

In Vitro Toxicology Lecture and Luncheon for Students

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SOT 53rd Annual Meeting and ToxExpo



Introduction

Helena Kandárová

- *MatTek Corp. & MatTek In Vitro Life Science Laboratories*



SOT 53rd Annual Meeting and ToxExpo

Phoenix, Arizona
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Searching for Reliable Replacement Models in Topical Toxicology: Focus on Skin and Eye Toxicity

Dr. Helena Kandárová

MatTek In Vitro Life Science Laboratories, Bratislava, Slovakia

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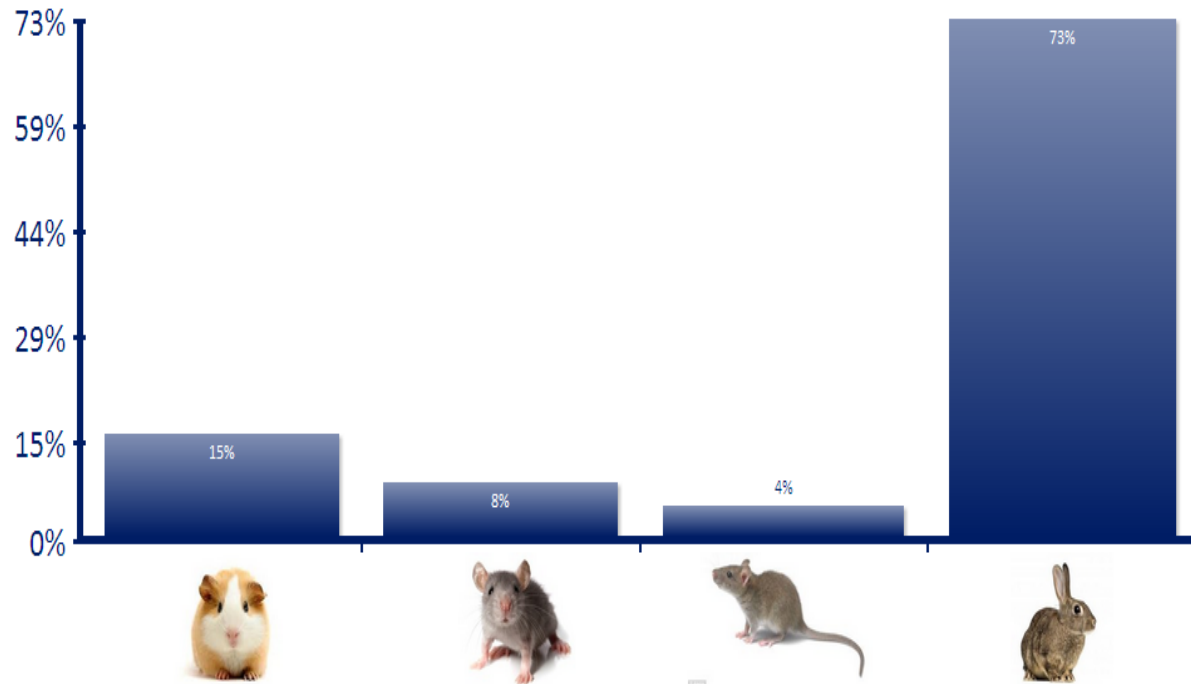


MatTek

IN VITRO
LIFE SCIENCE
LABORATORIES

Do you know which animal was selected by regulators for skin/eye irritation testing as being most sensitive and thus producing most protective results ?

i Start this poll to accept responses



A piece of history behind the rabbit skin and eye irritation test



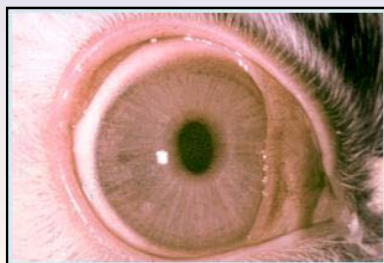
Fig. 1. To depict the need for governmental regulations over cosmetics, the U.S. Food and Drug Administration prepared this poster in 1933 from photographs of a 38-year-old Ohio woman. The patient's first photo was taken an hour before eyelash dyeing, and the other was obtained during the following month showing bilateral staphylococcal corneal ulcers that resulted in vision of light perception. (Reprinted from Lamb RdeF¹⁰⁹ with permission of Farrar and Reinhart.)



Fig. 3. John H. Draize, PhD, the FDA pharmacologist who developed methods for assessing the ocular and dermal toxicity of drugs and cosmetics (Courtesy of the U.S. Food and Drug Administration History Office).

To protect us, he selected the most sensitive animal !

IN VIVO SKIN AND EYE IRRITATION TEST



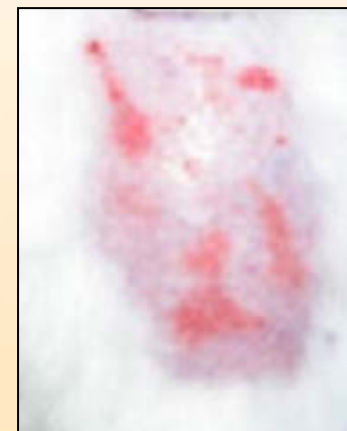
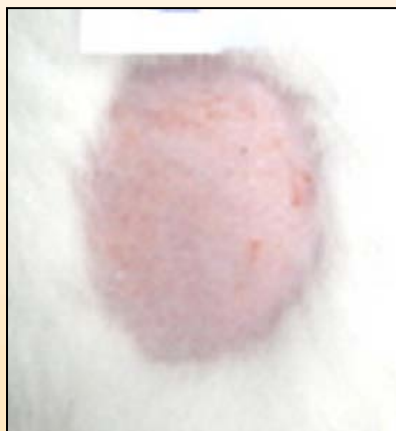
Pictures kindly provided by ZEBET, at the BfR

Application

No or minimal effect

Irritation


Corrosion

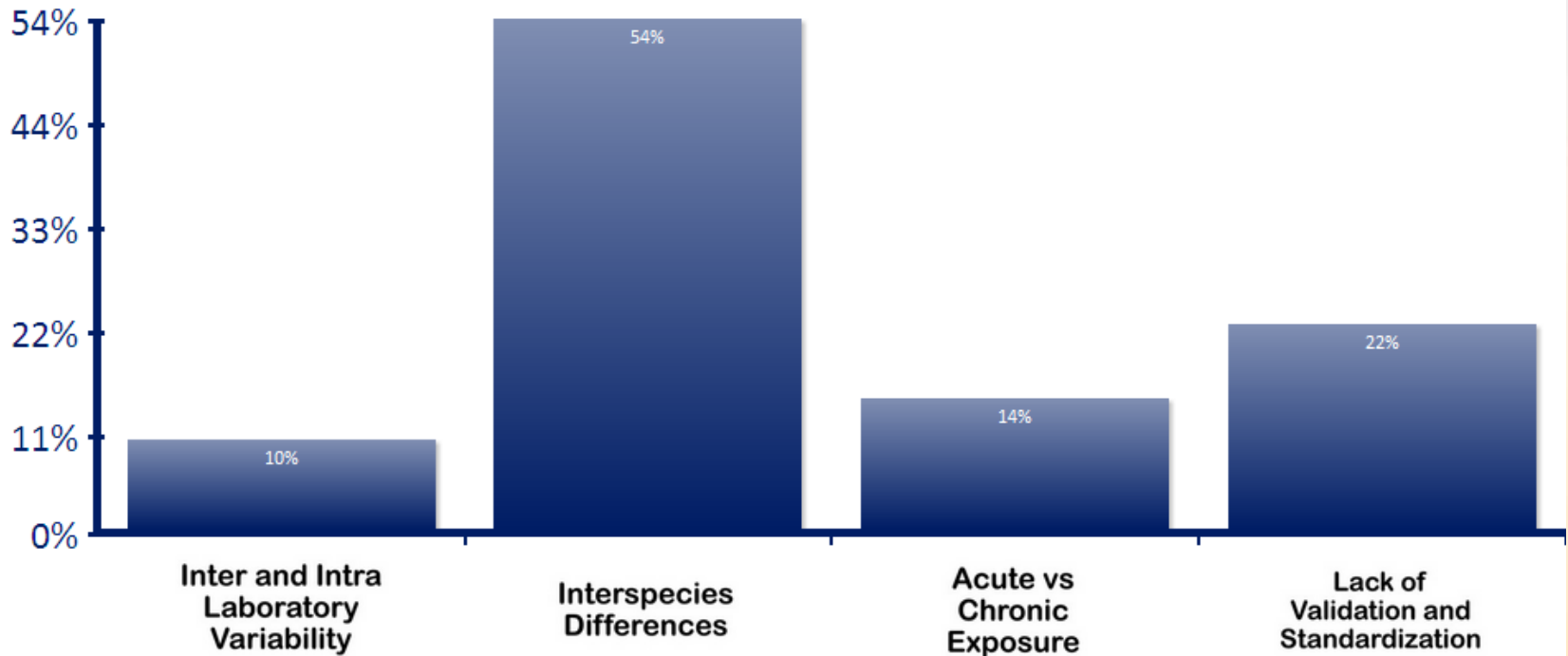


Picture Source: Kumar A. et al. (2007). International Journal of Pharmaceutics, Volume 345, Issues 1–2, 163-171.



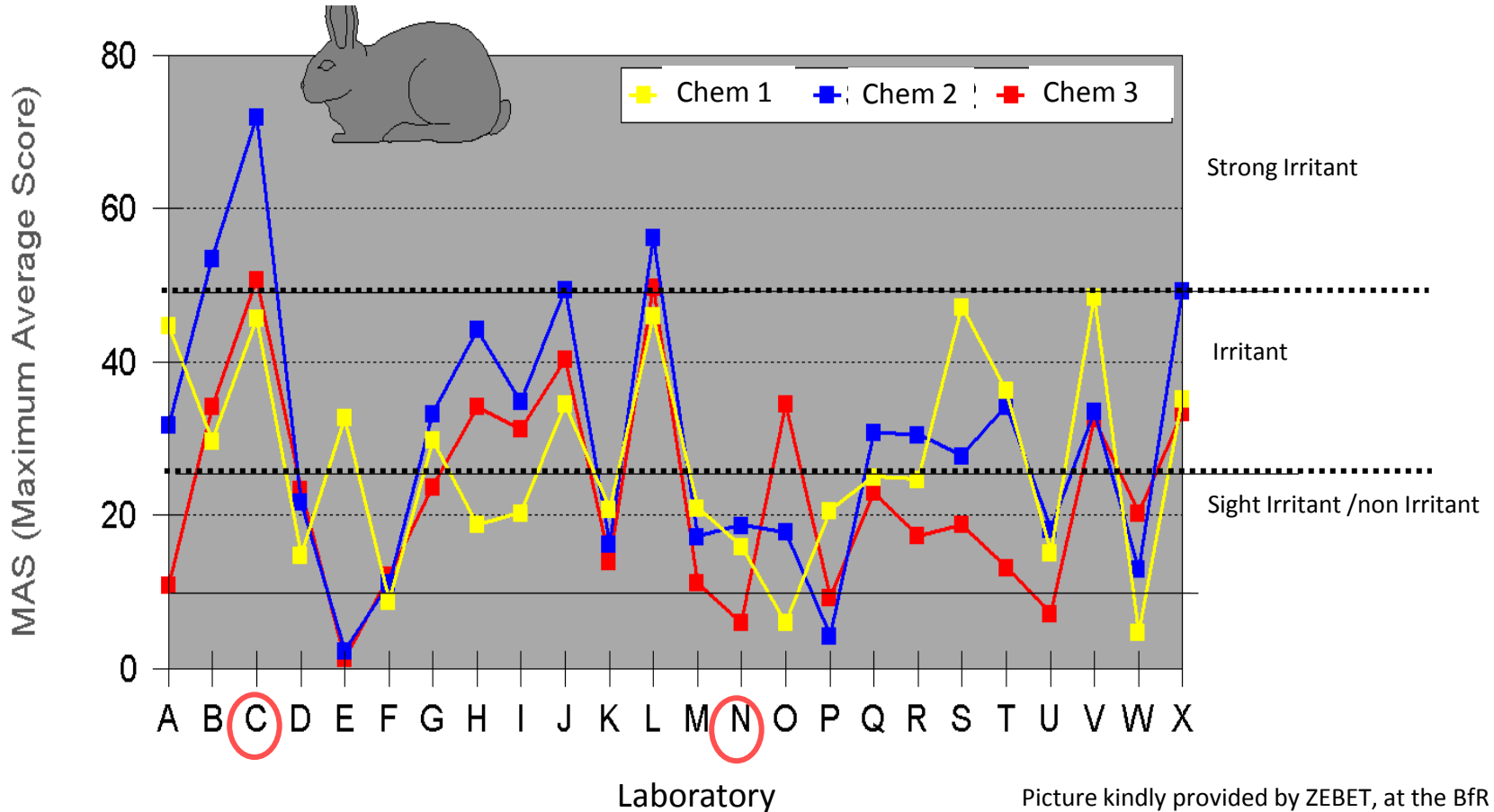
What is -in your opinion – the biggest problem in extrapolation of the results of in vivo animal assay towards man ?

 Start this poll to accept responses



PROBLEM 1: VARIABILITY OF RESPONSES

Inter-laboratory comparison of the observed effects
In vivo eye irritation—the same 3 substances in 24 laboratories



According to Weil and Scala: Toxicol. Appl. Pharmacol. 19:276-360 (1971)

PROBLEM 2: PREDICTIVE CAPACITY TOWARDS HUMAN

Contact Dermatitis 2004; 51: 1-4
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CONTACT DERMATITIS



Review Article

Determination of skin irritation potential in the human 4-h patch test

DAVID A. BASKETTER¹, MICHAEL YORK¹, JOHN P. MCFADDEN² AND MICHAEL K. ROBINSON³

¹Safety and Environmental Assurance Centre, Unilever Colworth Laboratory, Sharnbrook, Bedford, ²St John's Institute of Dermatology, St Thomas' Hospital, London, UK, and ³Procter and Gamble, Cincinnati, Ohio, USA

HUMAN SKIN IRRITATION DATABASE

3

Table 2. Materials tested in the human 4-h patch test (HPT)

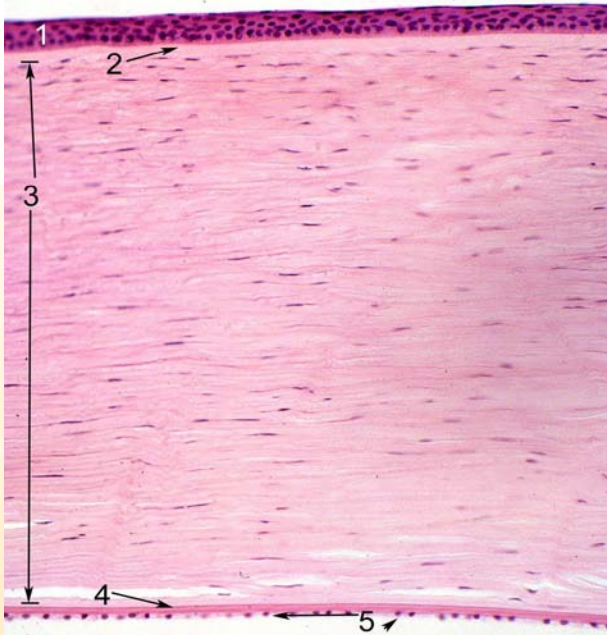
Test Material	Existing Class ¹	HPT Class ²	Positive reactions ³	Positive to SDS control ⁴
Acetic acid (10%)	R38	NC	6/63	45/64
Alcohol ethoxylate C ₁₁ /E3	R38	NC	1/32	26/32
Alcohol ethoxylate C ₁₁ /E7	R38	NC	0/31	12/31
Alcohol ethoxylate C ₁₂₋₁₅ /E3	R38	NC	0/32	24/32
Alcohol ethoxylate C ₁₂₋₁₅ /E5	R34	NC	1/32	23/33
Alcohol ethoxylate C ₁₆₋₁₈ /E5	R38	NC	0/27	14/27
Alcohol ethoxylate C ₁₆₋₁₈ /E14	R38	NC	0/27	14/27
Alkyl dimethyl betaine	R38	NC	3/32	12/32
Alkyl polyglucoside 600	NC	NC	1/30	28/31
Benzalkonium chloride (7.5%)	R38	R38	19/56	32/56
Benzalkonium chloride (10%)	R34	R38	4/29	8/27
Benzyl alcohol	NC	NC	1/31	17/32
Benzyl salicylate	NC	NC	0/30	20/31
Butan-1-ol	NC	NC	1/31	15/31
Butyl benzoate	NC	NC	0/30	14/30
Citronellol	R38	NC	0/30	20/31
Cocotrimethyl ammonium chloride	R38	NC	20/89	50/90
Diethylamine	R38	NC	0/31	0/30

50 % of chemicals classified as R38 were in 4h human patch test non irritating

See also recent review by Basketter et al, Interdiscip Toxicol. 2012; Vol. 5(2): 98–104

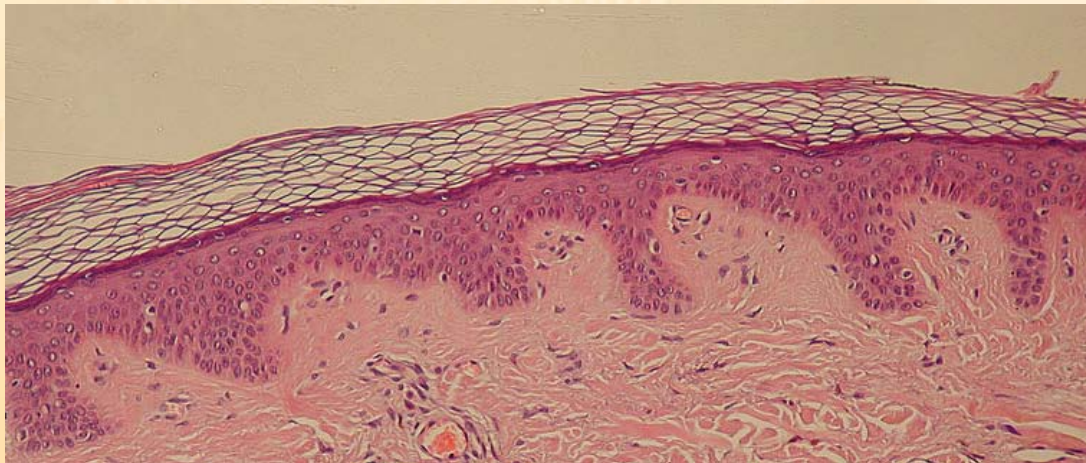
What we try to mimick by *in vitro* models ?

Light micrograph of the human cornea and human skin



The cornea lacks blood vessels and consists of five layers:

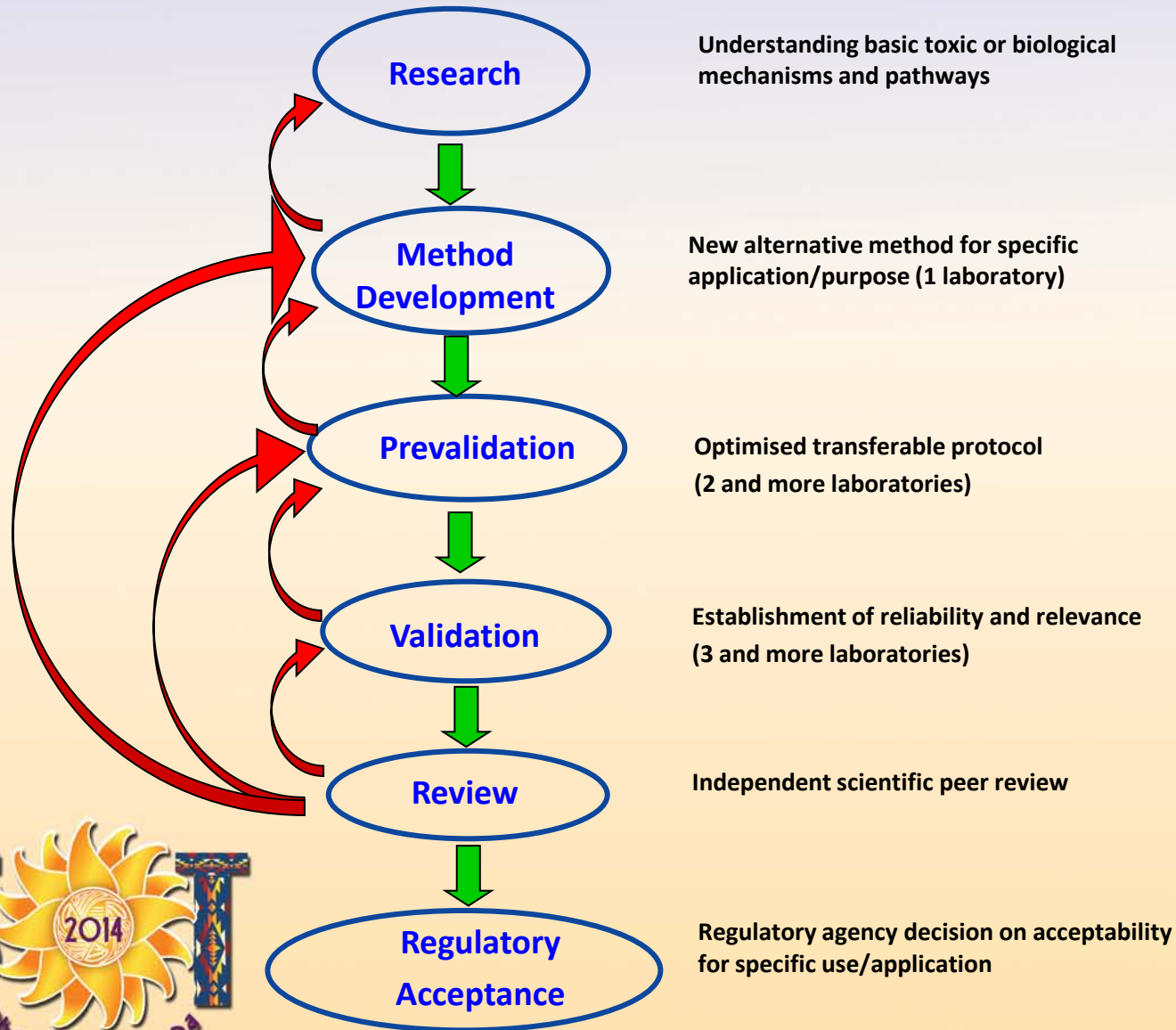
1. The outer surface (top) - non-keratinizing stratified **squamous epithelium** (about 50 μm).
2. It is supported by a **specialised basement membrane** called Bowman's membrane.
3. The bulk of the cornea (substantia propria) consists of a regular form of **dense collagenous connective tissue**.
4. The inner surface (bottom) is lined by a **layer of flattened endothelial cells**, which are supported by an elastic basement membrane called
5. **Descemet's membrane**



The skin consists of three layers:

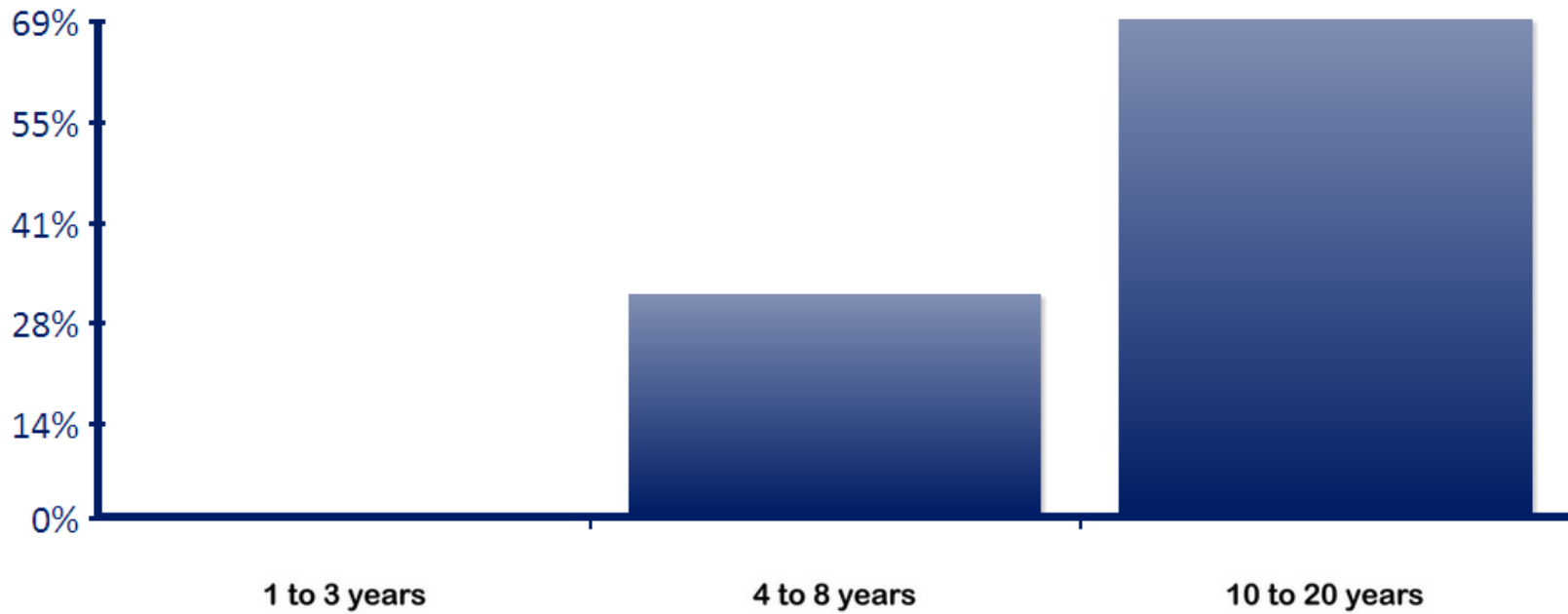
1. The outer surface (top) – keratinized stratum corneum and underlying stratum granulosum, spinosum and basale form together **Epidermis**.
2. **Dermis** is the middle layer of skin
The **Hypodermis** is the innermost and thickest layer of the skin

How can we assure that the *in vitro* models are as good or better than *in vivo* tests?

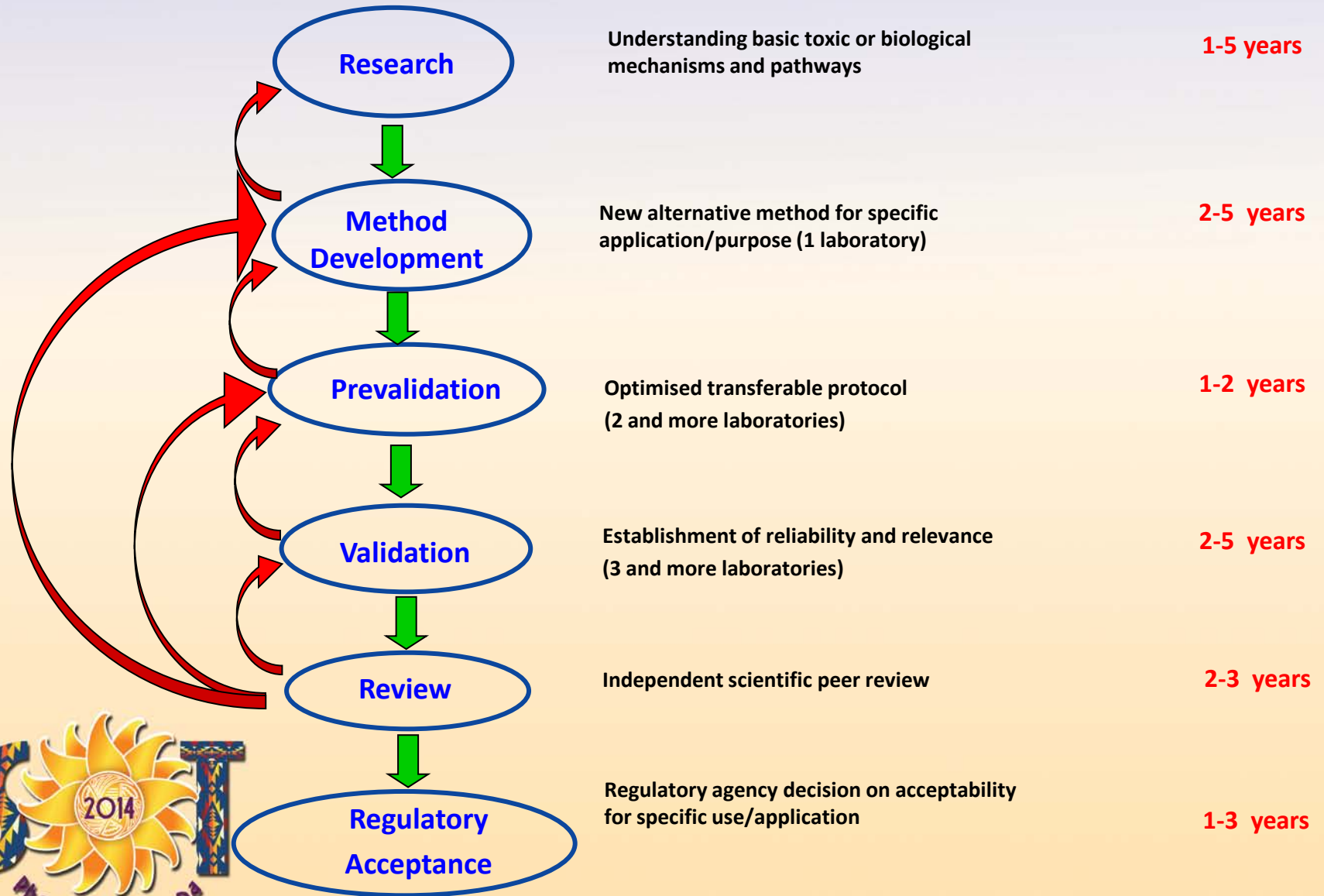


How long - in your opinion – does it take on average from the development of the alternative method to its regulatory acceptance ?

i Start this poll to accept responses



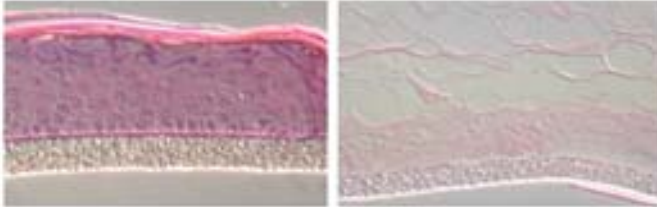
How can we assure that the *in vitro* models are as good or better than *in vivo* tests?



What is available as a replacement – SKIN TOX ?

SKIN CORROSION

OECD TG 431: In Vitro Skin Model Test (4 tissue models)



EpiDerm - H₂O - 60 minute exposure EpiDerm - KOH - 60 minute exposure

OECD TG 430: TER – Ex vivo rat skin test

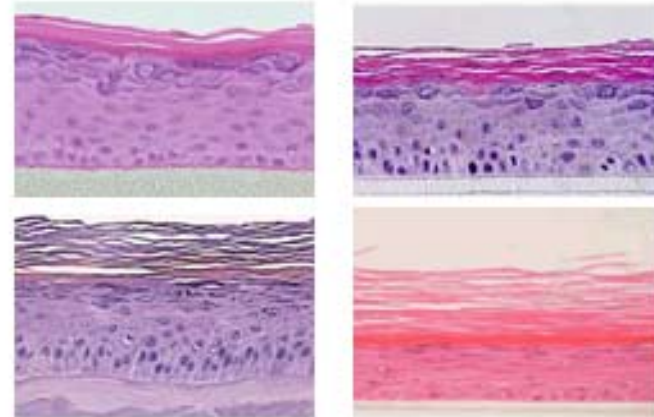


OECD TG 435: CORROSITEX – membrane test



SKIN IRRITATION

OECD TG 431: In Vitro Skin Model Test (4 tissue models)



What is available as a replacement – EYE TOX ?

Cytosensor™
Microphysiometer Cell Capsule, Sensor Chamber and Plunger
Labels: Fuel line, Fuel inlet, Capsule Gap, Capsule Inlet, Plunger, Plunger O-Ring, Capsule Spacer, Silicon Die, LCR

Neutral Red Assays
Treat with test article → Stain with dye → Measure uptake
Stain with dye → Treat with test article → Measure release

MatTek™ Epicuticular
Human Reconstructed Tissue Models

SkinEthic™ HCE

Fluorescein Leakage Assay
Treatment →

BCOP

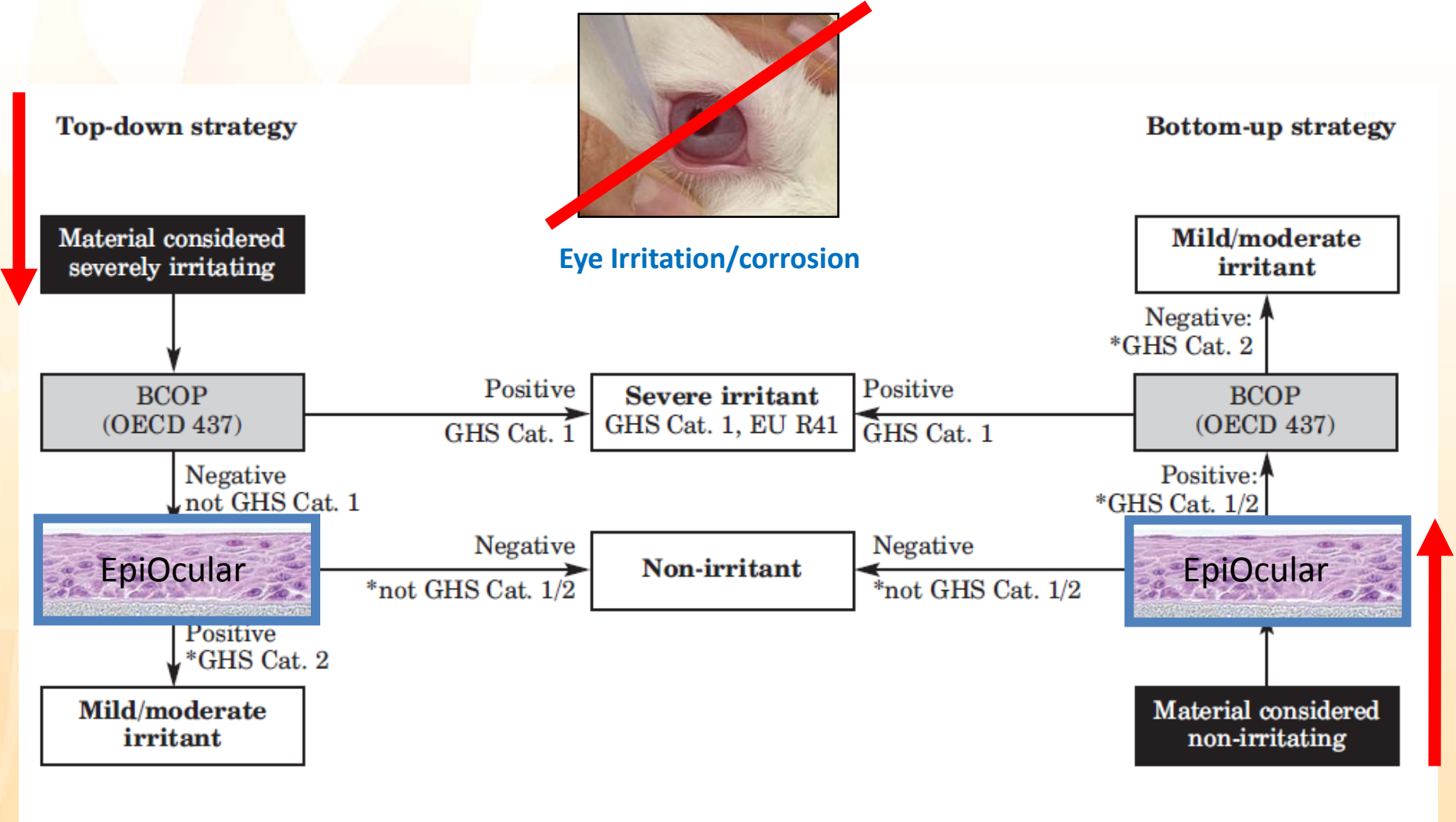
Isolated Chicken Eye Test

Red Blood Cell Assay

HET-CAM
Egg Shell, Yolk, CAM

Pictures taken from COLIPA/CosEU presentation, author – Dr. Pauline McNamee

(How) Can we use the different *in vitro* methods to avoid animal testing ?



In-house Validation of the EpiOcular™ Eye Irritation Test and its Combination with the Bovine Corneal Opacity and Permeability Test for the Assessment of Ocular Irritation. Kolle, S.N., Kandárová, H., Wareing, B. van Ravenzwaay, B., Landsiedel, R. (2011). ATLA 39, 365–387, 2011 (60 chemicals).

(How) Can we use the different *in vitro* methods to avoid animal testing ?



Skin Irritation/corrosion



Material considered as Corrosive

SKIN CORROSION TEST
RHE test (OECD TG 431)
Corrositex (OECD TG 435)
TER Test (OECD TG 430)

SKIN IRRITATION TEST
RHE test (OECD TG 439)

Label as Irritant
(consider sub-categorisation if required by legislation)

Label as Corrosive
(consider sub-categorisation if required by legislation)

Label as Non Irritant

Label as Irritant
(consider sub-categorisation if required by legislation)

SKIN CORROSION TEST
RHE test (OECD TG 431)
Corrositex (OECD TG 435)
TER Test (OECD TG 430)

SKIN IRRITATION TEST
RHE test (OECD TG 439)

Material considered as Non-Corrosive





Thank you for your attention !

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Scientist, Regulator, Advocate

You are the Expert!

- 
- 1) **Determine your role**
(**Scientist, Regulator, Advocate**)
 - 2) **Review** the scenario and discuss the data from the perspective of your role
 - 3) **Develop** your answers from the perspective of your role
 - 4) **Prepare** to report out and discuss with the group



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