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# *REACH - The Reality of Dermal Testing to Meet EU Regulations*

David J Esdaile, MSc., Scientific Director

LAB Research Ltd. (Hungary)



## *REACH - The Reality of Dermal Testing to Meet EU Regulations*

1. Regulatory Aspects
2. Corrosivity
3. Irritation
4. Sensitization
5. Other Potential Testing Requirements



## REACH

- *ALL substances manufactured, imported, transported, used as intermediates or placed on the market in the EU: this can be as a chemical, mixture, in preparations or any type of product.*
- *Exceptions: non-isolated intermediates, waste or materials that come under other legislation such as drugs, agrochemicals, biocides and radioactives. Even food materials that meet the definition of a substance, on its own or in a preparation, will be subject to REACH (however, such substances are largely exempted from Registration, Evaluation and Authorisation). Member States may exempt substances used in the interests of defence.*
- What is the Current Situation? .....



## *REACH*

- *Pre-registration was completed in 2008*
- It is difficult to estimate how many chemicals which should have been pre-registered have missed the required deadline.
- Currently, REACH testing in Contract Research Organisations is starting for “sole notification” companies. We see a delay is starting testing for most chemicals where groups of companies need to negotiate before testing can start.



## *REACH*

- *Testing requirements for REACH cover physical chemistry, environmental testing, genetic toxicology and animal toxicology.*
- *This short presentation will concentrate on the REACH testing that relates to Dermal Exposure to chemicals*



## CORROSIVITY TESTING

- REACH and the regulatory implications of Animal Experimentation and Alternatives
- No “potentially” corrosive material can be tested in live animals under REACH - Alternative approaches are obligatory
  - pH
  - In Vitro Human Reconstituted Skin Model (OECD 431)
  - Transcutaneous Electrical Resistance Assay (TER) (OECD 430)
  - In Vitro Membrane Barrier Test Method (OECD 435)



## What is Corrosion in Safety Testing?

- Dermal corrosion is the production of irreversible damage of the skin; namely, visible necrosis.

## Requirement for pH testing

(Extract From OECD guidelines)

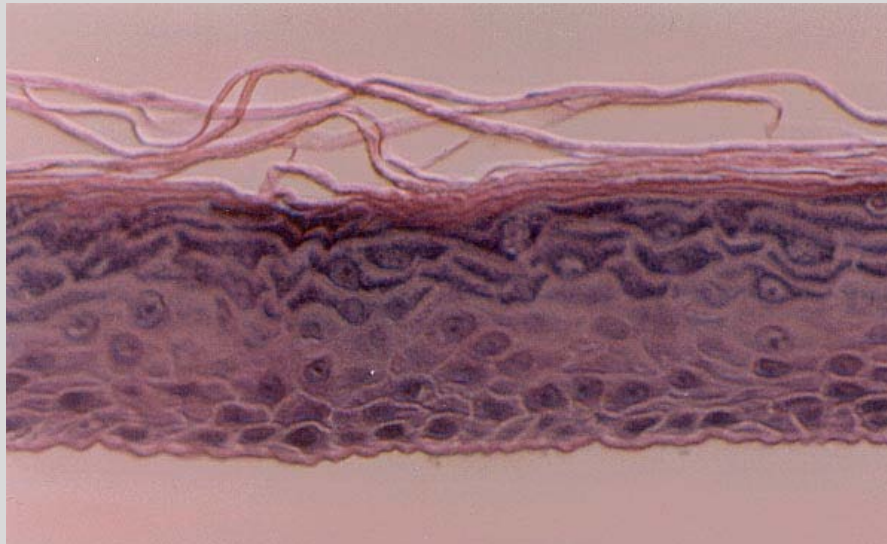
“Substances exhibiting pH extremes such as  $\leq 2.0$  and  $\geq 11.5$  may have strong local effects. If extreme pH is the basis for identifying a substance as corrosive to skin, then its acid/alkali reserve (or buffering capacity) may also be taken into consideration.

If the buffering capacity suggests that a substance may not be corrosive to the skin, then further testing should be undertaken to confirm this, preferably by the use of a validated and accepted *in vitro* or *ex vivo* test”

## In Vitro Corrosivity test Option 1 - OECD 431

### In Vitro Human Reconstituted Skin Model

Human Fibroblast cells extracted from neonatal foreskin material, are cultured to produce reconstituted human skin



- Test material applied to the skin

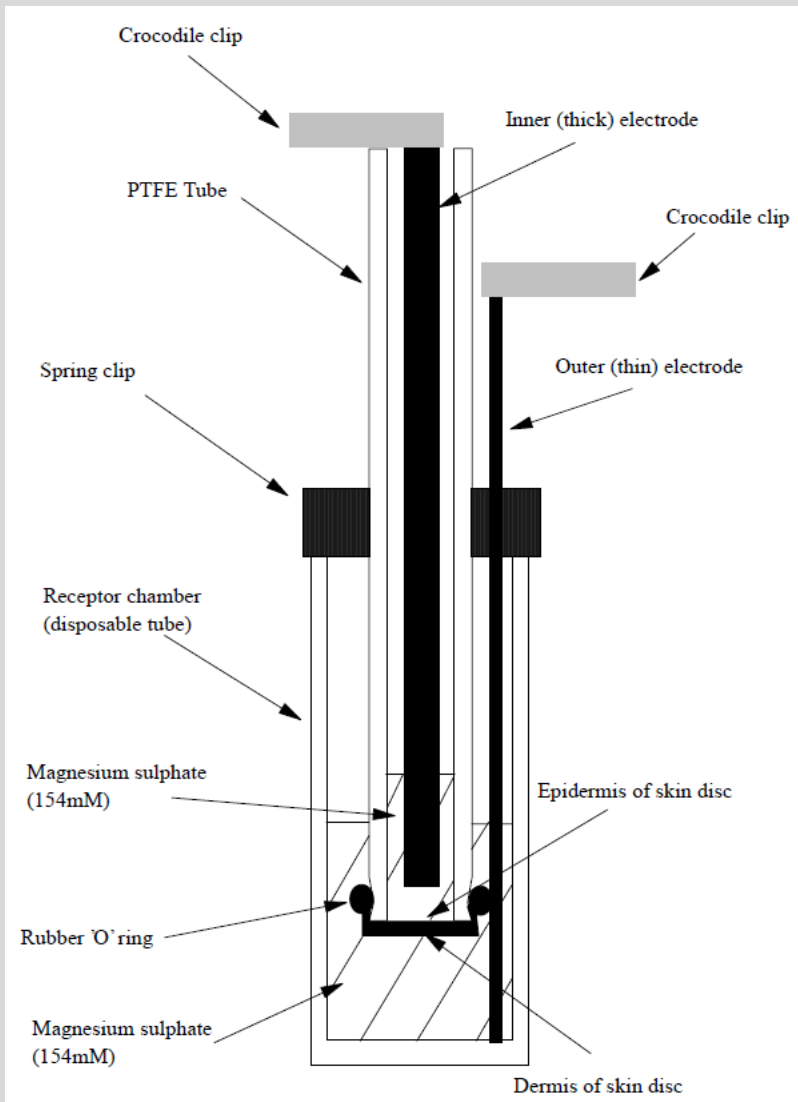
- Viability of the cells measured

Dead Cells=Corrosive



# Corrosivity

## In Vitro Corrosivity test Option 2 - OECD 430



### Transcutaneous Electrical Resistance Assay (TER)

Small disks of rat skin are prepared (many disks per skin) from specially prepared rats.

- Test material applied to the skin

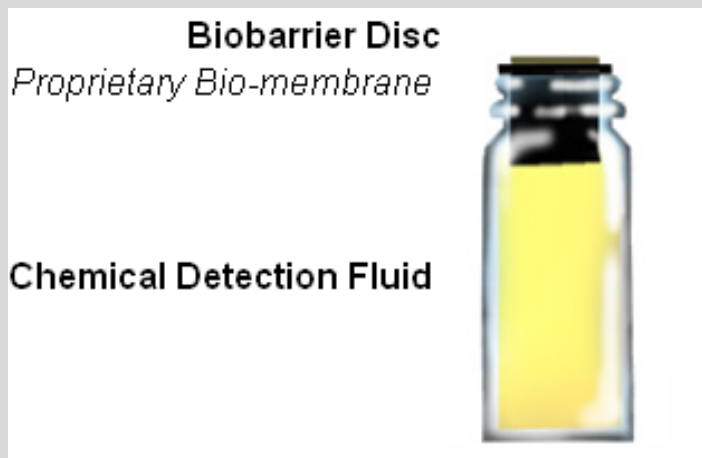
-Electrical Resistance of Skin measured

Low Resistance=Corrosive



## In Vitro Corrosivity test Option 3 - OECD 435

### In Vitro Membrane Barrier Test Method



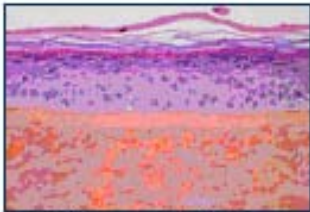
A commercial kit with a membrane barrier above a Chemical Detection Fluid is used

- Test material applied to the membrane
- Colour or other changes to the Fluid is measured

Change = Corrosive

## In Vitro Irritation Test Approaching Regulatory Acceptance

- EPISKIN is the first In Vitro test to receive approval from the ECVAM Scientific Advisory Committee (ESAC)
- Date of full EU regulatory acceptance not defined yet



Histology of the EpiSkin model after haematoxylin-eosin-safran staining





## In Vitro Irritation Test Approaching Regulatory Acceptance

(continued...)

### DRAFT EU Guideline B.46 *In Vitro* Skin Irritation: Reconstructed Human Epidermis (RHE) Model Test

“Regulatory requirements and needs in member countries will decide if this Test Methods will be used as a stand alone replacement test, as a screen, or as part of a testing strategy in combination with, if appropriate, a weight of evidence approach. In cases when a complete irritation categorization is required by regulation, the *in vivo* test should be considered.”



## In Vivo Rabbit Irritation Test

- For REACH, animal testing is only allowed if a chemical is negative for Corrosivity and the pH is  $>2$  &  $<11.5$
- For pH - if it can be shown that the pH is not likely to cause corrosive effects, then the rabbit test may be performed
- Initially, only one rabbit is tested, only if the results are not severe can a further 2 rabbits be used.

To Achieve a Label “Non-Irritant” - Currently a Rabbit Test is Required



## Cost For Corrosivity and Irritation Testing

- pH test plus Draize Rabbit Test used to be the basic EU test
- For REACH, normally Corrosivity testing is required before a rabbit test can be performed

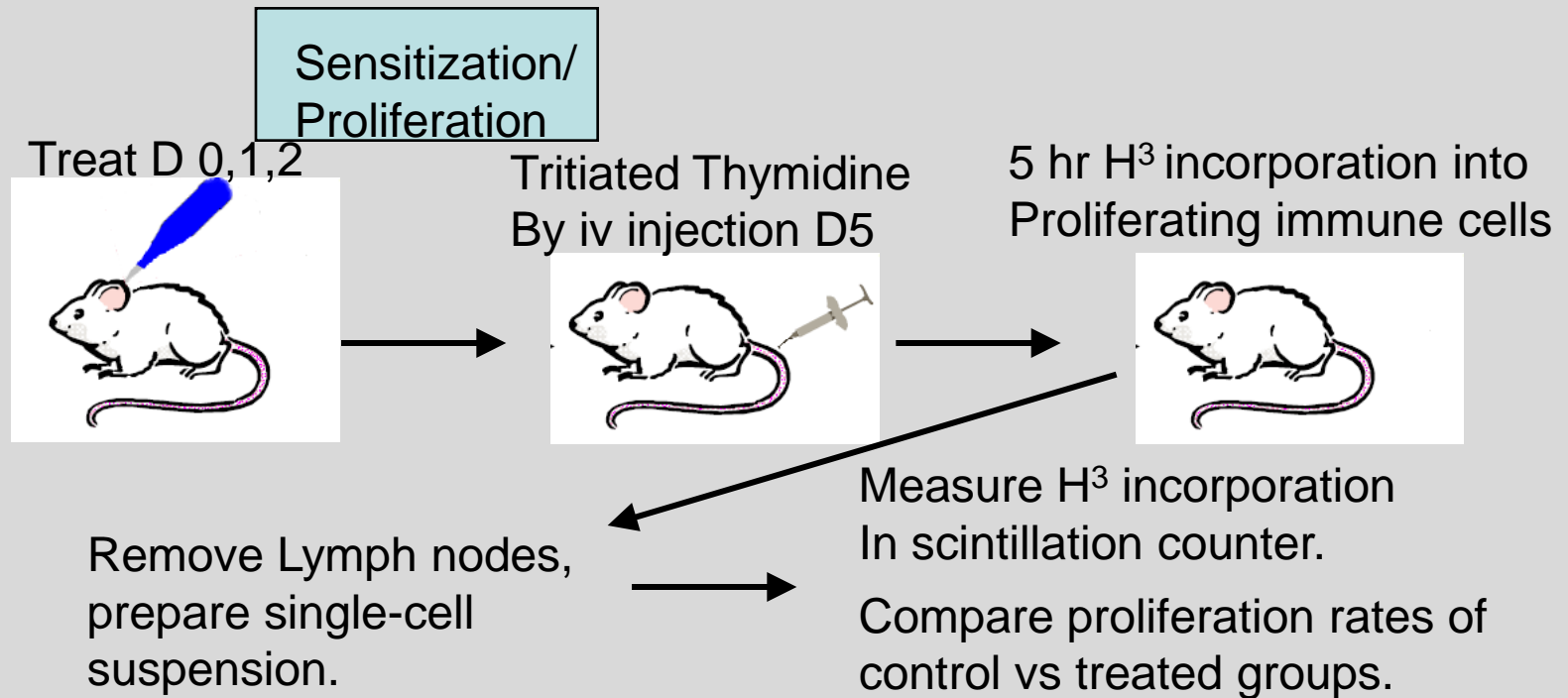
[Corrosivity plus Rabbit test = ~250% of the rabbit test alone]

- In the future, when (if) In Vitro Irritation replaces the In Vivo Rabbit test for REACH

[In Vitro Irritation = ~200% of the rabbit test alone]

## SENSITIZATION - SKIN CONTACT ALLERGY

The Sensitisation Test for REACH is generally the Local Lymph Node Assay (LLNA)



**The LLNA gives both scientific and animal welfare advantages over the guinea pig allergy tests.**



# SENSITIZATION TESTING

## Local Lymph Node Assay (LLNA):

### Cost and Modifications for REACH

-The standard LLNA for the EU uses 3 different concentrations testes in 4 mice each, plus controls.

-The REDUCED LLNA can test with just 4 mice, and several chemicals can be tested with a single common control group (BUT it can only be used if the preliminary test confirms suitability)

### **COSTS:**

The LLNA price is ~the same as the Guinea pig assay

The rLLNA cost per chemical is ~40% of a full test

REACH lists the specific required tests  
 - but also requires that detailed risk assessment is performed

- For a chemical with significant skin contact, and indications of significant toxicity, evidence of a lack of Skin Penetration may be required to maintain the current use of the chemical
- In Vitro Skin Penetration is one option (OECD 428)
- Mathematical calculations for Skin Penetration are not generally applicable to normal use.

REACH lists the specific required tests  
 - but also requires that any other relevant information is used

- If a chemical is known to have a high degree of dermal exposure, or if other information indicates specific dermal toxicity issues, then some of the REACH animal tests which are usually made by the oral route, may require dermal studies. Costs can increase by 50%.



REACH lists the specific required tests  
- but also requires that any other relevant information is used

- If a chemical has known dermal photo-toxicity, or if the chemical structure could indicate photo-toxicity, then an In Vitro Phototoxicity test may be required (OECD 432)



## THANK YOU

### LAB Research Canada

445 Armand Frappier Boulevard  
Laval, Quebec H7V 4B3  
Canada  
T: +1 450 973 2240  
F: +1 450 973 2259  
[busdevna@labresearch.com](mailto:busdevna@labresearch.com)

### LAB Research Denmark

Hestehavevej 36A, Ejby,  
DK-4623 LI. Skensved  
Denmark  
T: + 45 56 86 15 00  
F: + 45 56 82 12 02  
[Info@labresearch.dk](mailto:Info@labresearch.dk)

### LAB Research Hungary

Szabadságpuszta  
Veszprém, H-8200  
Hungary  
T: + 36 88 545 333  
F: + 36 88 545 301  
[busdevna@labresearch.com](mailto:busdevna@labresearch.com)