standard culture conditions

(37±1^{0}C , 5±1% CO_2 , 80+% RH)

Tissue Treatment

Refresh medium after 1 hour

Tissues are treated topically with test substances for 3 min, 1 hour and 4 hours)

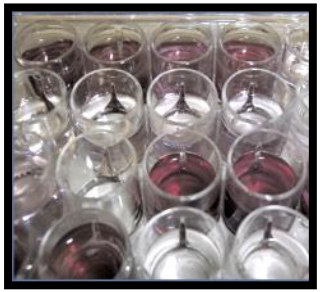
Tissue Rinsing

Rinse tissues after exposures to fully remove the control and test substances

MTT Reduction

Individual tissues are placed into wells containing MTT solution and incubated at standard culture conditions for 3 hours

Isopropanol Extraction



The tissues are placed in isopropanol at RT for 2 hours to extract the reduced MTT

Extracted MTT is thoroughly mixed and transferred to a 96-well plate

Spectrophotometric Quantification

Optical density (OD) at 570 nm (OD_{570}) is determined using a 96-well plate reader

OD values are used to calculate relative viability values relative to negative control tissue values

Images courtesy of Institute for In Vitro Sciences (IIVS)

RhE-Corrosion: Prediction Models

EpiSkin™ (SM) (discontinued)

Viability measured after exposure time points (3, 60 and 240 minutes)	Prediction to be considered UN GHS Category
< 35% after 3-minutes exposure	Corrosive: • Optional Sub-category 1A
≥ 35% after 3-minutes exposure **AND** < 35% after 60-minutes exposure **OR** ≥ 35% after 60-minutes exposure **AND** < 35% after 240-minutes exposure	Corrosive: • A combination of optional Sub-categories 1B and 1C
≥ 35% after 240-minutes exposure	Non-corrosive

EpiDerm™ (EPI-200)
SkinEthic™ RHE
epiCS®
LabCyte-EPI-MODEL24-SCT

Viability measured after exposure time points (3 and 60 minutes)	Prediction to be considered UN GHS Category
STEP 1	
< 50% after 3-minutes exposure	Corrosive
≥ 50% after 3-minutes exposure **AND** < 15% after 60-minutes exposure	Corrosive
≥ 50% after 3-minutes exposure **AND** ≥ 15% after 60-minutes exposure	Non-corrosive
STEP 2	
<25%; **18**%; **15**% after 3-minutes exposure	Optional Sub-category 1A
≥25%; **18**%; **15**% after 3-minutes exposure	A combination of optional Sub-categories 1B-and-1C

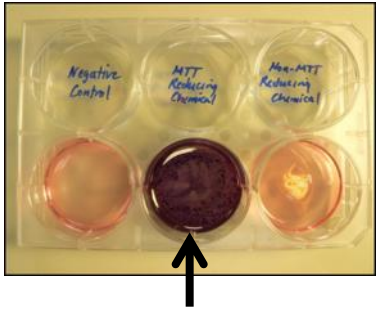
Desprez B., Barroso J., Griesinger C., Kandarova H., Alepee N., Fuchs H.W., Two novel prediction models improve prediction of skin corrosive sub-categories by test methods of OECD, Toxicology in Vitro, 29, 2055-2080 (2015) .

Technical Notes (Corrosivity Assay)

Pre-assay considerations

Q: Does the test substance reduce MTT directly?

Note: test needed to assess any interference of the test substance with the assay endpoint



Direct MTT reduction by test substance
Utilize freeze-killed tissue controls

Q: Is the test substance a **colorant** in the extractant?

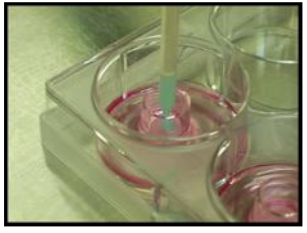
Test substance + isopropanol extractant → OD_{570}

If OD_{570} value >0.08, utilize colorant control tissues (viable tissues without incubation in MTT)

Assay conduct considerations

Specific dosing procedures for various test substance physical forms

Liquids

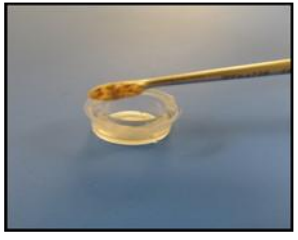


(positive displacement pipette)

Viscous

(dosing device)

Solids



(25 mg spoon)

Images courtesy of Institute for In Vitro Sciences (IIVS)

Evaluation of skin corrosion and irritation potential using *in vitro* assays validated for regulatory purposes

RhE Test Method – Skin Irritation Test (SIT – OECD TG 439)

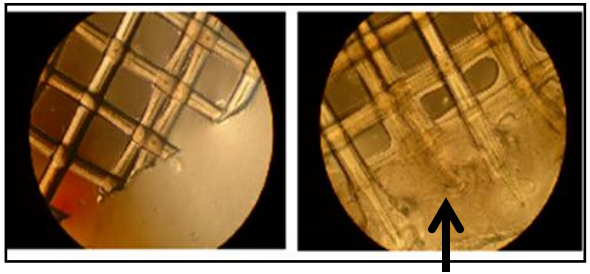
Technical Notes (specific to OECD 439)

Pre-assay considerations

Liquid test substances

Q: Does the test substance interact with the mesh?

Note: nylon mesh is needed to ensure spreading of the low volume of the test substance (30 µL) over the entire tissue surface



If interaction with the mesh is noted, no mesh is to be used

Assay conduct considerations

Rinsing of test substance / post-treatment incubation

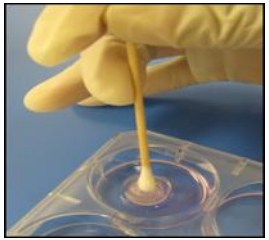
Rinse

(specific procedure)

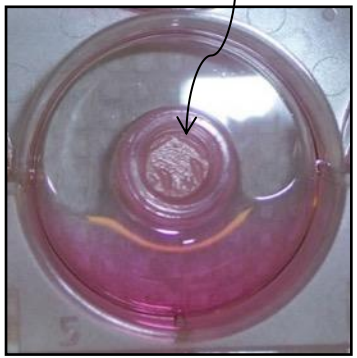
Observation

(residual test substance, any other unusual aspects of the tissues – blisters)

Removal of test substance residues



(careful handling not to affect the tissue; residues remaining on the tissues may over-predict irritation potential due to the long 42 hr post-exposure period)



Skin Irritation Test (SIT) – OECD Test Guideline 439

Tissue Receipt

Tissues are incubated in standard culture conditions

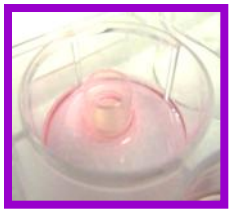
(37±1°C in humidified air containing 5±1% CO_2)

Tissue Treatment

Tissues are treated topically with control and test substances

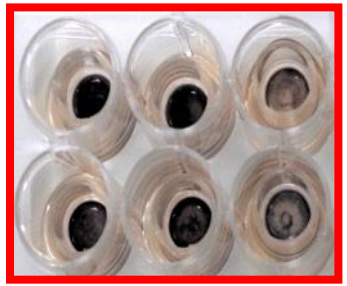
Tissue Rinsing

Post-treatment Expression Incubation



After exposure, tissues are rinsed and then placed in the incubator at standard culture conditions for a post-treatment incubation of 42±2 hours (to express delayed effects of test substances on the tissues)

MTT Reduction



Isopropanol Extraction

Spectrophotometric Quantification

Images courtesy of Institute for In Vitro Sciences (IIVS)

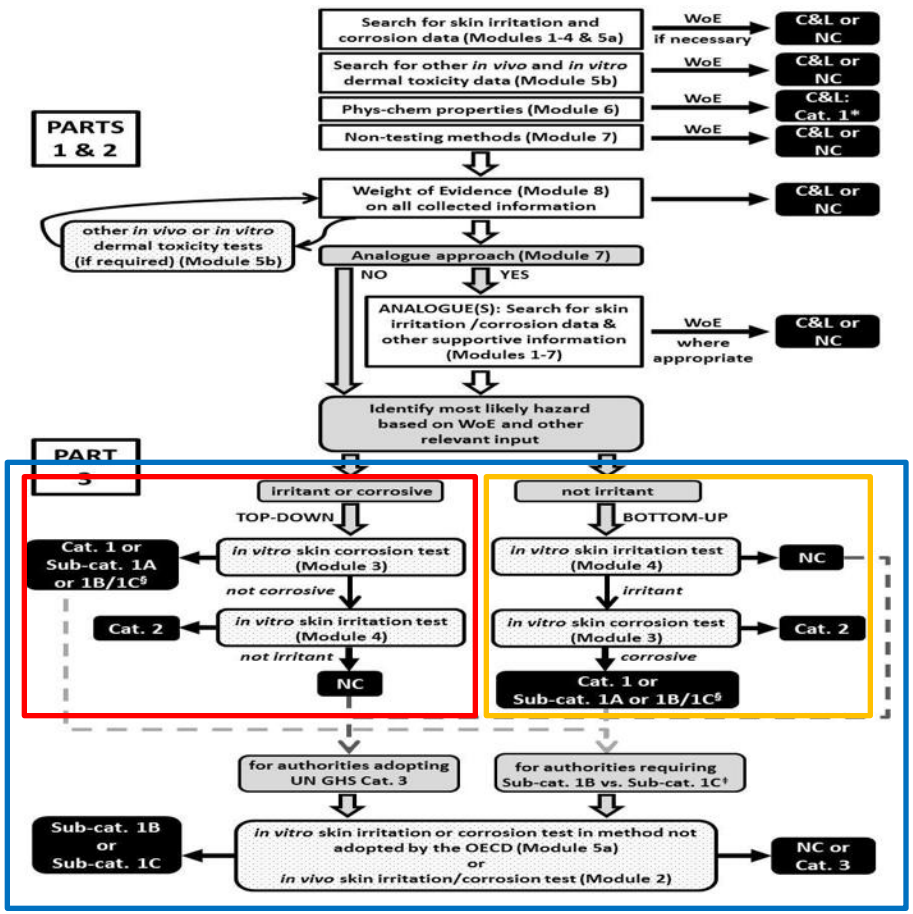
Skin Irritation Test

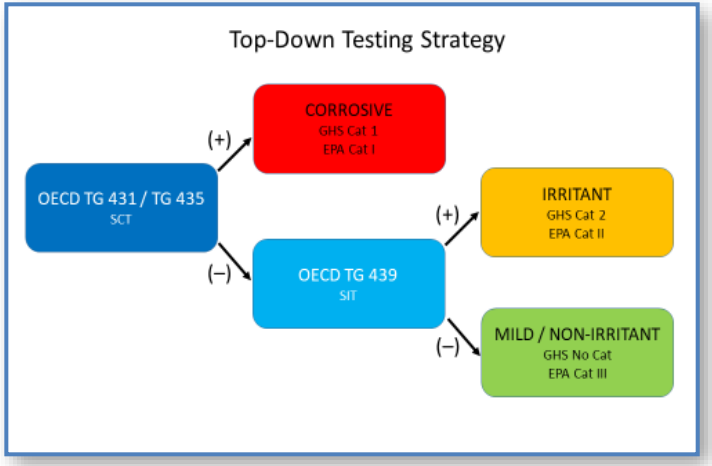
Prediction Model

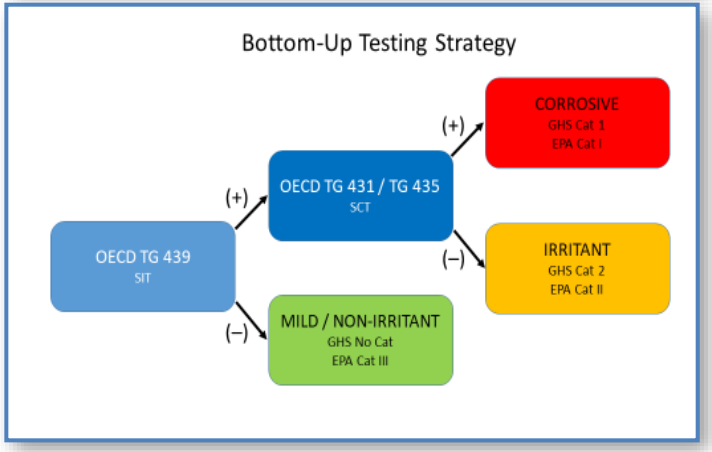
(OECD TG 439)

***In vitro* result**	***In vivo* prediction**	**UN GHS CATEGORY**
Mean tissue viability ≤ 50%	Irritant (I)	**Category 1 or 2** (*OECD 431 to be used for confirmation of Category 1*)
Mean tissue viability > 50%	Non-irritant (NI)	***No Category***

Tiered Testing Strategies for the Assessment of Skin Corrosion and Irritation Potential







Thank You for Your Participation!

For more information on New Approach Methodologies and non-animal test methods, please visit:

www.iivs.org

IIVS
Institute for In Vitro Sciences
Advancing Science & Animal Welfare Together