Use of *in vitro* skin irritation/corrosion test methods for toxicity assessment of Pesticides

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Outline of Presentation

- Introduction
- The impact of chemicals on human skin
- The animal test system used for skin corrosion and irritation assessment
- Alternatives, Validation
- Evaluation of skin corrosion and irritation potential using in vitro assays
- Summary

Pesticides

- Pesticides – Widely used,
- designed to be toxic to insects

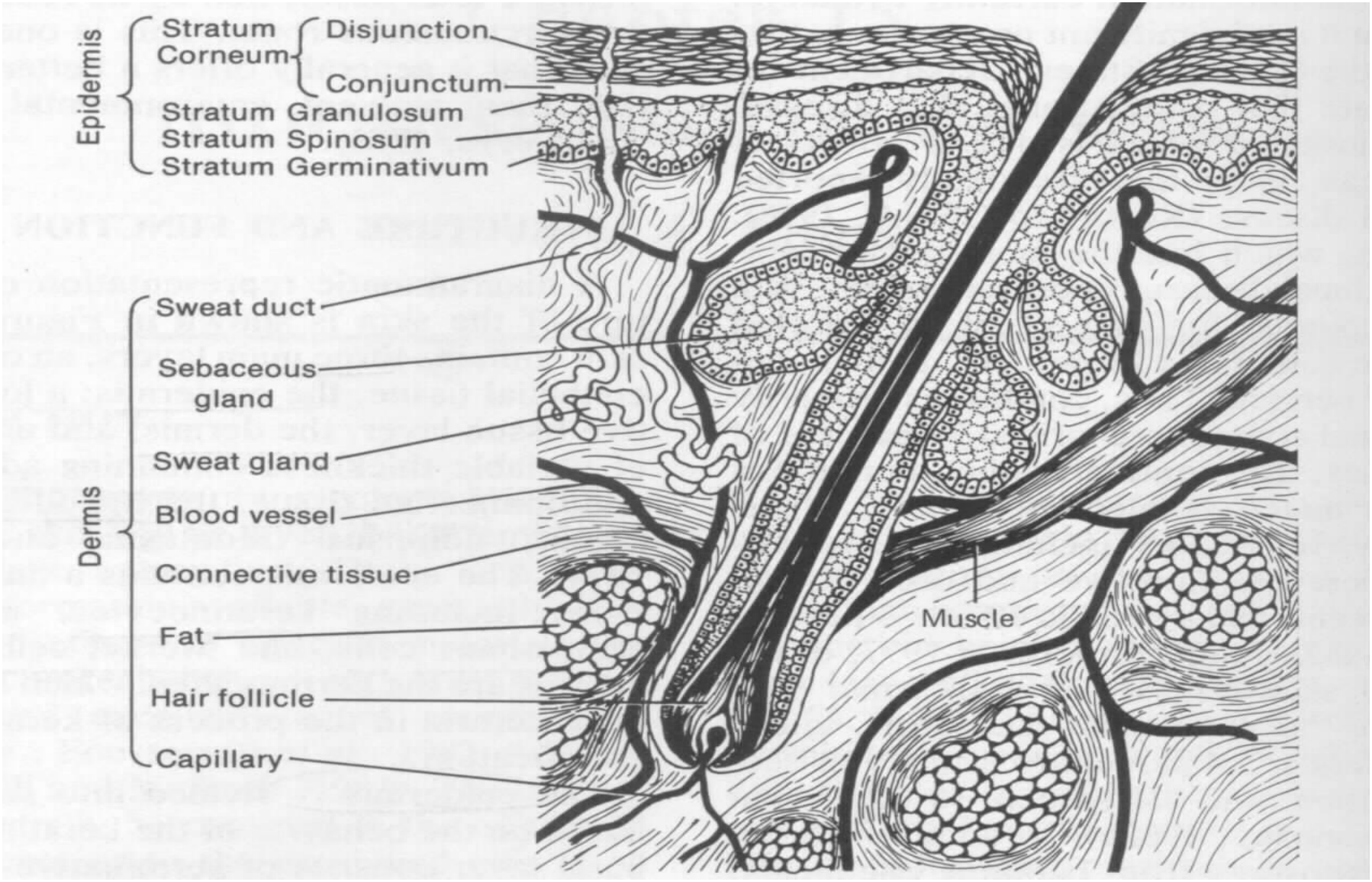
- Agrochemicals and household

- Public and environment come in contact with these substances – use, manufacture, distribution etc.

Skin Characteristics

- Skin largest organ in body
  - 10-15 % of body weight (4 - 12 kg)
- Metabolically active
  - Nearly same profile as liver (inducible)
- Nearly constant rate of cell growth
  - Keratinization
- Important functions
  - Protective
  - Thermoregulation
  - Sensory

Skin Structure



Local Toxicity

- Contact Dermatitis
  - Irritant contact dermatitis
  - Allergic contact dermatitis
- Corrosion/burns
- Phototoxicity
- Photosensitivity
- Photoallergy
- Specific syndromes
  - Acne, Urticaria, Hyper/Hypo-pigmentation, Granulomatous Disease
- Toxic Epidermal Necrolysis (TEN)
- Cancer

Regulatory requirement

- To safeguard humanity & environment, toxicological tests are required as per guidelines issued by Regulatory agencies.
  - OECD
  - EPA
  - CIB-RC
  - EU

Regulatory requirement

- Assessment of the potential of a substance to cause damage to the skin is a **basic endpoint** evaluated in regulatory toxicology.

- This endpoint is used to predict the **hazard**, i.e. the intrinsic properties, of a substance upon accidental or intentional contact with the skin.

Irritation & Corrosion

- **Dermal irritation:**
- reversible damage of the skin following the application of a test chemical for up to 4 hours.
- **Dermal corrosion:**
- irreversible damage of the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test chemical for up to four hours.
- Corrosive reactions are typified by ulcers, bleeding, bloody scabs
- discolouration due to blanching of the skin, complete areas of alopecia, and scars.

Irritation & Corrosion…..

- The extent of **severity** and **reversibility** of effects distinguishes skin **irritation** from skin **corrosion**.

- Irritant substances lead to a reversible local inflammatory reaction, while **corrosive substances irreversibly damage the skin through** the epidermis and into the dermis, beyond repair.

Irritant Contact Dermatitis (ICD)

- Common irritating agents
  - Surfactants
  - Soaps
  - **Pesticides**
  - Fuels
  - Hydrocarbons
  - Solvents

Predictive Animal Test

- Traditionally, the **Draize skin irritation test** has been used for many decades to predict skin irritation hazard (OECD, 2002, Draize *et. al*., 1944).

- In this test, the test material is applied topically onto the shaved skin of rabbits.

Draize Test

- Developed in 40's, mandated in 60's and modified by many agencies since
- 6 rabbits, application to clipped back, skin abraded, undiluted 0.5 ml of liquid, 0.5 gram of solids moistened, covered with gauze, trunk wrapped with rubberized cloth
- Persistent pressure to replace this test

Table 2. Comparison of skin irritation tests based on Draize method

	Draize	DOT	FHSA	OECD
Abrasion	Intact/abraded	Intact	Intact/abraded	Intact
Occlusion	Rubberized cloth	Yes	Impervious material	Semiocclusive
Exposure period	24 h	4 h	24 h	4 h
Observation time	24 and 72 h	4 and 48 h	24 and 72 h	0.5, 1, 24, 48, 72 h
Labeling criteria	—	Corrosion	PII > 5	Narrative/corrosion

Draize Test Scoring

Table 1. Grading scale typically used in performing Draize-type tests in albino rabbits

Description	Score assigned
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formations (injuries in depth)	4
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

Note. The scale is as defined by Draize and adopted by various regulatory agencies. The PII (primary irritation index) is calculated by averaging the erythema values and averaging the edema values, then combining the averages (maximum PII = 8).

Limitations

- The rabbit and human skin have different physiological properties and responses to test substances which may be more toxic to rabbits than to humans and vice versa.
- The Draize rabbit test has been criticized for over-prediction of human skin irritation.
- A debated ethical issue of the in vivo test concerns the animals' suffering and discomfort

Alternative Assays for Skin Irritation

- Traditionally, hazard assessments are conducted using the *in vivo* Draize skin test, but recently *in vitro* tests have gained regulatory acceptance.

Motives to develop alternatives:

Law

Ethics

Scientific

Economy

Public pressure

Alternatives

Russell and Burch in 1959 gave the concept of "THREE R's" i.e. reduction, replacement and refinement that contribute a lot in minimising the animal usage in the field of drug development and testing (Article in Science "The Principles of Humane Experimental Technique" 1959).

3 R

Replacement

Reduction

Refinement

Historical Background

- Marshall Hall was one of the first to address the issue of alternatives (1876)
- In 1969, the Fund for the Replacement of Animals in Medical Experimentation (FRAME) was founded in the UK
- 1981-extablishment of the John Hopkins Centre for Alternatives to Animal Testing (CAAT)

- 1990-The European Centre for the Validation of Alternative Methods (ECVAM) was established

- ECVAM started skin irritation validation study in Nov. 2003

- In April 2007, ECVAM approved 2 alternative tests, EpiSkin and EpiDerm Skin Irritation Tests as replacements of the in vivo rabbit skin irritation test

In light of the mandates passed by FDA, EPA and EU chemicals, *In Vitro* alternatives are of immense importance for the future of toxicity testing

US House passes legislation to end FDA animal testing mandate

FDA Modernization Act rider will spare animals, drive down drug prices and produce safer treatments and cures

By EP News Bureau — Last updated Jun 9, 2022

- **Revision of EU chemicals: Experts on New (non-animal) approach methodologies informed the Intergroup on animal Welfare that it is high time we moved away from using animals in laboratory testing.** 24 Oct 2022.
- Read more at: http://timesofindia.indiatimes.com/articleshow/93981623.cms?utm_source=contentofinterest&utm_medium=text&utm_campaign=cppst
- **Sept 4, 2022**

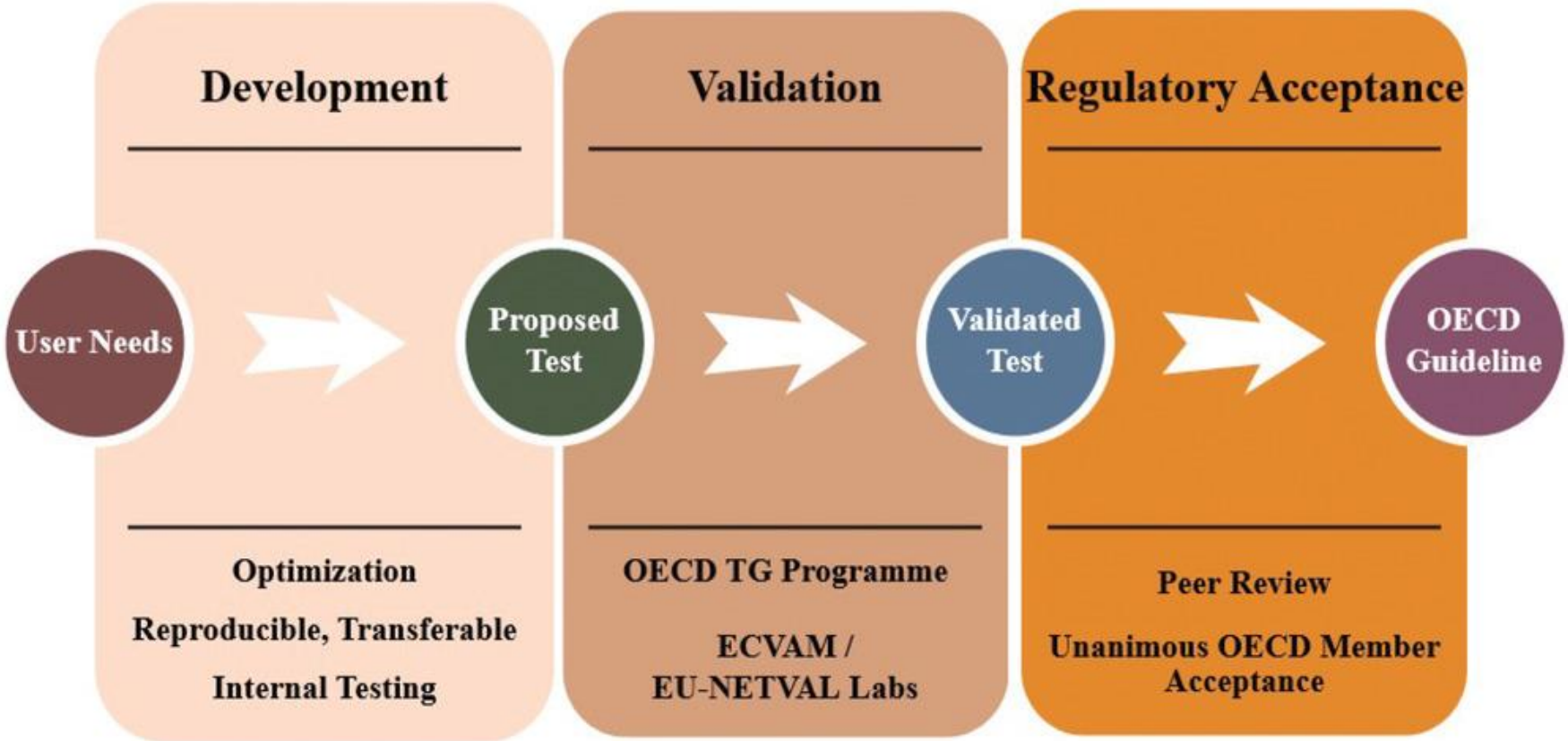
Regulatory Accepted *in vitro* Assays (Skin Irritation / Corrosion)

Guideline number	Study title
OECD 439	In Vitro Skin Irritation: Reconstructed Human Epidermis Test
OECD 430	In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test
OECD 431	In Vitro Skin Corrosion:Reconstructed Human Epidermis (RhE) Test Method
OECD 432	In Vitro 3T3 NRU Photo toxicity Test
OECD 428	Skin Absorption: In Vitro Method
ICCVAM	Recommended Protocol for the BALB/c 3T3 Neutral Red Uptake (NRU) Cytotoxicity Test - A Test for Basal Cytotoxicity

Validation

is the scientific process by which the **reliability** and **relevance** of a procedure are established for a **specific purpose**.

Understanding the Development, Standardization, and Validation Process of Alternative In Vitro Test Methods for Regulatory Approval

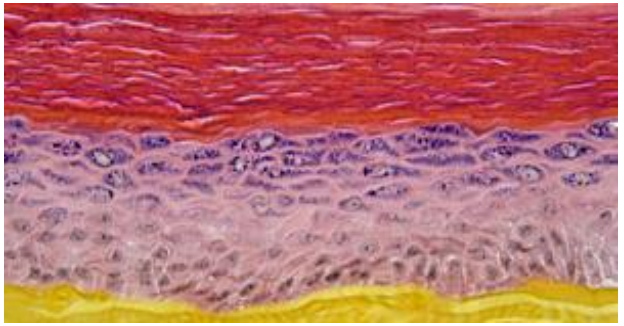


Small, Volume: 17, Issue: 15, First published: 22 January 2021, DOI: (10.1002/smll.202006027)

In Vitro Assays

- **EpiDerm, EpiSkin and SkinEthic** are validated for the purpose of classification and labelling

- **OECD TG 431**: In Vitro Skin Corrosion: Reconstructed Human Epidermis Test Method
- **OECD TG 435**: In Vitro Membrane Barrier Test Method for Skin Corrosion
- **OECD TG 439**: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method



Reconstructed Human Epidermis

GUIDANCE DOCUMENT ON TOXICOLOGY

- **FOR REGISTRATION OF PESTICIDES IN INDIA**

- 2017

- MINISTRY OF AGRICULTURE DEPARTMENT OF AGRICULTURE & CO-OPERATION CENTRAL INSECTICIDES BOARD & REGISTRATION COMMITTEE DIRECTORATE OF PLANT PROTECTION QUARANTINE & STORAGE NH-IV, FARIDABAD

CIB-RC Guideline

- **Primary Skin Irritation / Corrosion** Test in Rabbit (OECD 404)

- ***In Vitro* Skin Irritation**: Reconstructed Human Epidermis Test Method (OECD 439)
- As compared to classical Draize test (Rabbit test), in vitro models based on human cells predict better responses

In Vitro Assays....

- Currently, the three main commercial human skin equivalents used for skin irritancy testing are -

- **EPISKIN** (Imedex, Chaponost, France)

- **EpiDerm** (MatTek Corporation, Ashland, MA)

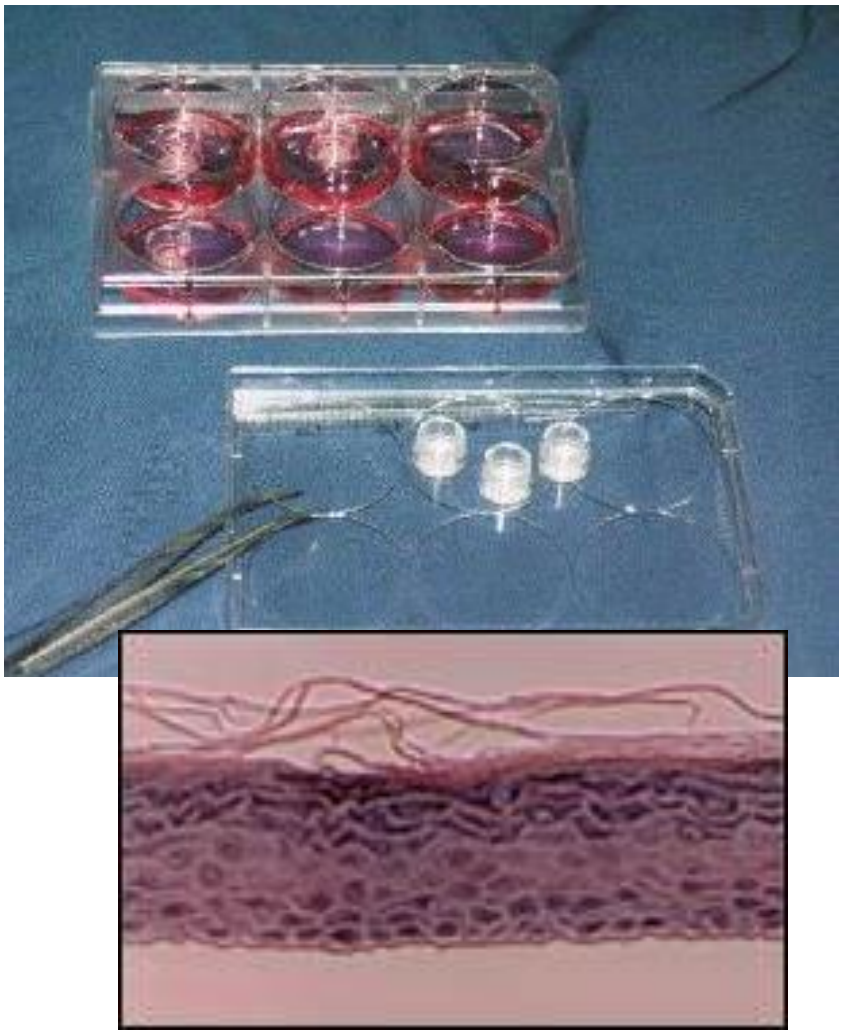
- **SKINETHIC** (SkinEthic Laboratories, Nice, France).

- The skin recombinant differentiated keratinocyte cultures are grown at the air-liquid interface on various substrates, thus resulting in a **stratified, differentiated epithelium**.

- The **EPISKIN cultures** consist of seeded adult human keratinocytes on a dermal support of collagens I and III, covered with a thin film of collagen IV.
- The **EpiDerm model** comprises normal human epidermal keratinocytes grown on permeable membranes to form a multilayered, differentiated epidermis.
- The **SKINETHIC cultures** consist of normal human adult keratinocytes on an inert polycarbonate filter at the air-liquid interface in modified and supplemented chemically defined medium.

EpiDerm™

- NHEK cultured on cell culture well inserts
- Test chemical added to surface
- MTT assay (tissue viability) is primary
- Vendor suggests histology, cytokine release or gene expression
- No blood flow or immune response

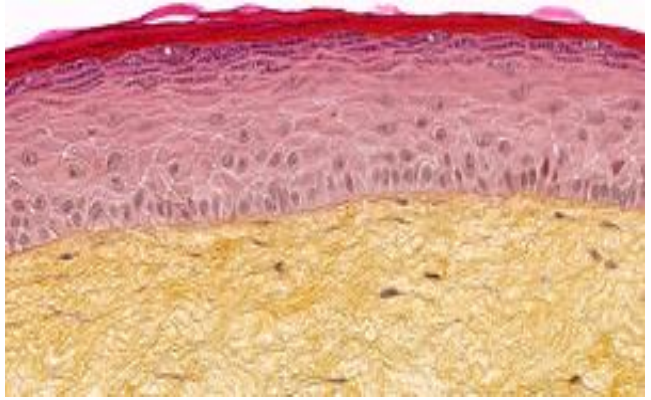


RHE Test Method (OECD TG 431)

- • **Test system**:
- 3-D RHE models [EpiDermTM (EPI-200), EpiSkin™ (SM), SkinEthic™ RHE, epiCS®]

- • **Applicability**:
- the results can be used for regulatory purposes for distinguishing corrosive from non-corrosive test substances

- Reconstructed Human Full Thickness Skin Model



RHE Test Method (OECD TG 439)

- **Test system**:
- 3-D RHE models [EpiDermTM (EPI-200), EpiSkin™ (SM), SkinEthic™ RHE, LabCyte EPI-MODEL24]

- **Applicability**:
- Reconstructed Human Epidermis Test Method (RHE) is an *in vitro* assay that allows distinction between irritants and substances not classified.
- the results can be used for regulatory purposes to determine the skin irritancy of test substances either as a **stand-alone replacement** for in vivo skin irritation testing or as **partial replacement** test within a tiered testing strategy •

Advantages - *in vitro* systems

Brandon *et. al.* (2003) reviewed "pros and cons" of *in vitro* methods -

- no interactions with other organs
- increased sensitivity
- better control conditions
- better experimental flexibility
- clearer interpretation
- large sample capacity
- small amount of test substance needed

Summary

- The skin is an exciting and interesting organ!
- Irritation / corrosion of the skin is significant health problem
- Predictive tests for irritation/corrosion are rapidly changing (from animal testing to in vitro alternatives)

  In vitro alternative tests have been validated and accepted by regulatory authorities for safety assessment of pesticides.

Summary….

- Full replacement of animal studies for prediction of skin irritation / corrosion potential are available
- However, alternative assay are being used for priority setting and screening purposes. Not accepted by **All** regulatory authorities
- Advancements are being made every day, and hopefully, **very soon animals will not have to be used for prediction of skin irritation / corrosion potential.**


Thank you
What is my Fault ?