3D for 3Rs:
Apports des tissus humains reconstruits en toxicologie

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Scientific Director of EPISKIN Academy

Ingéniérie tissulaire,
Introduction aux applications industrielles des modèles de tissus reconstruits en 3D

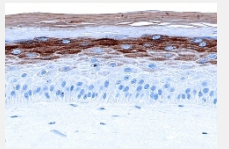
Mardi 30 Mars 2021

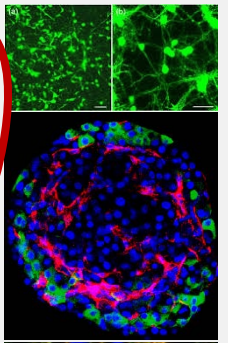
3D MODELS: TISSUE ENGINEERING

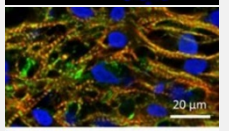
Tissue engineering

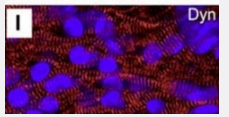
- Medical application
  - Replace or repair tissue/organ *in vivo*

- Research & industrial application
  - Mimic a function for toxicological, pharmacological studies *in vitro*
    - Organotypic tissue/organoid
      - Epithelia
        - skin
        - cornea
        - Vaginal, oral
        - intestinal
        - Respiratory
      - Liver
      - Nervous system
      - Cardiac
    - Spheroid
    - Organ on a chip









INDUSTRIAL MODELS

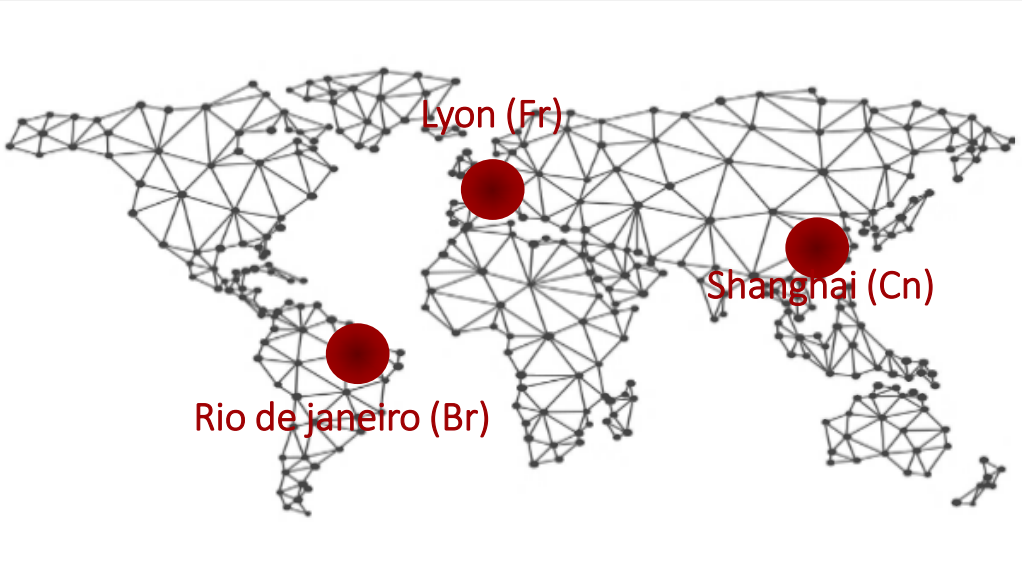
EPISKIN SKINETHIC TISSUE CULTURE LABORATORIES 1997 1992

EPISKIN EPISKIN ACADEMY 2014 2012

SHANGHAI EPISKIN 2015

BRAZIL EPISKIN 2019

EPISKIN



- Created in 1997 by L'Oréal
- 3670 m² net area
- 1260 m² ISO 6 & ISO 7 clean rooms
- 100 000+ tissues produced per year

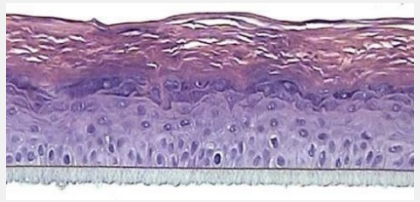
afaq ISO 9001 Qualité AFNOR CERTIFICATION

OECD

ISO

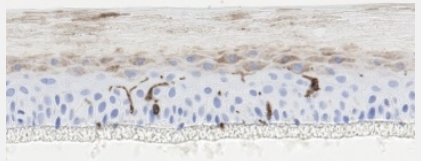
EPISKIN ACADEMY

EPITHELIAL MODELS

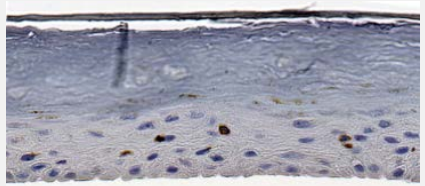


Epidermis
EpiSkin - RHE

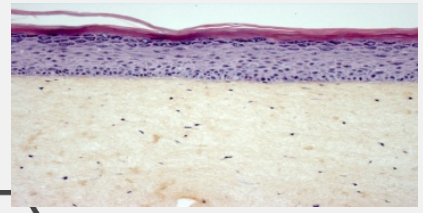
Pigmented
epid. RHPE



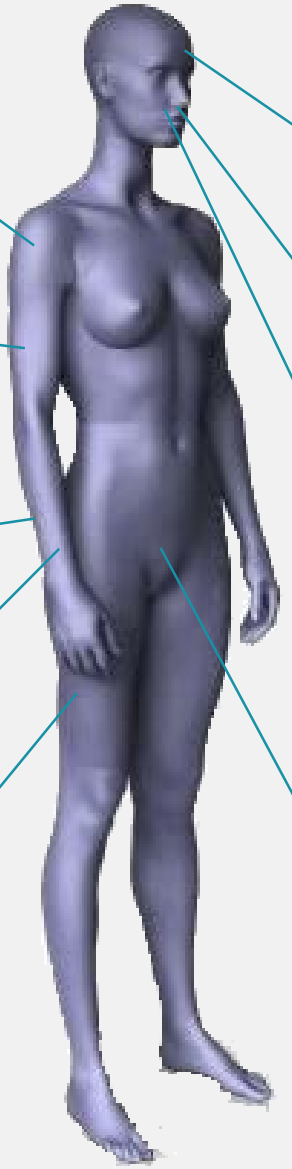
Epidermis +
Langerhans cells



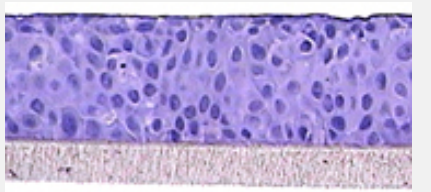
Cell migratory
models (CMM)



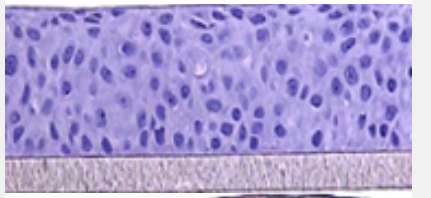
Epidermis
+ Dermis



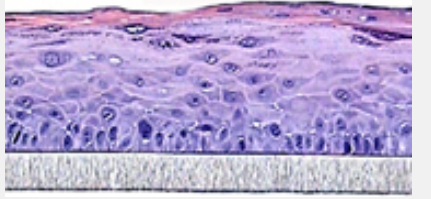
Corneal
HCE



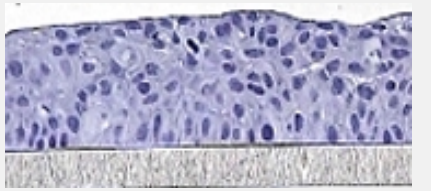
Oral
HOE



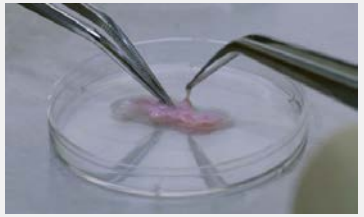
Gingival
HGE

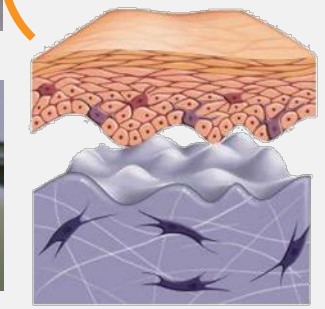


Vaginal
HVE

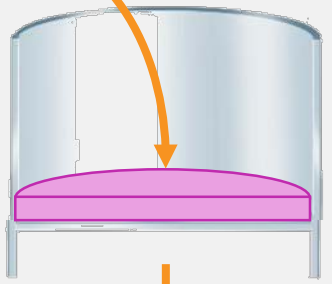


3D MODELS: TISSUE ENGINEERING

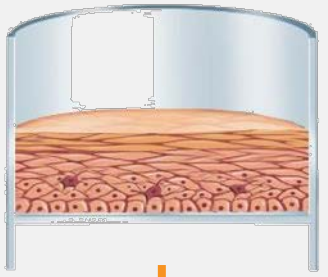




Keratinocytes

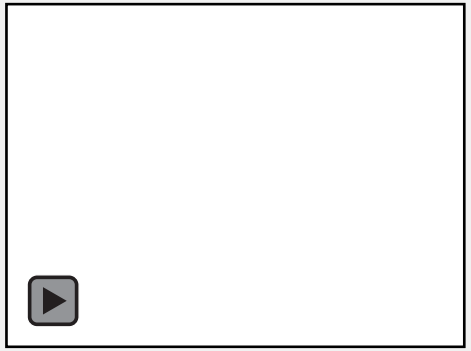


Seeding of keratinocytes in Inserts 0.5 cm2 square with polycarbonate membrane



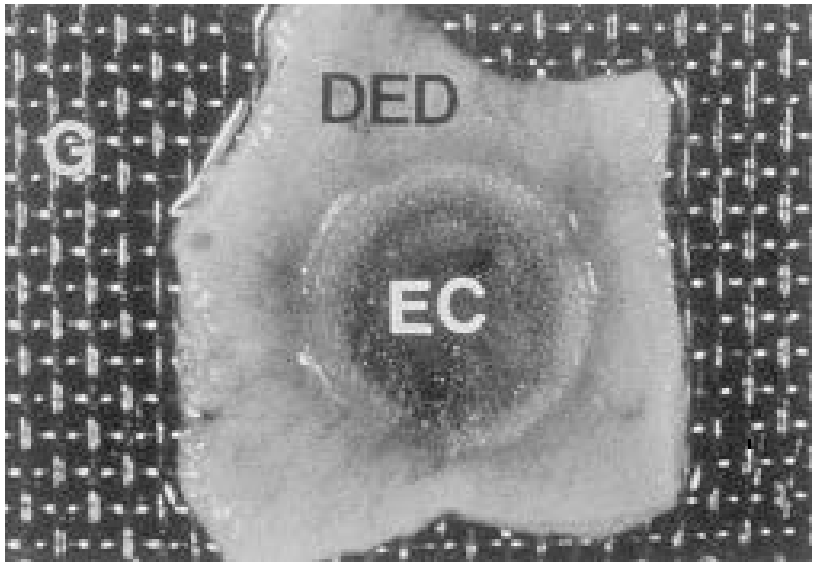
Proliferation/differentiation

Human skin explan



SkinEthic RHE in 24 well plates

FROM RESEARCH TO INDUSTRIAL MODELS

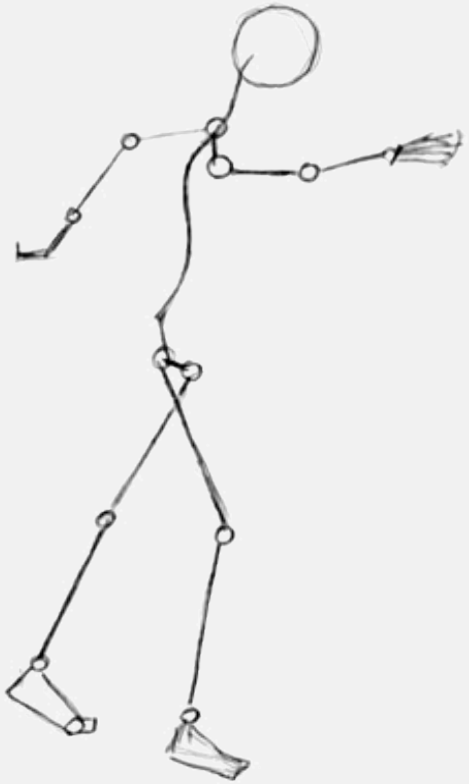


1979 -1983

1st epidermis (Pruniéras, Régnier)

3D MODELS

2D



Human

All models are wrong but some are useful – Georges E.P. Box (1919-2013)

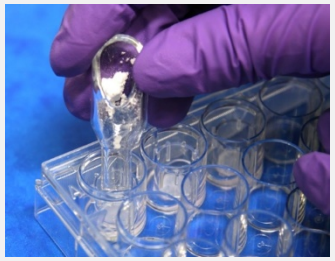
3D MODELS: PHYSIOLOGICAL SITUATION

Characteristics	2D cell culture	3D cell culture	References
Morphology	Cells grow on a flat surface and have flat or stretched shape	Cells grow naturally into 3D aggregates/spheroids in a 3D environment and natural shape retained	Huang H, Ding Y, Sun XS, Nguyen TA. Peptide hydrogelation and cell encapsulation for 3D culture of MCF-7 breast cancer cells. PLoS One. 2013;8:59482
Cell shape	Single layer	Multiple layers	Edmondson R, Broglie JJ, Adcock AF, Yang L. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay and Drug Development Technologies. 2014;12:207-218
Cell to cell contact	Limited cell to cell contact, only on edges	Physiologic cell to cell contact similar to *in vivo*	Li Z, Cui Z, et al. Three-dimensional perfused cell culture. Biotechnology Advances. 2014;32:243-254
Distribution of medium	Cells receive an equal amount of nutrients and growth factors from the medium during growth.	Cells do not receive an equal medium during growth. The core cell receive less growth factors and nutrients from the medium and tend to be in a hypoxic state, which is very similar to *in vivo* tissues, especially in tumors	Bell CC, Hendriks DF, Moro SM, et al. Characterization of primary human hepatocyte spheroids as a model system for drug-induced liver injury, liver function and disease. Scientific Reports. 2016;6:25187 Li Z, Cui Z, et al. Three-dimensional perfused cell culture. Biotechnology Advances. 2014;32:243-254
Cell proliferation	Generally, cells proliferate at a fast rate than *in vivo*	Cells proliferate faster or slower depending on the type of cell or 3D system used	Chitcholtan K, Sykes P, Evans J. The resistance of intracellular mediators to doxorubicin and cisplatin are distinct in 3D and 2D endometrial cancer. Journal of Translational Medicine. 2012;10:1-16 Fallica B, Mafia JS, Villa S, Makin G, Zaman M. Alteration of cellular behavior and response to PI3K pathway inhibition by culture in 3D collagen gels. PLoS One. 2012;7:48024 Luca AC, Mersch S, Deenen R, et al. Impact of the 3D microenvironment on phenotype, gene expression, and EGFR inhibition of colorectal cancer cell lines. PLoS One. 2013;8:e59689 Comparative Assay of 2D and 3D Cell Culture Models: Proliferation, Gene Expression and Anticancer Drug Response April 2018Current Pharmaceutical Design 24(15)
Protein/gene expression	Protein and gene expression profiles differ compared with *in vivo*models	Protein and gene expression profiles more similar to *in vivo*models	Price KJ, Tsykin A, Giles KM, et al. Matrigel basement membrane matrix influences expression of microRNAs in cancer cell lines. Biochemical and Biophysical Research Communications. 2012;427:343-348
Cell differentiation	Moderately differentiated	Properly differentiated	Chitcholtan K, Asselin E, Parent S, Sykes PH, Evans JJ. Differences in growth properties of endometrial cancer in three dimensional (3D) culture and 2D cell monolayer. Experimental Cell Research. 2013;319:75-78
Response to stimuli	Poor response to mechanical stimuli of cells	Good response to mechanical stimuli of cells	Li Y, Huang G, Li M, et al. An approach to quantifying 3D responses of cells to extreme strain. Scientific Reports. 2016;6:19550
Viability	Sensitive to cytotoxin	Greater viability and less susceptible to external factors	Elkayam T, Amitay-Shaprut S, Dvir-Ginzberg M, Harel T, Cohen S. Enhancing the drug metabolism activities of C3A-a human hepatocyte cell line--by tissue engineering within alginate scaffolds. Tissue Engineering. 2006;12:1357-1368
Drug sensitivity	Cells are more sensitive to drugs and drug show high efficacy	Cells are more resistant to drugs and drug show low potency	Bokhari M, Carnachan RJ, Cameron NR, Przyborsk SA. Culture of HepG2 liver cells on three dimensional polystyrene scaffolds enhances cell structure and function during toxicological challenge. Journal of Anatomy. 2007;211:567-576
Cell Stiffness	High stiffness	Low stiffness	Dieter SM, Ball CR, Hoffmann CM, et al. Distinct types of tumor-initiating cells form human colon cancer tumors and metastases. Cell Stem Cell. 2011;9:357-365
Sub-culturing time	Allows cell to be grown in culture for up to 1 week	Allows cells to be grown in culture for almost 4 weeks	Baker BM, Chen CS. Deconstructing the third dimension—How 3D culture microenvironments alter cellular cues. Journal of Cell Science. 2012;125:3015-3024

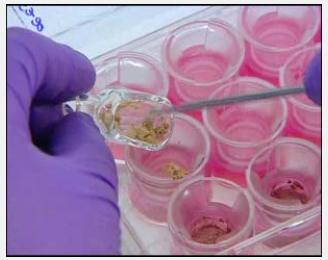
Table from Two-Dimensional (2D) and Three-Dimensional (3D) Cell Culturing in Drug Discovery By Jitcy Saji Joseph, Sibusiso Tebogo Malindisa and Monde Ntwasa, *Open access peer-reviewed chapter November 28th 2018 - DOI: 10.5772/intechopen.81552*

3D MODELS: APPLICABILITY DOMAINS

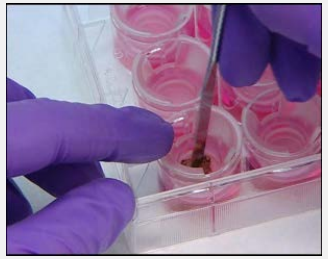
Application of products closer mimics real life



Topical or systemic application, diffusion, gradient of C°, metabollisation…



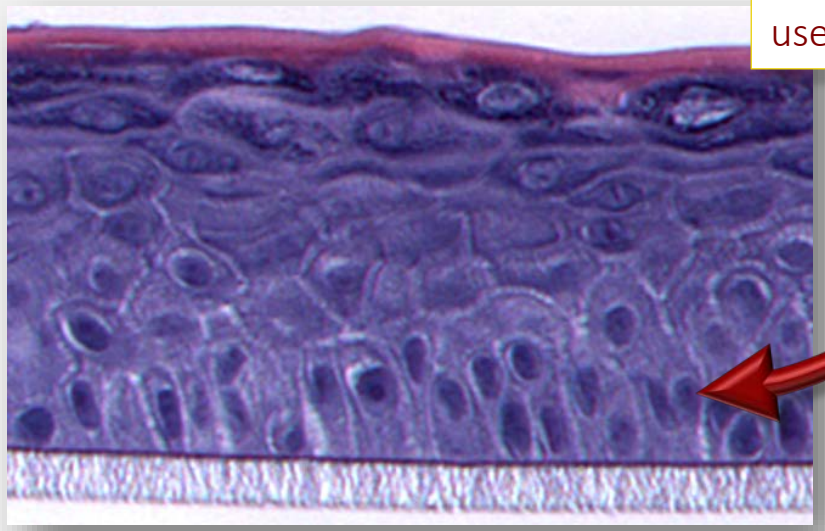
Soluble and not soluble substances



Substances, whatever their physicochemical forms (liquid, powder, gel ...)

Tested products
-Small amounts
-Powders, liquide, pastes…
-Hydro & lipo soluble substances

Barrier function
Topical or systemic application
Mimics the real conditions of use and exposure

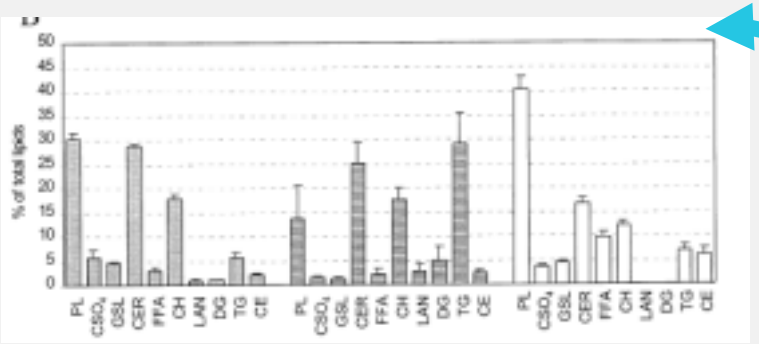


Metabolism
- Xénobiotics (activation, deactivation)
-Stéroids

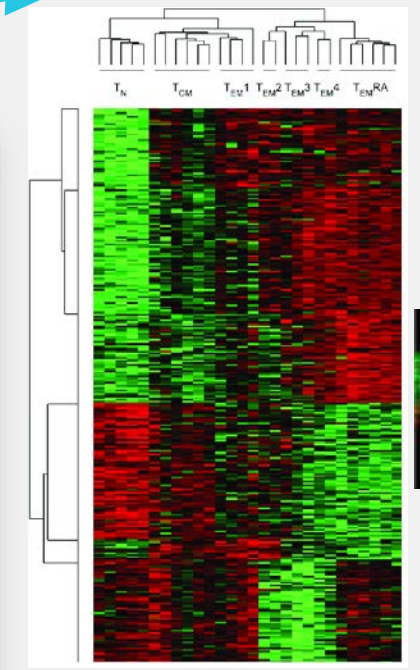
Diversity of measurable parameters
Histology, Enzymatic studies, Biochemistry, Imaging, Transcriptomics/ Toxicogenomic signature, Proteomic studies…

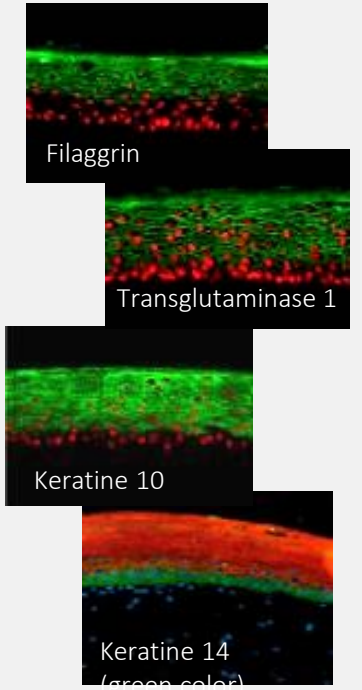
CHARACTERIZATION OF RHE MODELS

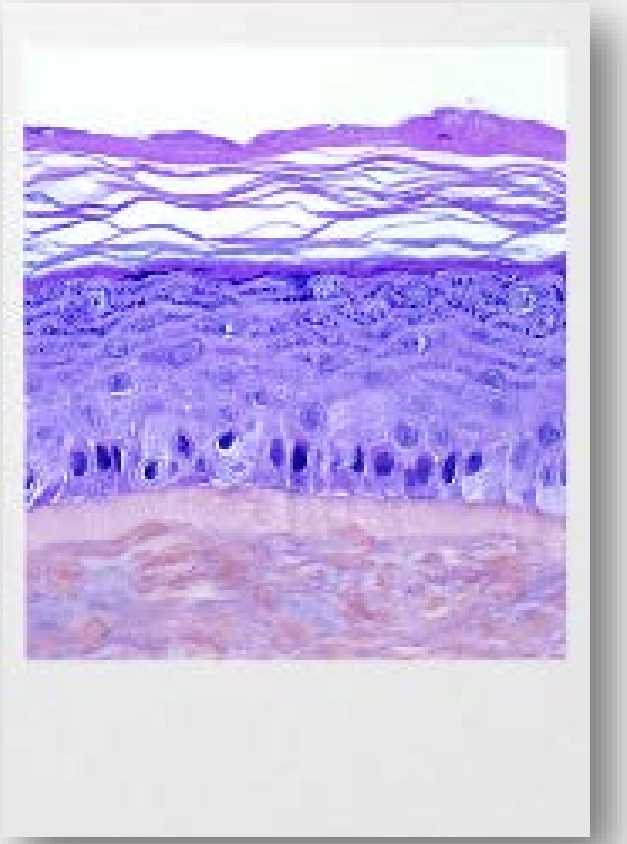
Lipids composition



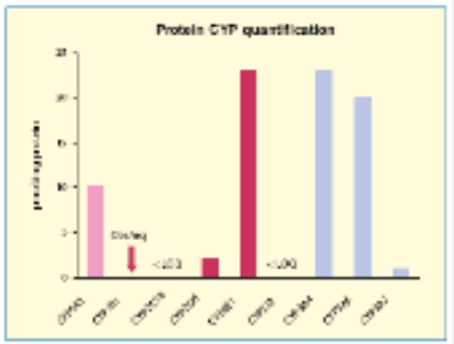
Differentiation/biomarkers

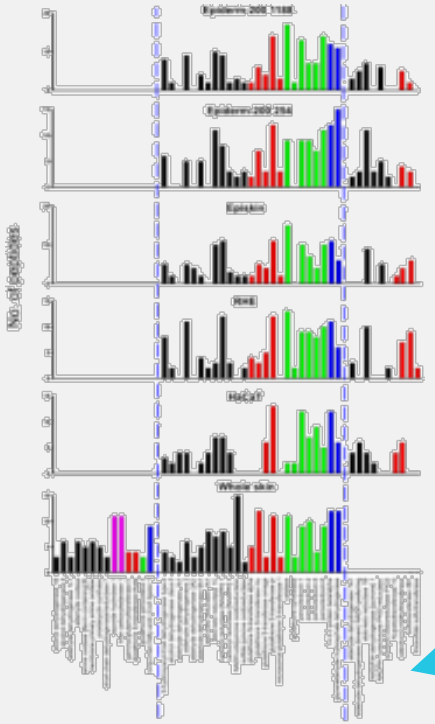






Xenobiotic metabolism





Barrier function

EPISKIN ACADEMY

3D SKIN MODELS FOR …

Basic research
- Homeostasis
- UV effects
- Aging
- Microbiota

Screening safety
- Penetration
- ADME
- Phototoxicity OCDE

Formulation optimization
- Penetration
- bioavailability

Screening efficacy
- Anti-aging
- Anti-inflammatory
- Photoprotection
- Microbiota modulation

Regulatory toxicology: hazard
- Skin corrosion/irritation OCDE ISO
- Eye irritation OCDE
- Skin sensitization OCDE
- Genotoxicity OCDE

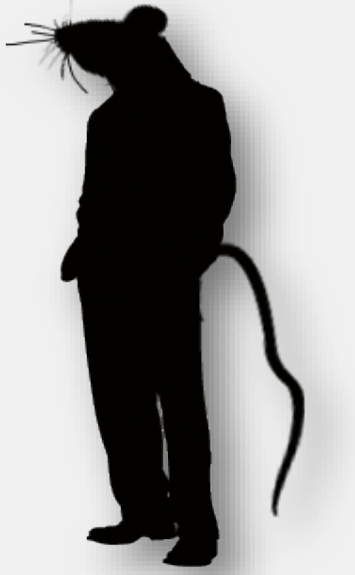
Tolerancy
- Cream/gel
- Shampoo
- Perfume

EPISKIN ACADEMY

NEW PARADIGM IN TOXICOLOGY

Animals

Hu-mal

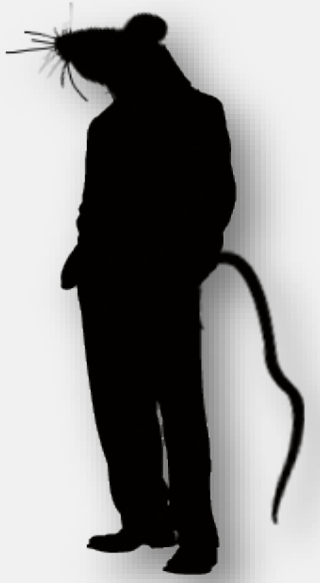


Human

NEW PARADIGM IN TOXICOLOGY

Animals

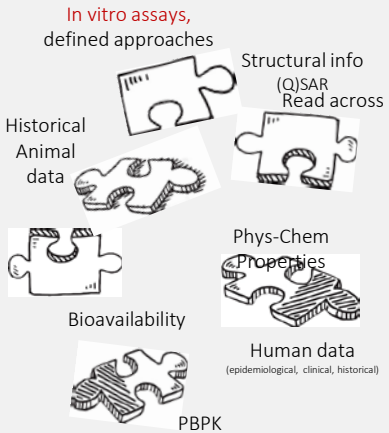
Hu-mal



MECHANISTICS APPROACHES VALIDATION

NAMs

- ✓ Ex vivo methods
- ✓ In vitro methods
- ✓ In chemico methods
- ✓ In silico methods



Human


Nature Reviews | Drug Discovery

REVIEWS

FIRST DOSE OF POTENTIAL NEW MEDICINES TO HUMANS: HOW ANIMALS HELP

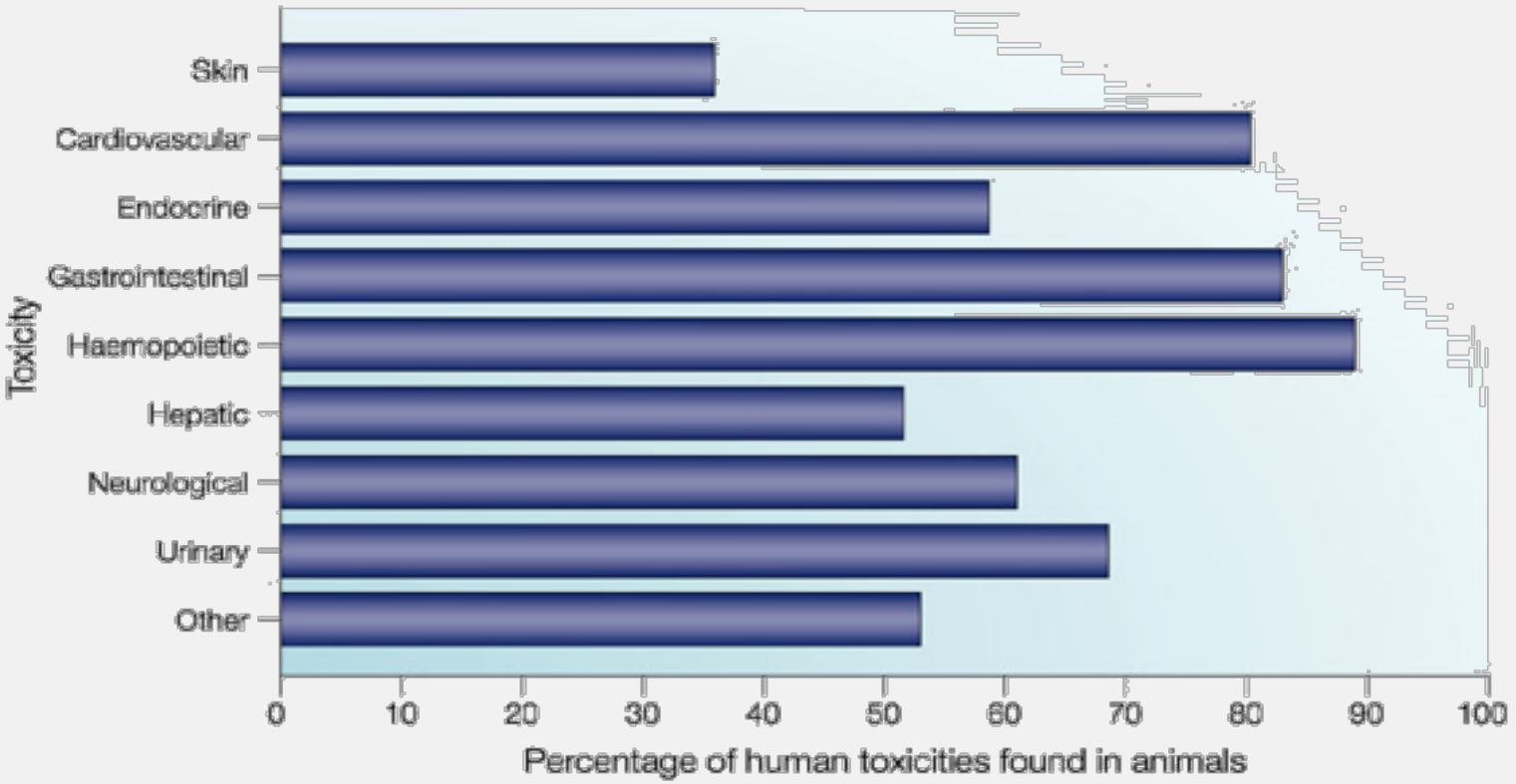
Peter Greaves, Andrew Williams‡ and Malcolm Eve§*

The need for careful testing of new drugs in animal models before study in humans has been recognised by physicians since the First World War. Now, first human studies on new drugs are subject to detailed government guidelines, which in the European Union are presently being reinforced through the wide-ranging *Clinical Trials Directive*. However, despite their long history and widespread application, these guidelines are empirical and have been formulated with a paucity of critical scientific evidence. Here, we review the principles and the available, albeit limited, evidence that support the design and conduct of preclinical studies in a way that permits effective and safe first-dose studies of potential new medicines in humans.

First dose of potential new medicines to humans: How animals help

Nature Reviews Drug Discovery , Volume 3, Issue 3, March 2004, Pages 226-236 - **Greaves P**, Williams A, Eve M.
http://www.nature.com/nrd/journal/v3/n3/full/nrd1329.html

Figure 4 | **Percentage concordance between animal and human toxicities, grouped by organ.**



Similarly to data on anticancer drugs, correlation is better for toxicities in the gastrointestinal tract, and haemopoietic and cardiovascular systems.

Modified, with permission, from Olson et al. 2000 (Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals)

REGULATORY TOXICOLOGY

Toxicological endpoints (human safety) generally used in safety assessment

Local toxicity

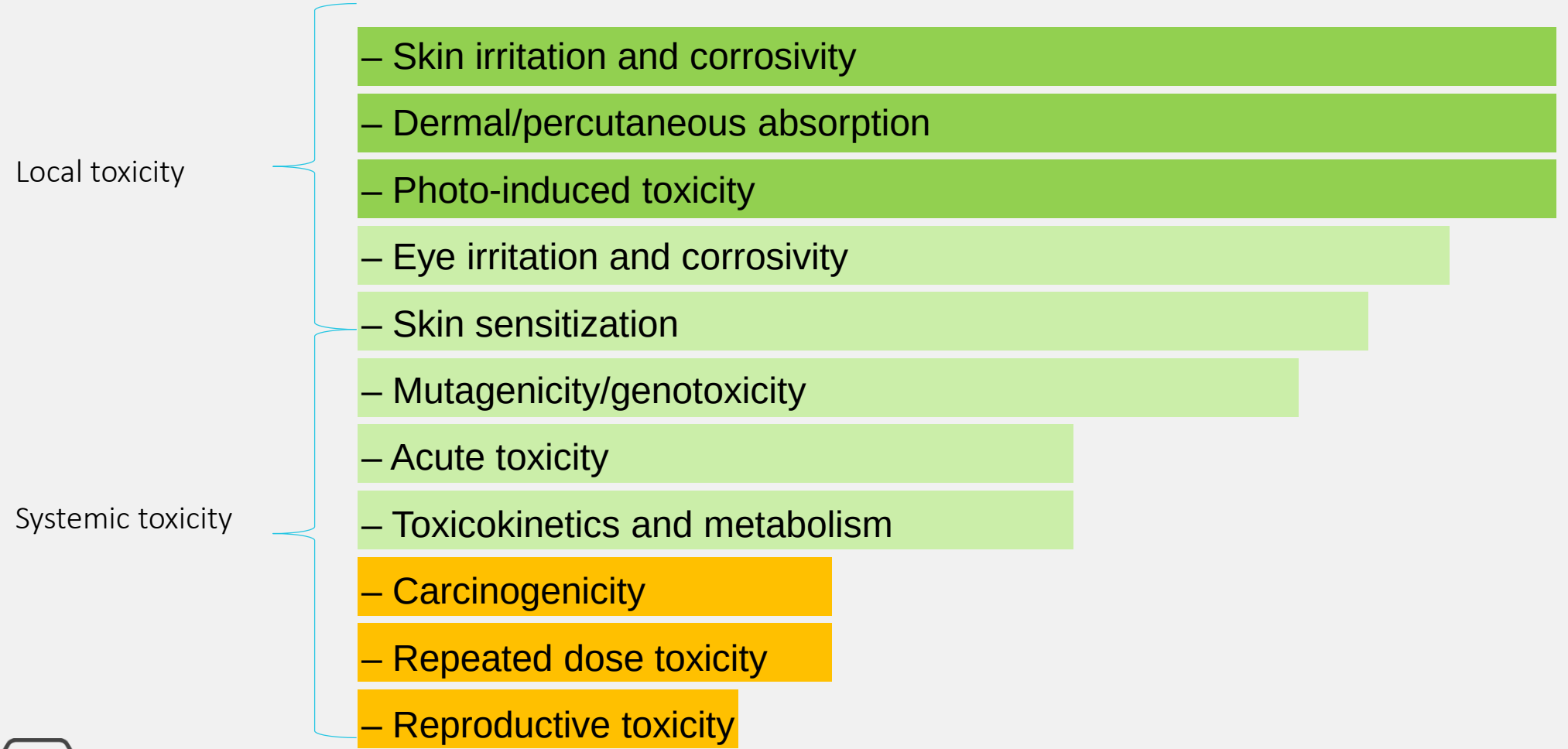
- Skin irritation and corrosivity
- Dermal/percutaneous absorption
- Photo-induced toxicity
- Eye irritation and corrosivity
- Skin sensitization

Systemic toxicity

- Mutagenicity/genotoxicity
- Acute toxicity
- Toxicokinetics and metabolism
- Carcinogenicity
- Repeated dose toxicity
- Reproductive toxicity

NON ANIMAL METHODS

Toxicological endpoints (human safety) generally used in safety assessment



NON ANIMAL METHODS & 3D MODELS

Toxicological endpoints (human safety) generally used in safety assessment

Local toxicity

– Skin irritation and corrosivity

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– Photo-induced toxicity

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– Skin sensitization

– Mutagenicity/genotoxicity

Systemic toxicity

– Acute toxicity

– Toxicokinetics and metabolism

– Carcinogenicity

– Repeated dose toxicity

– Reproductive toxicity

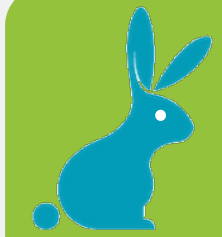
3D MODELS USED IN VALIDATED OECD METHODS

		EPISKIN	Cellink (MatTek)	J-TEC	Henkel (CellSystem)	Biosolution	Sterlab
		France, China, Brazil	US, Slovenia	Japan	Germany	Korea	France
Skin corrosion	TG431	EpiSkin™ SkinEthic™ RHE	EpiDerm™ SCT	LabCyte EPI-MODEL24 SCT	epiCS		
Skin irritation	TG439	**EpiSkin™ (VRM)** SkinEthic™ RHE	EpiDerm™ SIT (EPI-200)	LabCyte EPI-MODEL24 SIT	epiCS		Skin+ ®
Eye irritation	TG492	**SkinEthic™ HCE EIT (VRM 2)**	**EpiOcular™ EIT (VRM 1)**	LabCyte CORNEA-MODEL24 EIT		MCTT HCE™ EIT	
	Project 4.143: TTT* Time To Toxicity	**SkinEthic™ HCE**					
Skin sensitization	Project 4.107: SENS-IS*	EpiSkin™ SkinEthic™ RHE					
Genotoxicity	Project 4.139: Micronucleus & comet*	*EpiSkin™*	EpiDerm™		Phenion		
Phototoxicity	Project 4.138: In Vitro Phototoxicity*	SkinEthic™ RHE	EpiDerm™				

*https://www.oecd.org/chemicalsafety/testing/Test_Guidelines_Workplan_2020.pdf

SKIN IRRITATION OF CHEMICALS

- "**Skin irritation or dermal irritation** is defined as <u>reversible damage</u> of the skin following the application of a test substance for up to 4 hours“



HISTORICAL ANIMAL TEST

Draize Test, OECD TG 404 (1944)

VALIDATED ALTERNATIVE METHOD

OECD TG 439 In vitro method for skin irritation(2009)

Validation

Skin Model

Test Method

Scientific recognition

External Validation

Use for Safety Assessment

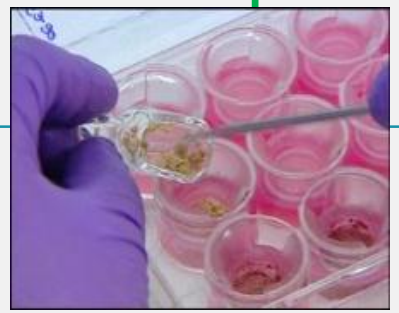
OECD TG 439 IN VITRO SKIN IRRITATION

Preincubation

Application
Time 0

Exposure

15' or 42'
Model specific

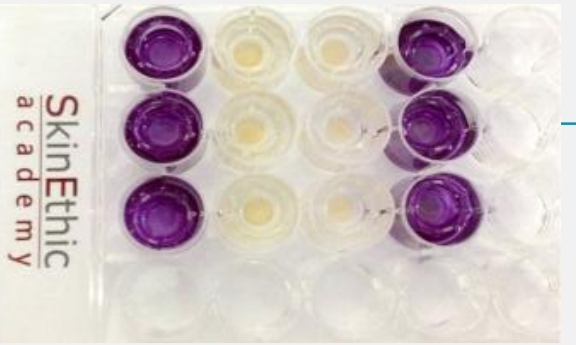


Wash

Recovery

42 hr

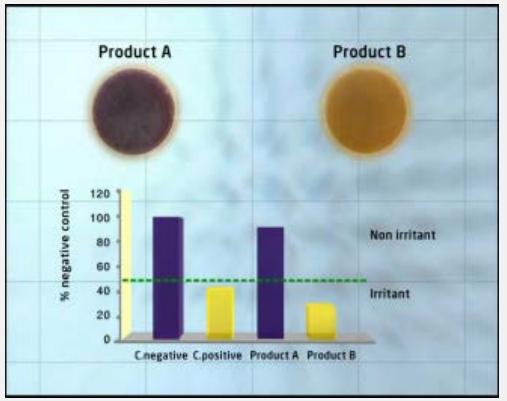
Viability = MTT



OECD

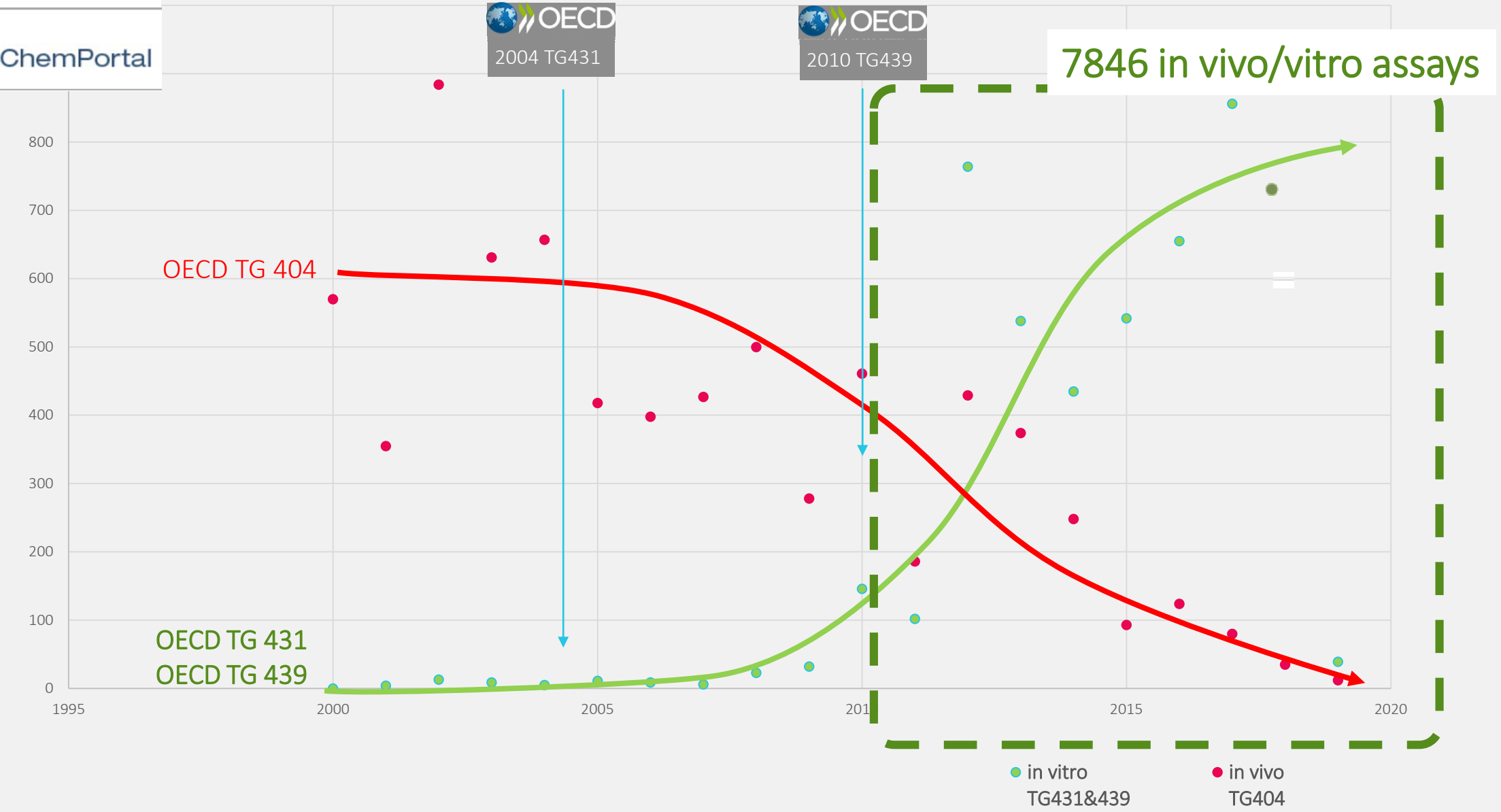
Mean tissue viability is > 50% Non irritant (No Cat)

Mean tissue viability is ≤ 50% - Irritant (Cat 2)



EPISKIN ACADEMY

IMPACT OF TG439 IN THE REAL WORLD



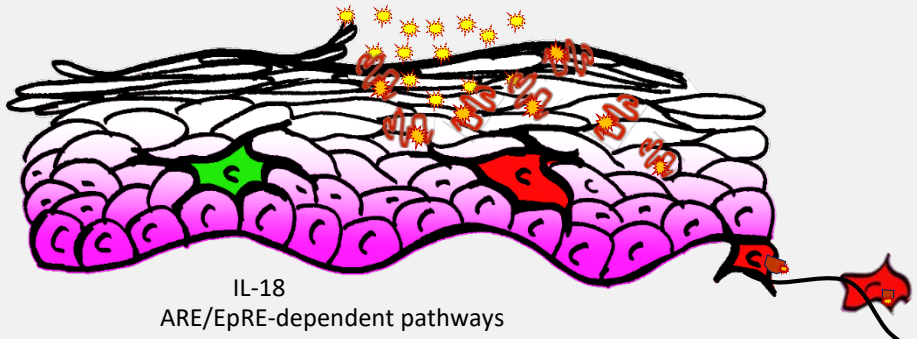
SKIN SENSITIZATION

- Prevalence of contact allergy in the general population (Alinaghi et al. 2019)
- 28 studies covering 20107 patch tested individuals
- Overall, the pooled prevalence of contact allergy was **20.1%.**
- The most common allergen was
  - nickel 11.4%,
  - followed by fragrance mix I 3.5%,
  - cobalt 2.7%,
  - *Myroxylon pereirae* 1.8%,
  - chromium 1.8%,
  - *p*-phenylenediamine 1.5%,
  - methylchloroisothiazolinone/methylisothiazolinone 1.5%,
  - and colophonium 1.3%.

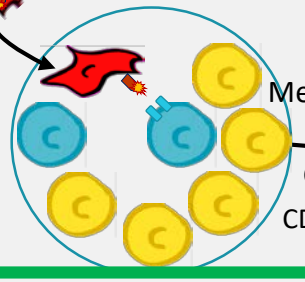
Alinaghi, F, Bennike, NH, Egeberg, A, Thyssen, JP, Johansen, JD. Prevalence of contact allergy in the general population: A systematic review and meta-analysis. *Contact Dermatitis*. 2019; 80: 77–85. https://doi.org/10.1111/cod.13119

SKIN SENSITIZATION

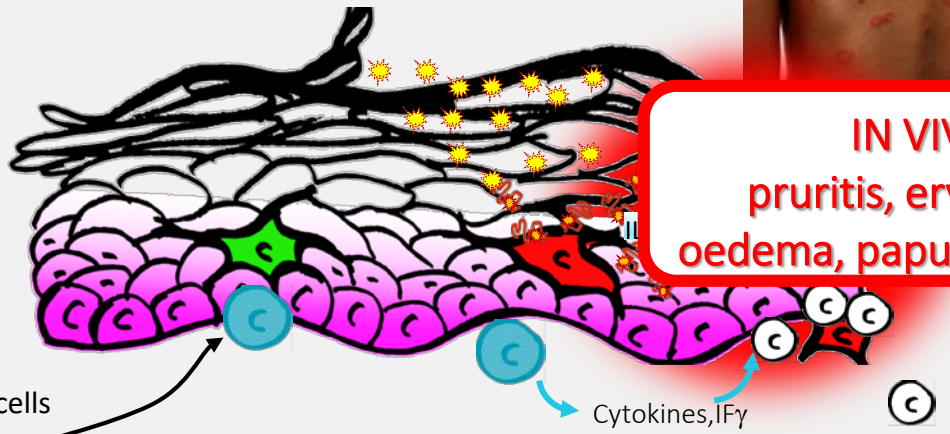
INDUCTION



Local Lymph Node



ELICITATION



IN VIVO
pruritis, erythema, oedema, papules, vesicles

IN VITRO: MECHANISTIC APPROACH

2012: The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins

Chemical structure & properties

1. Skin Penetration

2. Electrophilic substance: directly or via auto-oxidation or metabolism

Molecular initiating event

3-4. Haptenation: covalent modification of epidermal proteins — Ke1

Cellular response

5-6. Activation of epidermal keratinocytes & Dendritic cells — Ke2, Ke3

Organ response

7-8. Presentation of haptenated protein by Dendritic cell resulting in activation & proliferation of specific T cells — Ke4

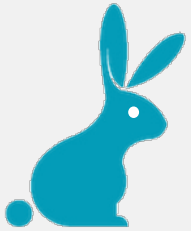
Organism response

9-11. Allergic Contact Dermatitis: Epidermal inflammation following re-exposure to substance due to T cell-mediated cell death

EPISKIN ACADEMY

SKIN SENSITIZATION

HISTORICAL ANIMAL TESTS



Regulatory adopted animal-based tests, which are part of Council Regulation No 440/2008, include:

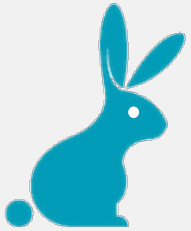
- 1965 - **Buehler** occluded patch test in the guinea pig (OECD TG 406)
- 1969 - **GPMT**, by Magnusson & Kligman (OECD TG 406)
- 1999 - **LLNA** (OECD TG 429), and its non-radioactive modifications, LLNA-DA (OECD TG 442A) and LLNA-BrdU Elisa (OECD TG 442B)

OECD

EPISKIN ACADEMY

SKIN SENSITIZATION

HISTORICAL ANIMAL TESTS



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VALIDATED ALTERNATIVE METHODS

OECD tests guidelines and define approaches (DA)

OECD guideline	Methods	AOP
OECD TG 442C	Direct Peptide Reactivity Assay (**DPRA**)	KE1: protein binding
OECD TG 442D	ARE-Nrf2 Luciferase Test Method: **KeratinoSens**	KE2: keratinocyte activation
OECD TG 442E	1) human Cell Line Activation Test (**h-CLAT**) 2) U937 cell line activation test (**U-SENS**) 3) Interleukin-8 Reporter Gene Assay (**IL-8 Luc assay**)	KE3: dendritic cell activation
OECD Project 4.106:	New TG: Genomic Allergen Rapid Detection test for skin (**GARD™skin**) test: An in vitro method for identification of skin sensitizers based on a genomic interpretation of the impact of chemicals on human dendritic cell-like cells (AOP key event 3).	KE3: dendritic cell activation
OECD Project 4.107:	New TG: Toxicogenomic analysis on 3D reconstituted epidermis for measuring skin sensitization potency – **the SENS-IS assay.**	KE1: protein binding KE2: keratinocyte activation

LLNA results of an Independent Peer Review Evaluation Coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Center for the Evaluation of Alternative Toxicological Methods (NICEATM) (1999)

The Murine Local Lymph Node Assay:
A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds

The Results of an Independent Peer Review Evaluation Coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

February 1999
NIH Publication No. 99-4494

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

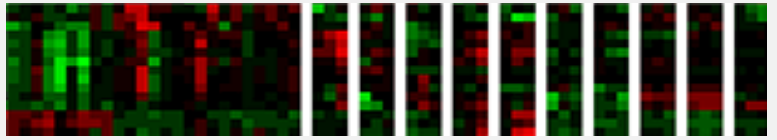
Table 2. Comparative Evaluation of the PRP's Revised LLNA Database[1]

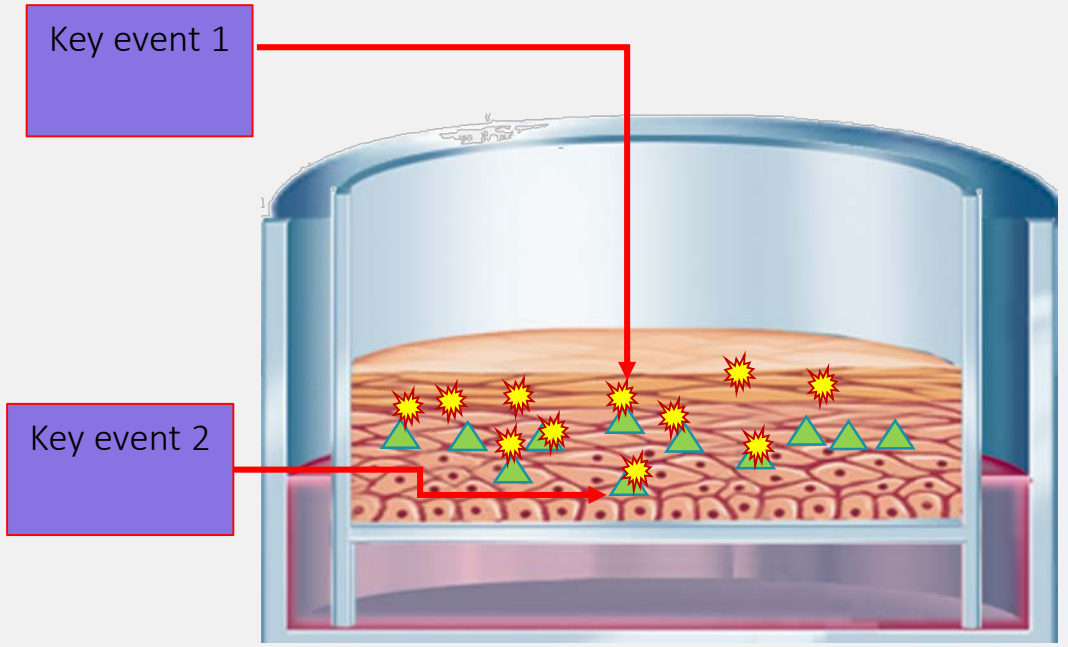
Comparison	Number of Comparisons	Sensitivity[2]		Specificity[3]		Positive Predictivity[4]		Negative Predictivity[5]		Accuracy[6]	
		%	Number	%	Number	%	Number	%	Number	%	Number
LLNA vs GPMT/BA	97	91%	(62/68)	83%	(24/29)	93%	(62/67)	80%	(24/30)	89%	(86/97)
LLNA vs GPT	126	87%	(81/93)	82%	(27/33)	93%	(81/87)	69%	(27/39)	86%	(108/126)
LLNA vs HUMAN	74	72%	(49/68)	67%	(4/6)	96%	(49/51)	17%	(4/23)[7]	72%	(53/74)
GPMT/BA vs HUMAN	57	70%	(38/54)	100%	(3/3)	100%	(38/38)	16%	(3/19)[7]	72%	(41/57)
GPT vs HUMAN	62	71%	(42/59)	100%	(3/3)	100%	(42/42)	16%	(3/20)[7]	73%	(45/62)

Abbreviations: LLNA = Local Lymph Node Assay; GPMT = Guinea Pig Maximization Test; BA = Buehler Assay; GPT includes nonstandard Guinea pig tests; HUMAN = Human Maximization Test (HMT) plus Human Patch Test Allergen (HPTA)

Number of comparisons refers to the number of substances tested in both systems.
Numbers in parentheses indicate actual number of comparisons for each analysis.

SENS-IS ASSAY: TOXICOGENOMIC

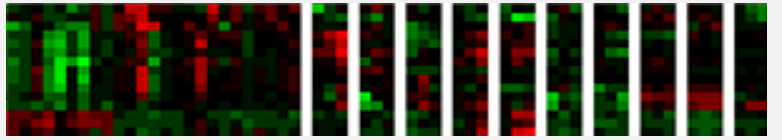




Sensitization assays with RhEs could overcomes some limitations of 2D cell assays:

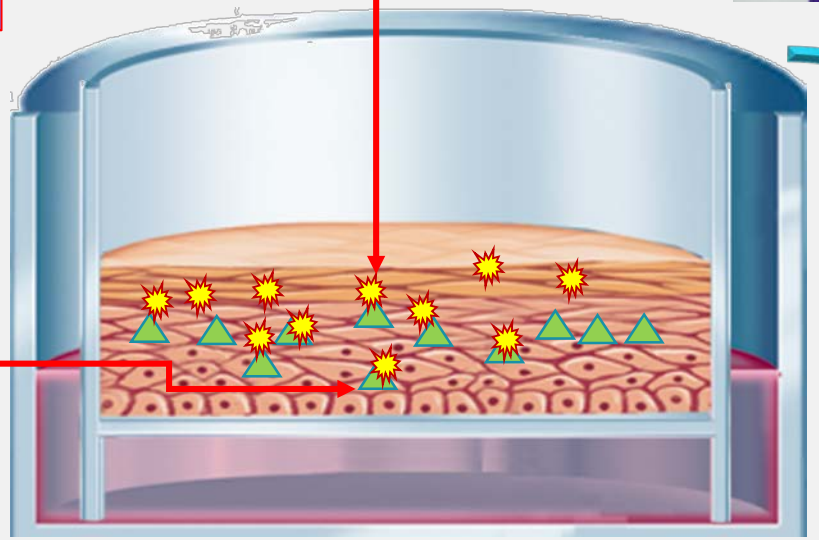
➔ complexe test systems, solubility, bioactivity

SENS-IS ASSAY: TOXICOGENOMIC



Key event 1

Key event 2



application

washing

postincubation

lysing

RT-PCR quantification

62 biomarkers classified into 3 groups : irritation, ARE and SENS-IS genes

Validation assay by analysis of:
- negative control (Propylene Glycol)
- irritant control (5% SLS)
- two sensitizer controls (50% HCA, 1% TNBS)

Sensitization: positive if at least:
- **7/17 genes** in ARE genes group (REDOX group)

and/or

- **7/21genes** in SENS-IS genes group are significantly induced

Potency assessment :
-positive at 0,1% : extreme
-positive at 1% : strong
-positive at 10% : moderate
-positive at 50% : weak

Sensitization assays with RhEs could overcomes some limitations of 2D cell assays:

➔ complexe test systems, solubility, bioactivity

Non-animal methods to predict skin sensitization (I): the Cosmetics Europe database

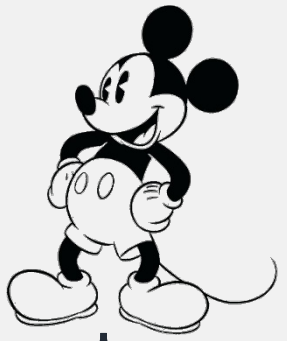


Table 3. Skin sensitization potential predictivity of individual test methods and the mechanistic domains compared to both human and LLNA reference data, incl

		Human				LLNA			
Test method	Sample size	Specificity	Sensitivity	Accuracy	Balanced accuracy	Specificity	Sensitivity	Accuracy	Balanced accuracy
LLNA	128	50.0%	85.2%	74.2%	67.6%	–	–	–	–
DPRA	124*	74.4%	72.9%	73.4%	73.6%	67.7%	66.7%	66.9%	67.2%
KeratinoSens™	128	77.5%	75.0%	75.8%	76.3%	66.7%	67.4%	67.2%	67.0%
h-CLAT	127°	52.5%	89.7%	78.0%	71.1%	51.5%	86.2%	77.2%	68.9%
U-SENS™	105#	44.7%	95.5%	77.1%	70.1%	48.0%	90.0%	80.0%	69.0%
SENS-IS	126"	47.5%	93.0%	78.6%	70.3%	50.0%	90.4%	80.2%	70.2%
Mechanistic reaction domain	122**	75.0%	86.6%	82.8%	80.8%	77.4%	81.3%	80.3%	79.4%

Hoffmann S, Kleinstreuer N, Alépée N, Allen D, Api AM, Ashikaga T, Clouet E, Cluzel M, Desprez B, Gellatly N, Goebel C, Kern PS, Klaric M, Kühnl J, Lalko JF, Martinozzi-Teissier S, Mewes K, Miyazawa M, Parakhia R, van Vliet E, Zang Q, Petersohn D., Crit Rev Toxicol. 2018 May;48(5):344-358. doi: http://10.1080/10408444.2018.1429385

CONCLUSION

- Tissue engineering: Closer to human physiological situation
- Toxicology: Shift from vivo to vitro is engaged in chemicals, cosmetics, pesticides, medical devices…
- Validated 3D models for replacement of the animal in local endpoints
- Promising application for systemical endpoints

Validation is a must have but acceptance is also based on trust and confidence, importance of collaborative approaches, scientific rigor, transparency, communication and education

EPISKIN ACADEMY