

# The Biocompatibility Trail

*a challenging hike towards animal-free testing of Medical Devices*

PETER CORNELIS



CONFIDENTIAL

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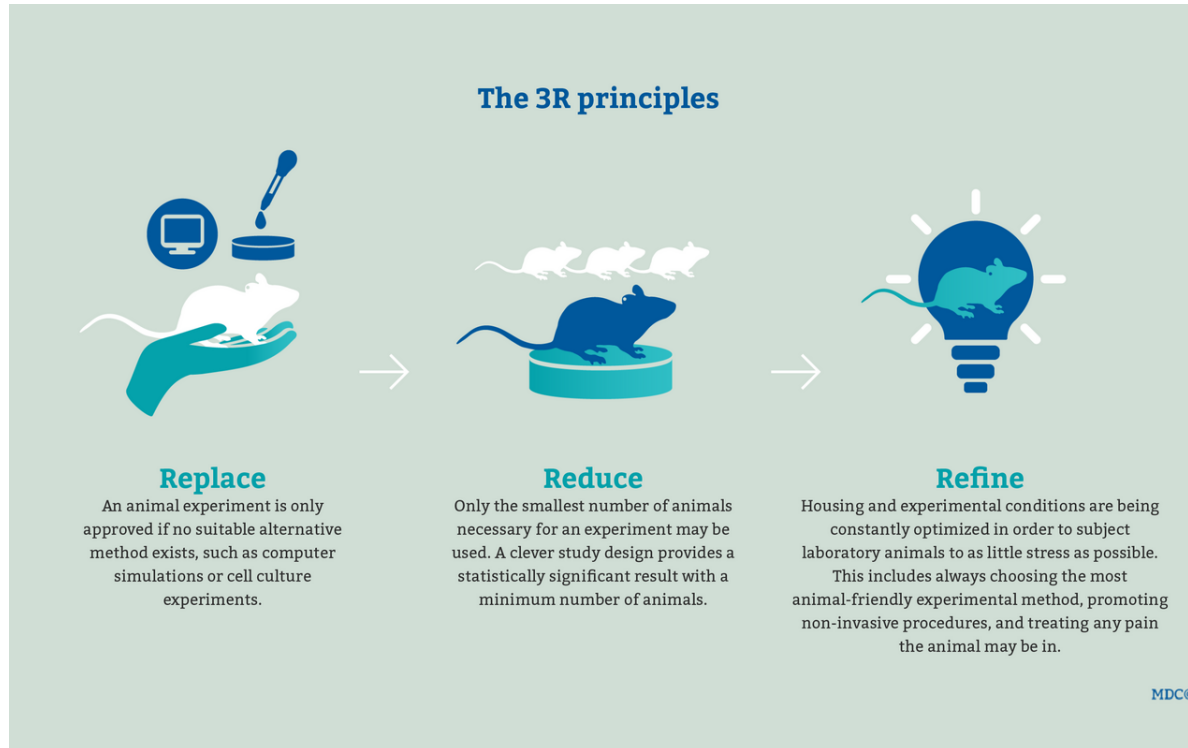
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# Biological evaluation of a Medical Device

- Evaluate the risk of using a medical device
- ISO 10993
- Initially full assesement > 100 animals required

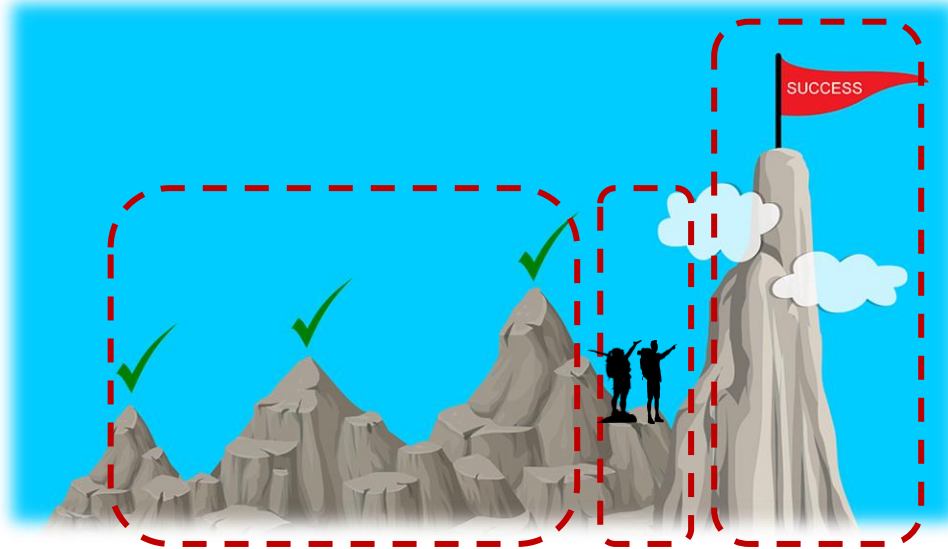


# Trail map: Principle of the 3R's for Animal experimentation (1959)



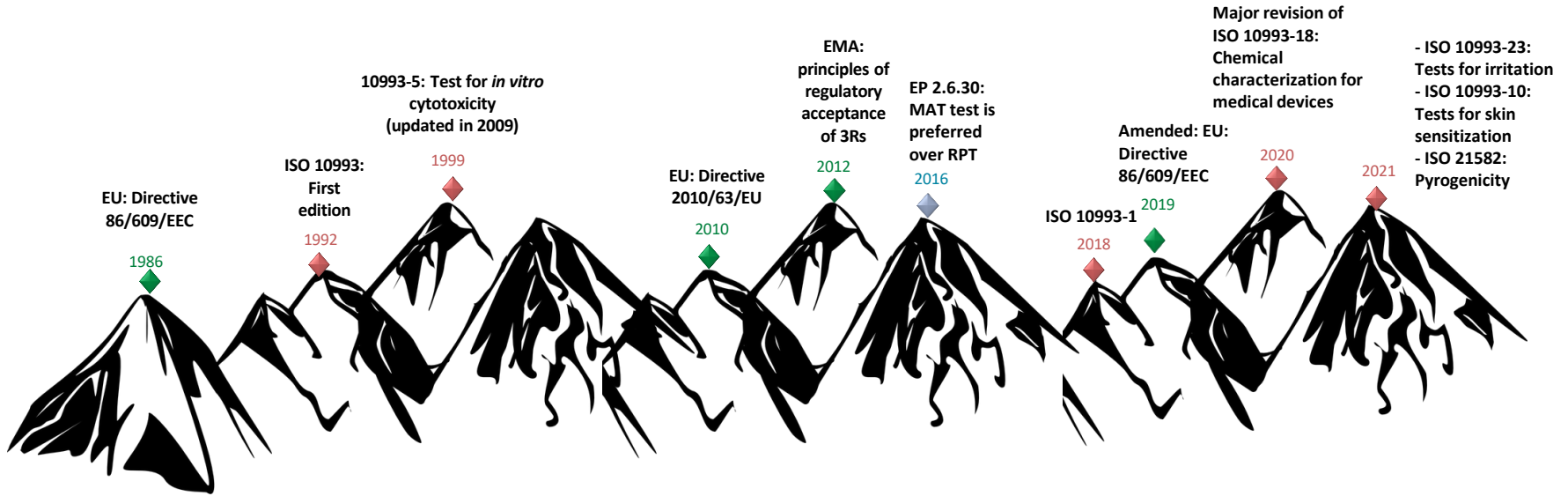
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# Our hike so far



1. The road behind us
2. Which current in-vitro (alternate) trails can we follow?
3. How do we prepare for our ultimate animal-free hike?

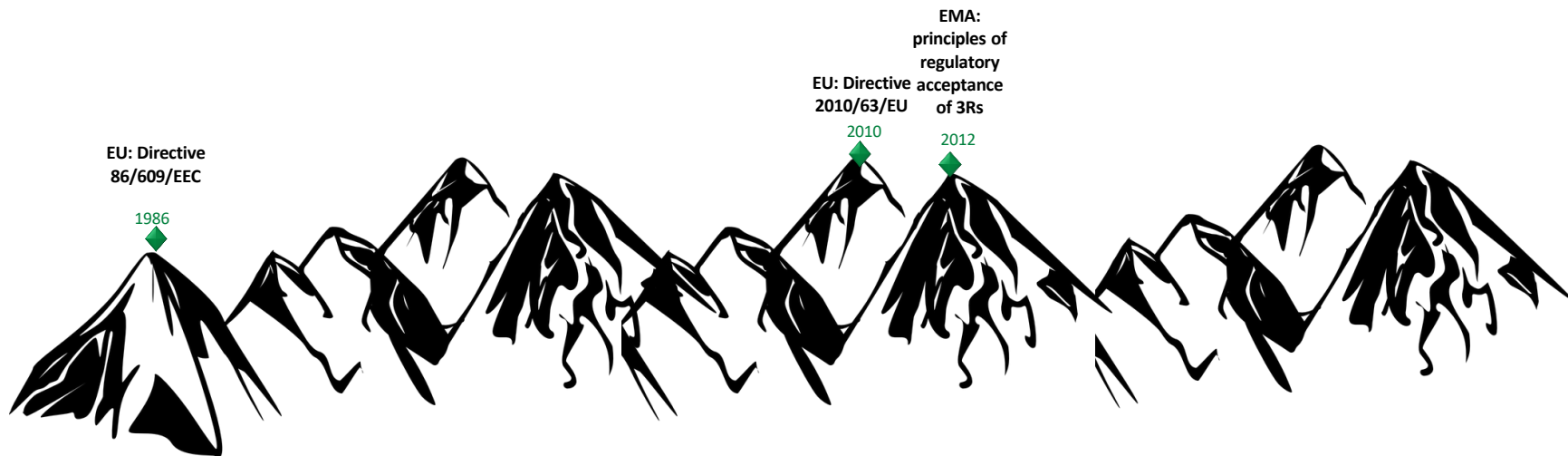
# The regulatory trail



# The regulatory trail



- Anchor & regulatory principles of 3 Rs in EU legislation
  - Reduce
  - Refine
  - Replace



- Regulates & Limit use of lab animals +
- Member States should actively support the development, validation and acceptance of methods which could reduce, refine or replace the use of laboratory animals.



# The ISO 10993 trail



10993-5: Test for *in vitro* cytotoxicity (updated in 2009)

ISO 10993:  
First edition  
1992

1999

ISO 10993-1  
2018

Major revision of ISO 10993-18:  
Chemical characterization for medical devices

2020

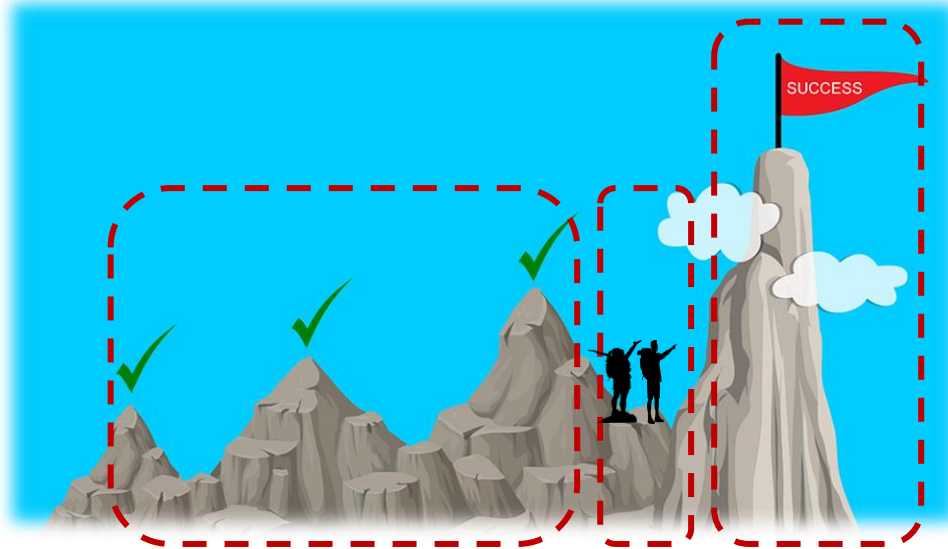
- ISO 10993-23:  
Tests for irritation  
- ISO 10993-10:  
Tests for skin sensitization  
- ISO 21582:  
Pyrogenicity

2021

Medical device categorization by			Endpoints of biological evaluation															
Nature of body contact		Contact duration	Physical and/or chemical information	Cytotoxicity	Sensitization	Irritation or intra cutaneous reactivity	Material mediated pyrogenicity <sup>a</sup>	Acute systemic toxicity <sup>b</sup>	Subacute toxicity <sup>b</sup>	Subchronic toxicity <sup>b</sup>	Chronic toxicity <sup>b</sup>	Implantation effects <sup>bc</sup>	Hemocompatibility	Genotoxicity <sup>d</sup>	Carcinogenicity <sup>d</sup>	Reproductive/developmental toxicity <sup>de</sup>	Degradation <sup>f</sup>	
Category	Contact	A - limited (≤24 h) B - prolonged (>24 h to 30 d) C - Long term (>30 d)																
Surface medical device	Intact skin	A	Xg	E <sup>h</sup>	E	E												
		B	X	E	E	E												
		C	X	E	E	E												
	Mucosal membrane	A	X	E	E	E							E					
		B	X	E	E	E		E	E				E					
		C	X	E	E	E		E	E	E	E		E		E			
	Breached or compromised surface	A	X	E	E	E	E	E										
		B	X	E	E	E	E	E	E				E					
		C	X	E	E	E	E	E	E	E	E		E		E	E		
Externally communicating medical device	Blood path, indirect	A	X	E	E	E	E	E						E				
		B	X	E	E	E	E	E	E				E					
		C	X	E	E	E	E	E	E	E	E		E		E	E		
	Tissue/bone/dentin <sup>i</sup>	A	X	E	E	E	E	E										
		B	X	E	E	E	E	E	E				E		E			
		C	X	E	E	E	E	E	E	E	E		E		E	E		
	Circulating blood	A	X	E	E	E	E	E						E	E			
		B	X	E	E	E	E	E	E				E	E	E			
		C	X	E	E	E	E	E	E	E	E		E	E	E	E		

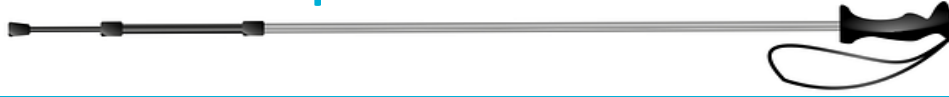


# Our hike so far



1. The road behind us
2. **Which current *in vitro* (alternate) trails can we follow?**
3. How do we prepare for our ultimate animal-free hike?

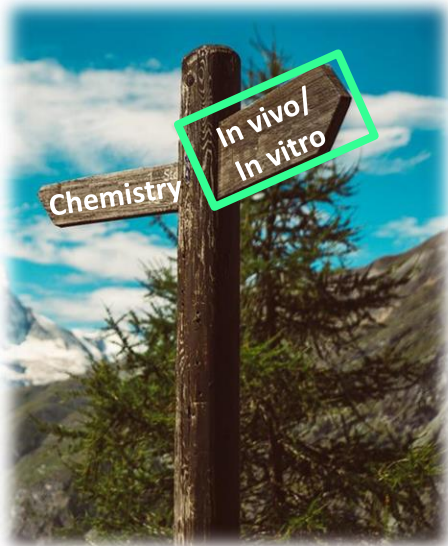
## Table A1 simplified – easier for our trail



# Endpoints of Biological evaluation

Physical and/or Chemical information
Cytotoxicity
Sensitization
Irritation
Material Mediated Pyrogenicity
Acute Systemic toxicity
Sub acute toxicity
Sub chronic toxicity
Chronic toxicity
Implantation effects
Hemocompatibility
Genotoxicity
Carcinogenicity
Reproductive developmental toxicity
Degradation

# Two trails to evaluate long-term toxicities

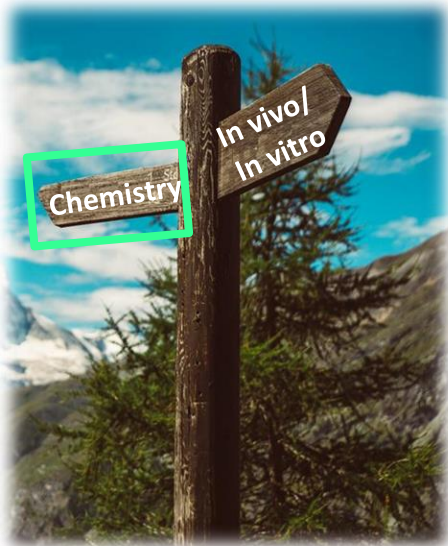


## Endpoints of Biological evaluation

Physical and/or Chemical information	Cytotoxicity	Sensitization	Irritation	Material Mediated Pyrogenicity	Acute Systemic toxicity	Sub acute toxicity	Sub chronic toxicity	Chronic toxicity	Implantation effects	Hemocompatibility	Genotoxicity	Carcinogenicity	Reproductive/developmental tox	Degradation
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Endpoint	# of animals	duration
Acute Systemic Toxicity	3-5	3 days
Subacute Systemic Toxicity	6-10	14 days
Subchronic Systemic Toxicity	8-20	28 days
Chronic Systemic Toxicity	30	6 months +
Genotoxicity	0	12 weeks
Carcinogenicity	Custom	1-2 years
Repro./developmental tox	Custom	Custom

# Two trails to evaluate long-term toxicities



## Endpoints of Biological evaluation

Physical and/or Chemical information	Cytotoxicity	Sensitization	Irritation	Material Mediated Pyrogenicity	Acute Systemic toxicity	Sub acute toxicity	Sub chronic toxicity	Chronic toxicity	Implantation effects	Hemocompatibility	Genotoxicity	Carcinogenicity	Reproductive/developmental tox	Degradation
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Endpoint	# of animals	duration
Acute Systemic Toxicity	0	~12-16 weeks
Subacute Systemic Toxicity		
Subchronic Systemic Toxicity		
Chronic Systemic Toxicity		
Genotoxicity		
Carcinogenicity		
Repro./developmental tox		



Use Chemistry testing and a toxicological risk assessment

# Chemistry: fast track towards animal-free testing.

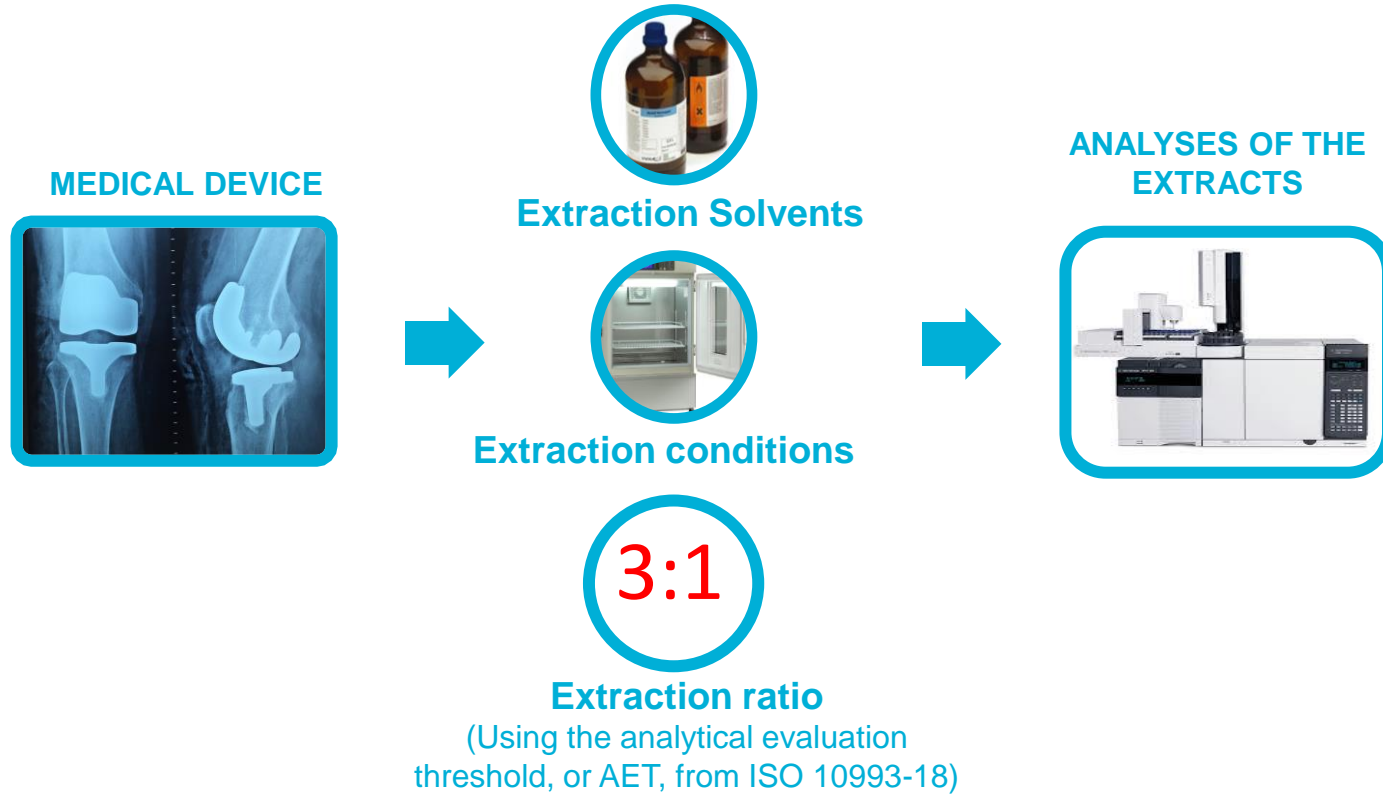
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**Warning:**

**You need to know how to!**

# Design of extractables/leachables (E&L) testing



# Approach to E&L

Volatile  
organic  
compounds

HS-GC/MS  
**Screening**



Semi-volatile  
organic  
compounds

GC/MS  
**Screening**



Non-volatile  
organic  
compounds

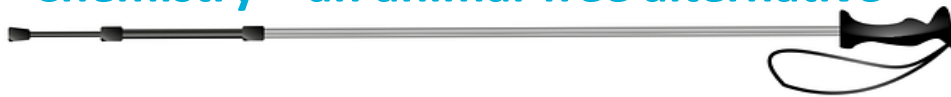
UPLC/MS  
**Screening**









ICP/OES  
**Target**



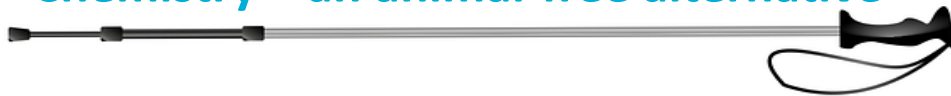
*Analytically*, we cast a **wide net**, looking for essentially everything










## Endpoints of Biological evaluation

Physical and/or Chemical information														
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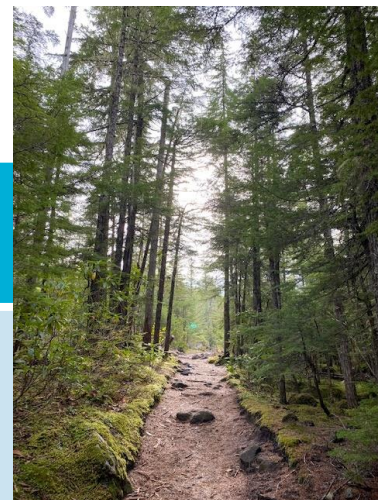
## Endpoints of Biological evaluation

Physical and/or Chemical information	Cytotoxicity	Sensitization	Irritation	Material Mediated Pyrogenicity	Acute Systemic toxicity	Subacute toxicity	Subchronic toxicity	Chronic toxicity	Implantation effects	Hemocompatibility	Genotoxicity	Carcinogenicity	Reproductive developmental toxicity	Degradation	
															

















## Endpoints of Biological evaluation

Physical and/or Chemical information	
Cytotoxicity	
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Carcinogenicity	
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Degradation	

















# Endpoints of Biological evaluation

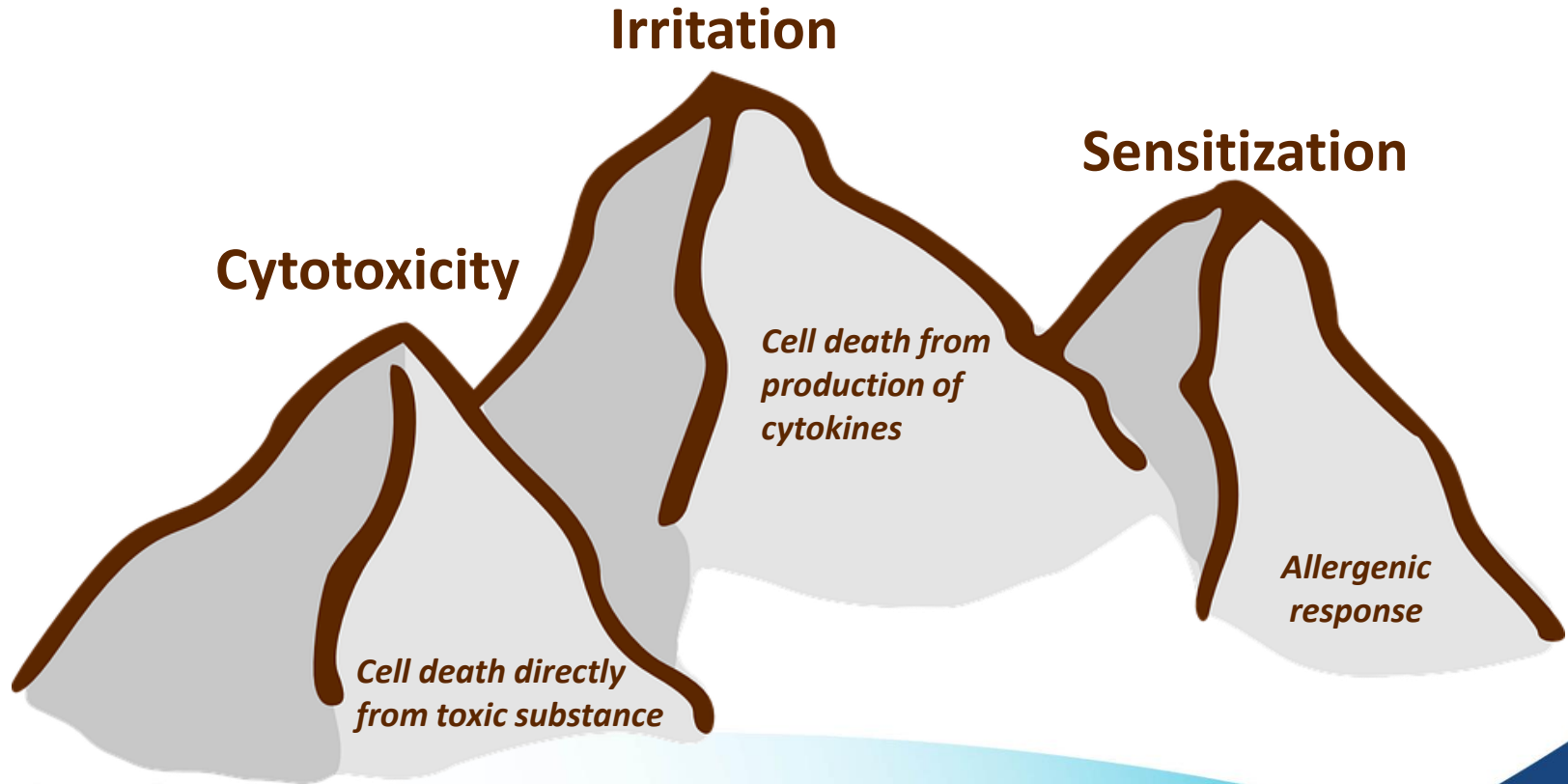
Physical and/or Chemical information	Cytotoxicity	Sensitization	Irritation	Material Mediated Pyrogenicity	Acute Systemic toxicity	Sub acute toxicity	Sub chronic toxicity	Chronic toxicity	Implantation effects	Hemocompatibility	Genotoxicity	Carcinogenicity	Reproductive/developmental tox	Degradation
														



## Endpoints of Biological evaluation

Physical and/or Chemical information	Cytotoxicity	Sensitization	Irritation	Material Mediated Pyrogenicity	Acute Systemic toxicity	Sub acute toxicity	Sub chronic toxicity	Chronic toxicity	Implantation effects	Hemocompatibility	Genotoxicity	Carcinogenicity	Reproductive developmental toxicity	Degradation
														

# The Big Three



# The Big Three



**Irritation**



**Cytotoxicity**

**Sensitization**

*Cell death from  
production of  
cytokines*

*Allergenic  
response*

*Cell death directly  
from toxic substance*

## ISO 10993-23 : Skin Irritation

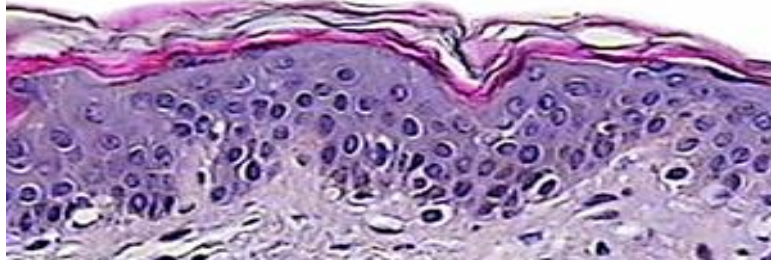
Skin irritation is defined as the production of reversible damage of the skin following the application of a test substance for up to 4 hours



# ISO 10993-23: Skin Irritation

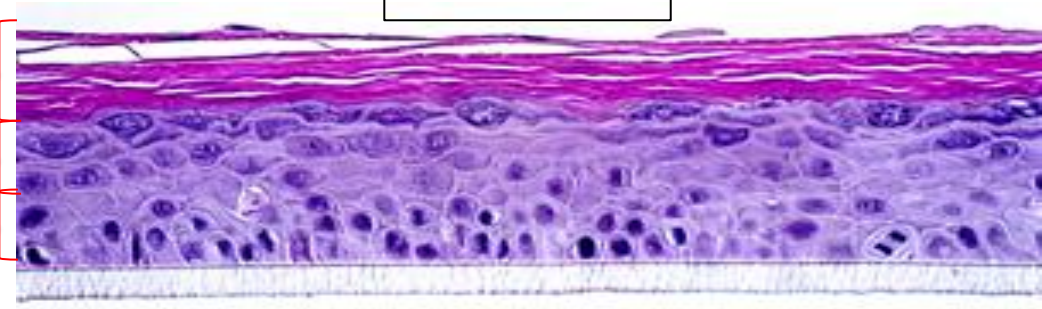
*In vitro* assay is based on using a Reconstructed Human Epidermis (RhE)

*In vivo*



Evaluate cell viability (MTT) of tissue after exposure to extract of product

*In vitro*



Stratum corneum

Stratum granulosum

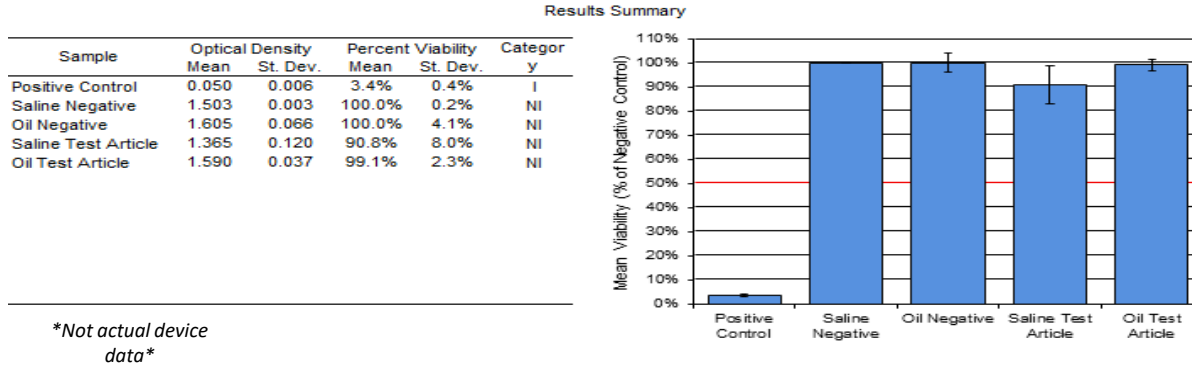
Stratum spinosum

*RhE Tissue*



# ISO 10993-23: Skin Irritation

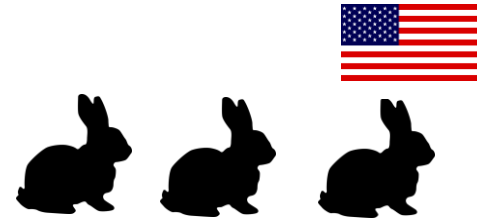
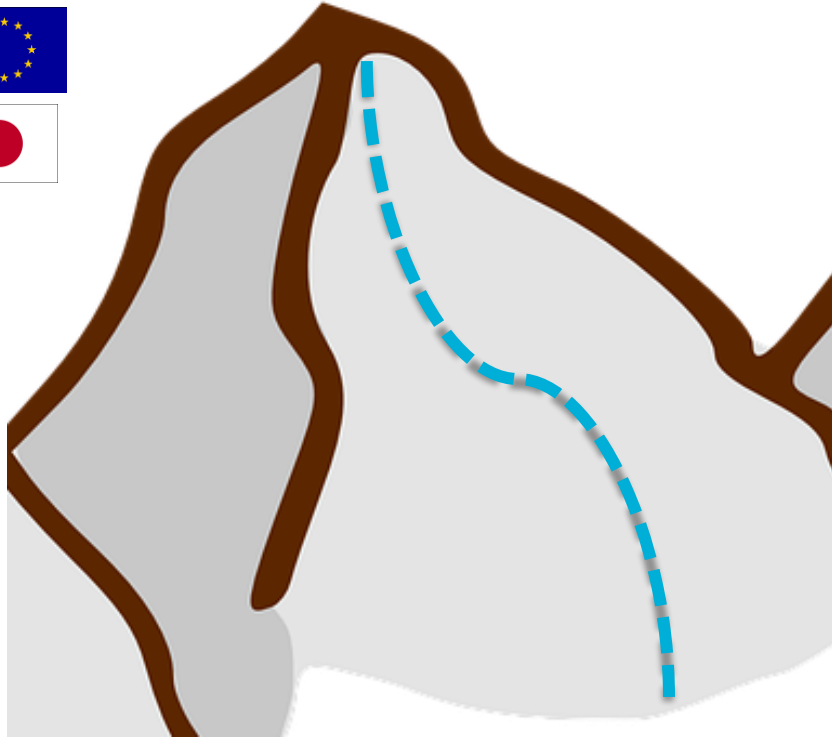
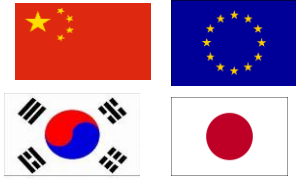
$$\text{Percent viability(\%)} = [\text{OD}_{\text{PC/TA}} / \text{Mean OD}_{\text{NC}}] \times 100$$



**Table 1 — Classification of test sample**

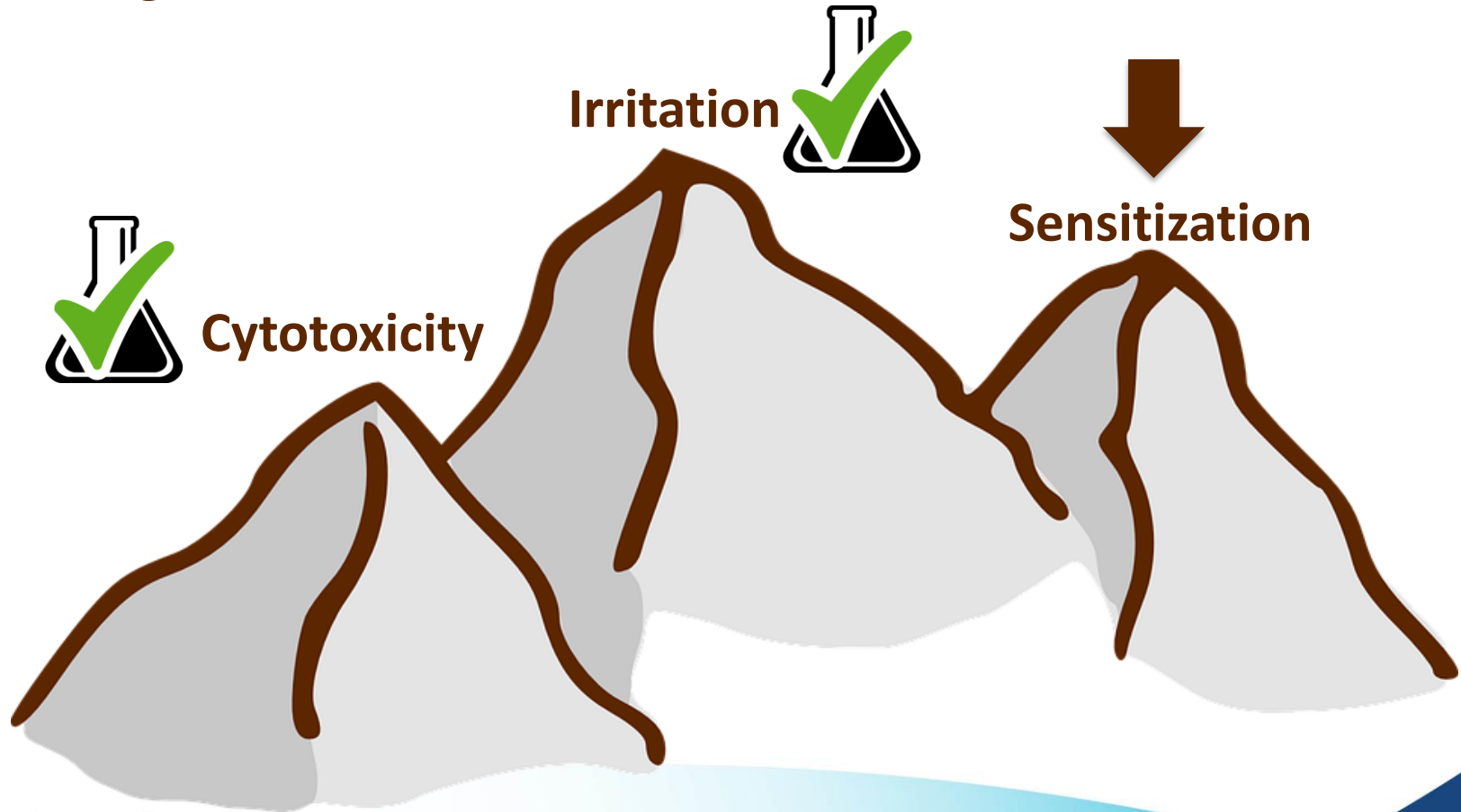
Criteria for <i>in vitro</i> interpretation	Classification
Mean tissue viability is ≤50 % in at least one extraction vehicle	Irritant (I)
Mean tissue viability is >50 % in the two extraction vehicles	Non-irritant (NI)

# *In vitro* reconstructed human epidermis (RhE) model → ISO 10993-23

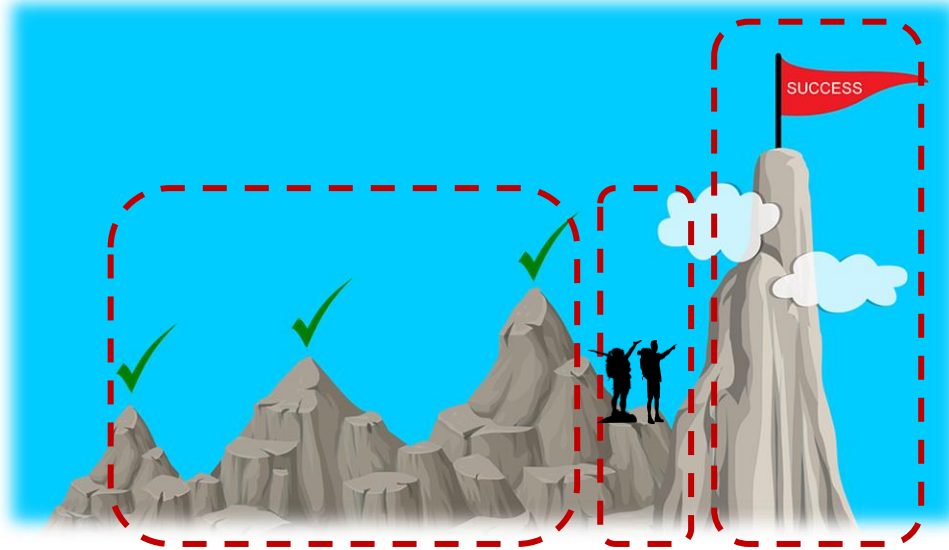


*In vivo* irritation test by skin exposure/by intracutaneous administration → ISO 10993-23

# The Big Three



# Our hike so far

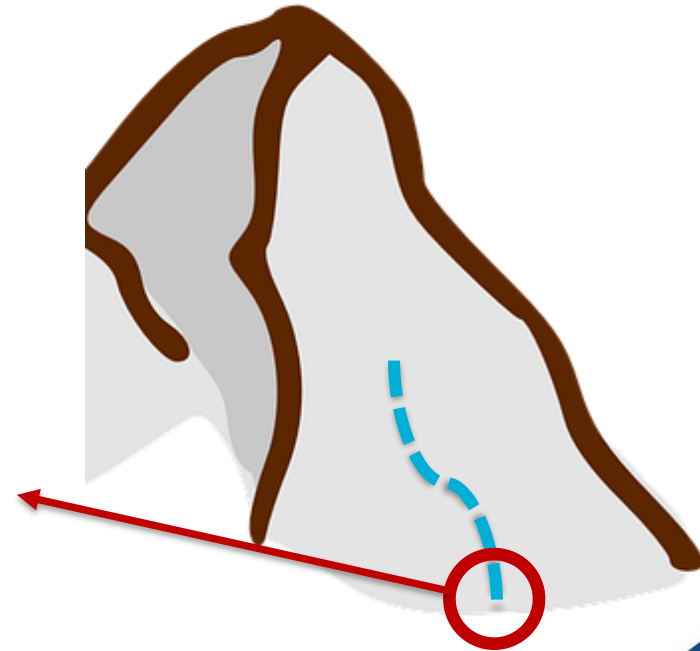


1. What have we learned from the previous hikes?
2. Which current *in vitro* (alternate) trails can we follow?
3. **How do we prepare for our ultimate animal-free hike**

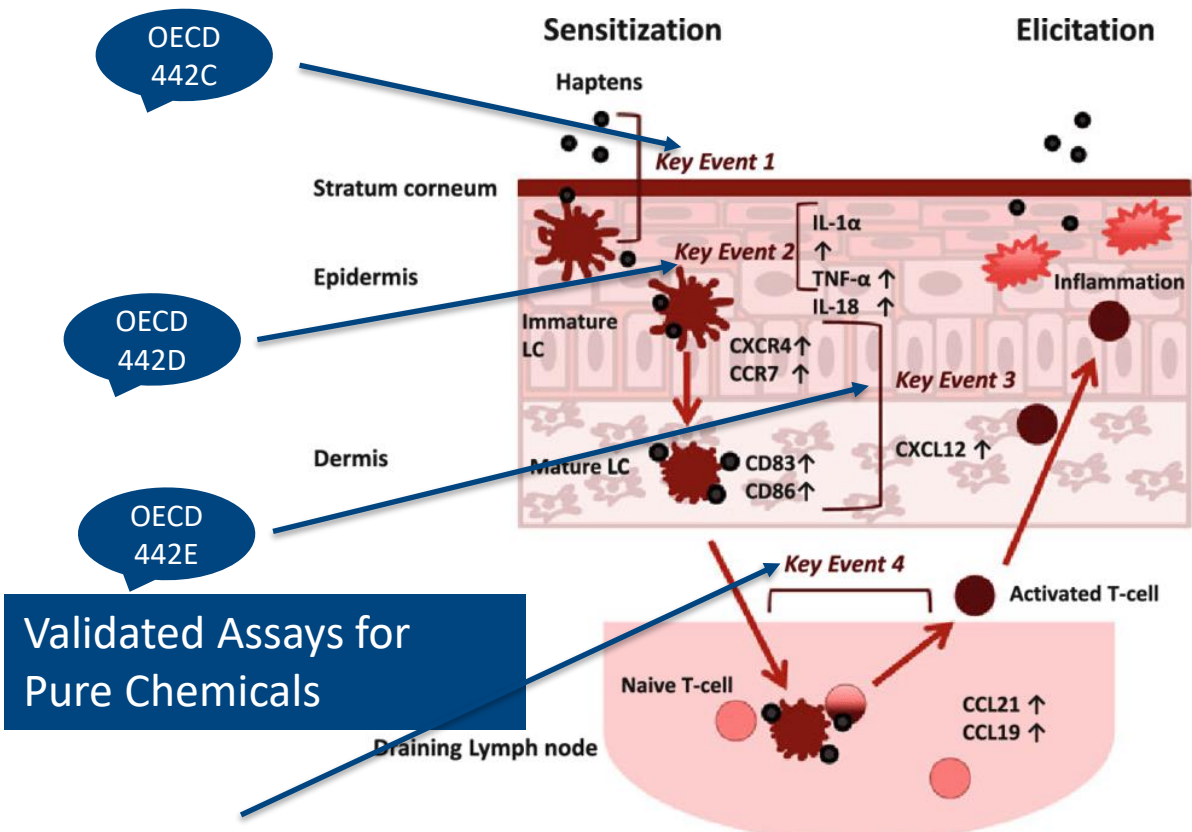
# Skin sensitization

Def.: Skin sensitization is defined as allergic response to a substance after skin contact.

Test Method	Device Contact	# animals	Data
Guinea Pig Maximization	Indirect	35	Qualitative
Local Lymph Node Assay (LLNA)	Indirect	5	Quantitative
Buehler	Direct	35	Qualitative



# Adverse Outcome Pathway (AOP) for Skin Sensitization (OECD)



Adverse Outcome

Not a single *in vitro* assay can replace the *in vivo* sensitisation assay. A combination of several assays is required

Validated Assays for Pure Chemicals

AOP



### KEY event 1

OECD 442 C (2015): Direct Peptide Reactivity Assay (HPLC)

### Key Event 2

OECD 442 D (2018): **Keratinosense method**

### Key Event 3

OECD 442 E (2018): H-Clat – **Usense** – IL8 assayGard

### Key event 4

No validated assay available.

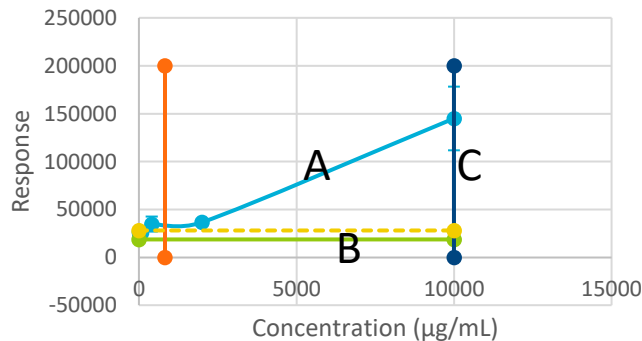
Tests are validated for **pure** chemicals – not for more complex extracts from medical devices!!!

## R&D at Nelson Labs

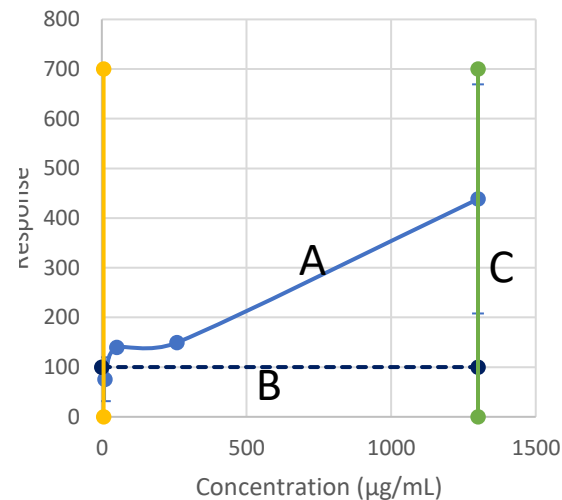


20 different known sensitizers spiked to extracts of medical devices in 2 methods

### Keratinosense



### U-sense



A) Concentration response of the known sensitizer

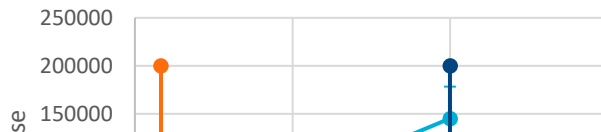
B) Response above this line is considered a sensitizer

C) Sensitizing concentration for the animal test

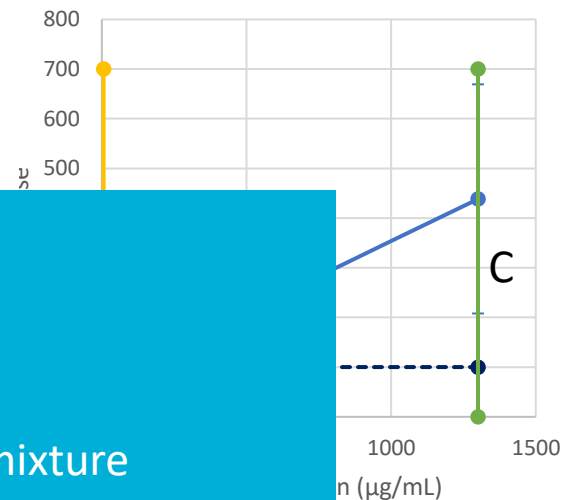


## R&D at Nelson Labs

### Keratinosense



### U-sense



### Conclusions from several years of research:

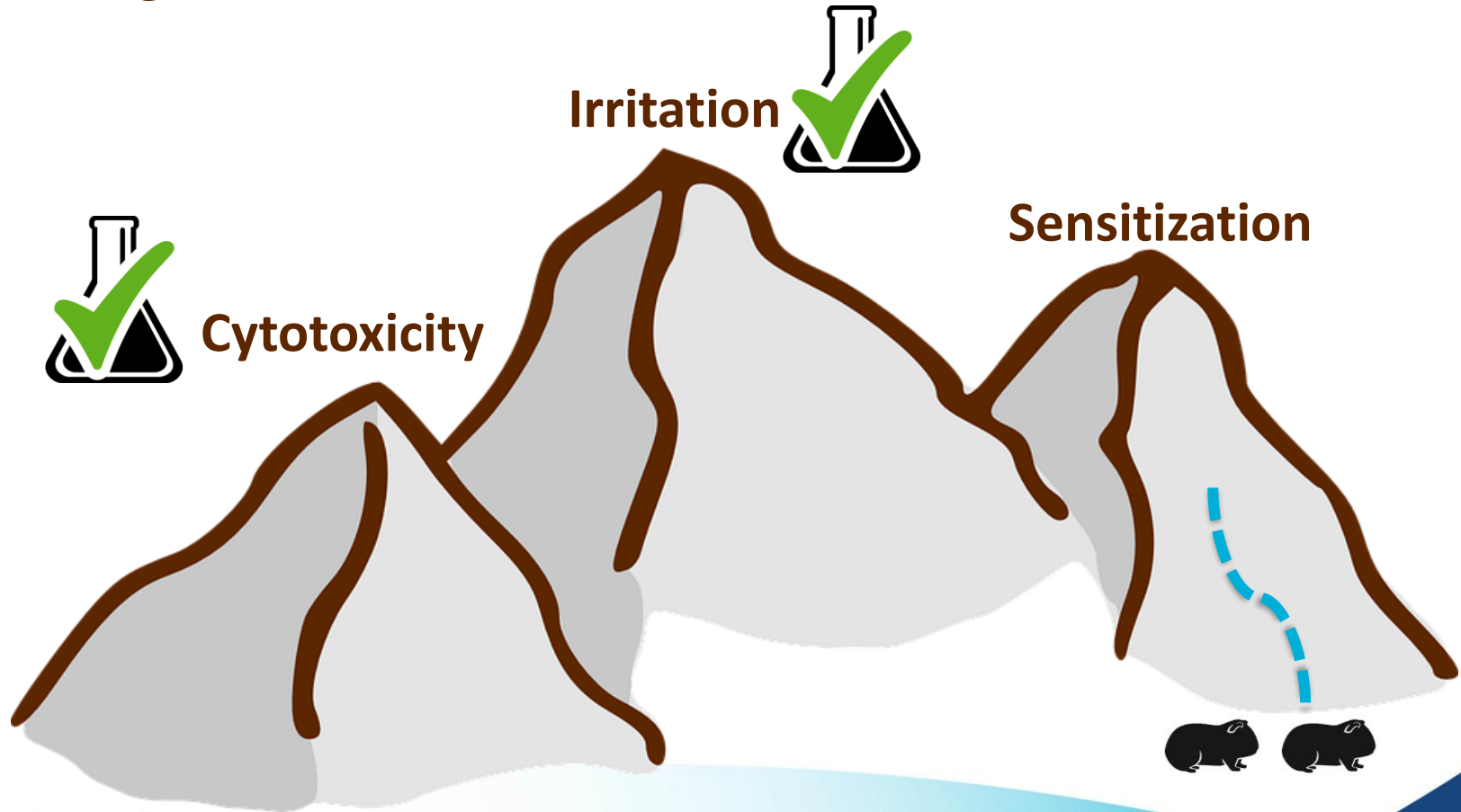
1. Success with apolar solvents
2. Little or no interference from extraction mixture
3. More sensitive than animal tests

20 different known sensitizers spiked to extracts of medical devices in 2 methods

line is considered a sensitizer B

C) Sensitizing concentration for the animal test

# The Big Three

















# A review of our trail



Which peaks do we still need to conquer?



# Endpoints of Biological evaluation

Physical and/or Chemical information	Cytotoxicity	Sensitization	Irritation	MAT? Material Mediated Pyrogenicity	Acute Systemic toxicity	Sub acute toxicity	Sub chronic toxicity	Chronic toxicity	Implantation effects	Blood loop? Hemocompatibility	Genotoxicity	Carcinogenicity	Reproductive developmental toxicity	Degradation
														

# FINAL THOUGHTS

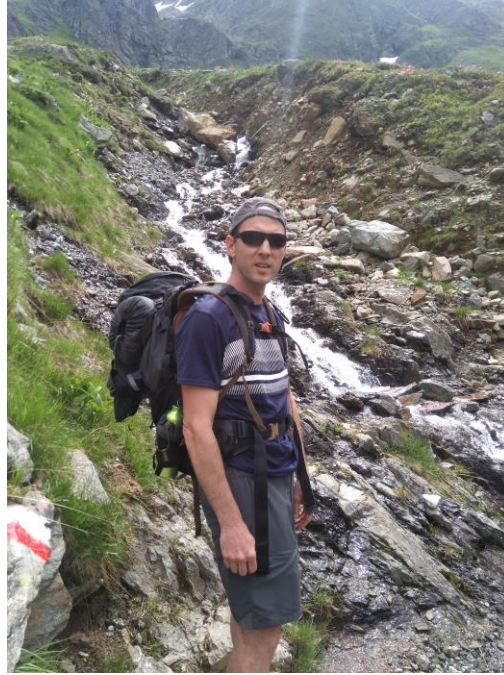


- ✓ Safe medical devices
- ✓ No animal testing



**We are not there yet but we are close!**

# THANK YOU!



Peter Cornelis  
pcornelis@nelsonlabs.com



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