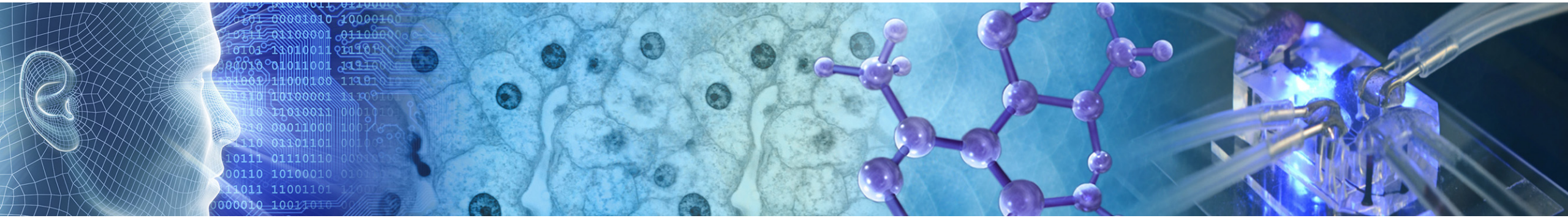
NTP
National Toxicology Program

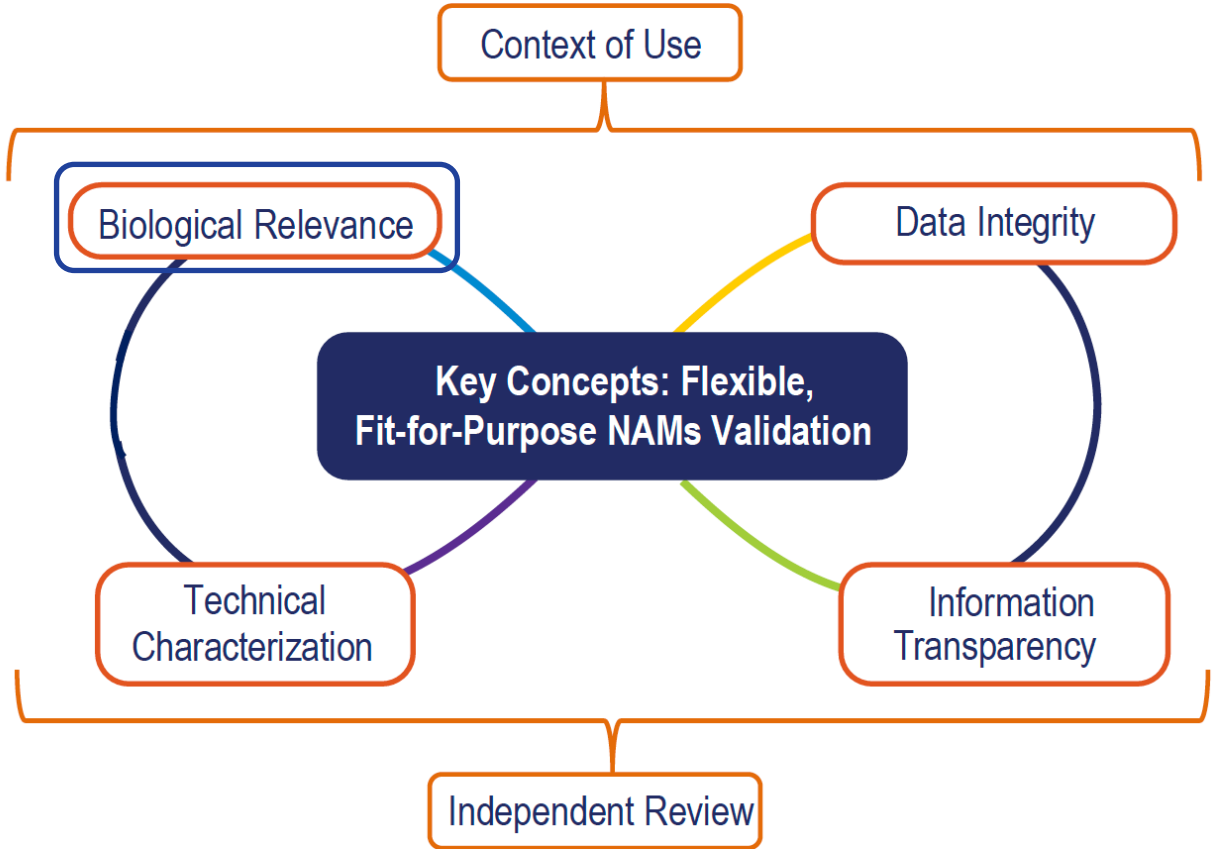


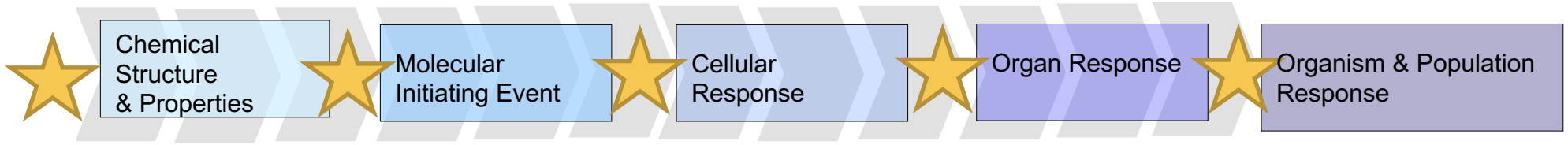
Biological Relevance as a Better Benchmark

2022 SACATM Meeting

Nicole Kleinstreuer
Acting NICEATM Director
NIEHS/DTT/PTB

Assessing Alternatives: A New Gold Standard





Ongoing NICEATM and ICCVAM Projects

- Integrated Chemical Environment
- OPERA (QSAR/QSPR)
- Computational Chemistry
- Quantitative IVIVE
- Reference data curation
- Variability of in vivo data
- Acute Systemic Toxicity
- Dermal absorption
- Skin sensitization
- Eye and skin irritation
- Developmental Toxicity
- DNT Testing Battery
- Cardiovascular Toxicity
- Carcinogenesis
- Ecotoxicology
- Zebrafish models
- Animal-free affinity reagents
- Microphysiological Systems
- Evolving Process of Validation

- ICCVAM Biennial Report – PDF and web format >
- Summarizes US agency activities to promote alternatives or reduce animal use

Report for 2020-2021 is out now!

https://ntp.niehs.nih.gov/go/2021iccvamreport

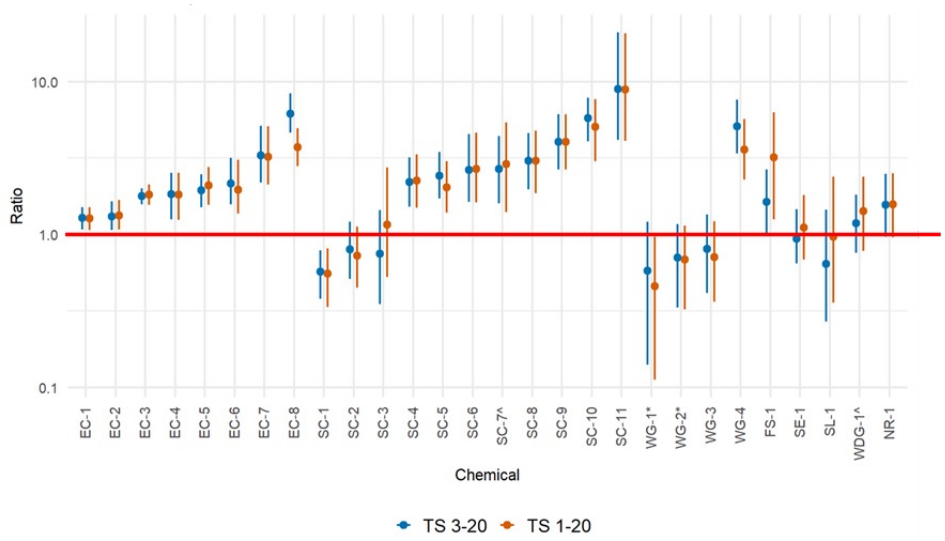
Subscribe to NICEATM News email list

https://ntp.niehs.nih.gov/go/niceatm

Dermal Absorption Analyses

Allen et al. 2021 ALTEX

- Absorption through in vitro human skin was found to be similar to, or less than, that observed in rat skin (in vitro and in vivo) for all formulations.
- The human in vitro assay provided a similar or higher estimate of dermal absorption than the triple pack
- For human health risk assessment, in vitro assays using human skin would be preferable. Such tests would be directly relevant to the species of interest (humans) and avoid any overestimation of dermal absorption using rat models.

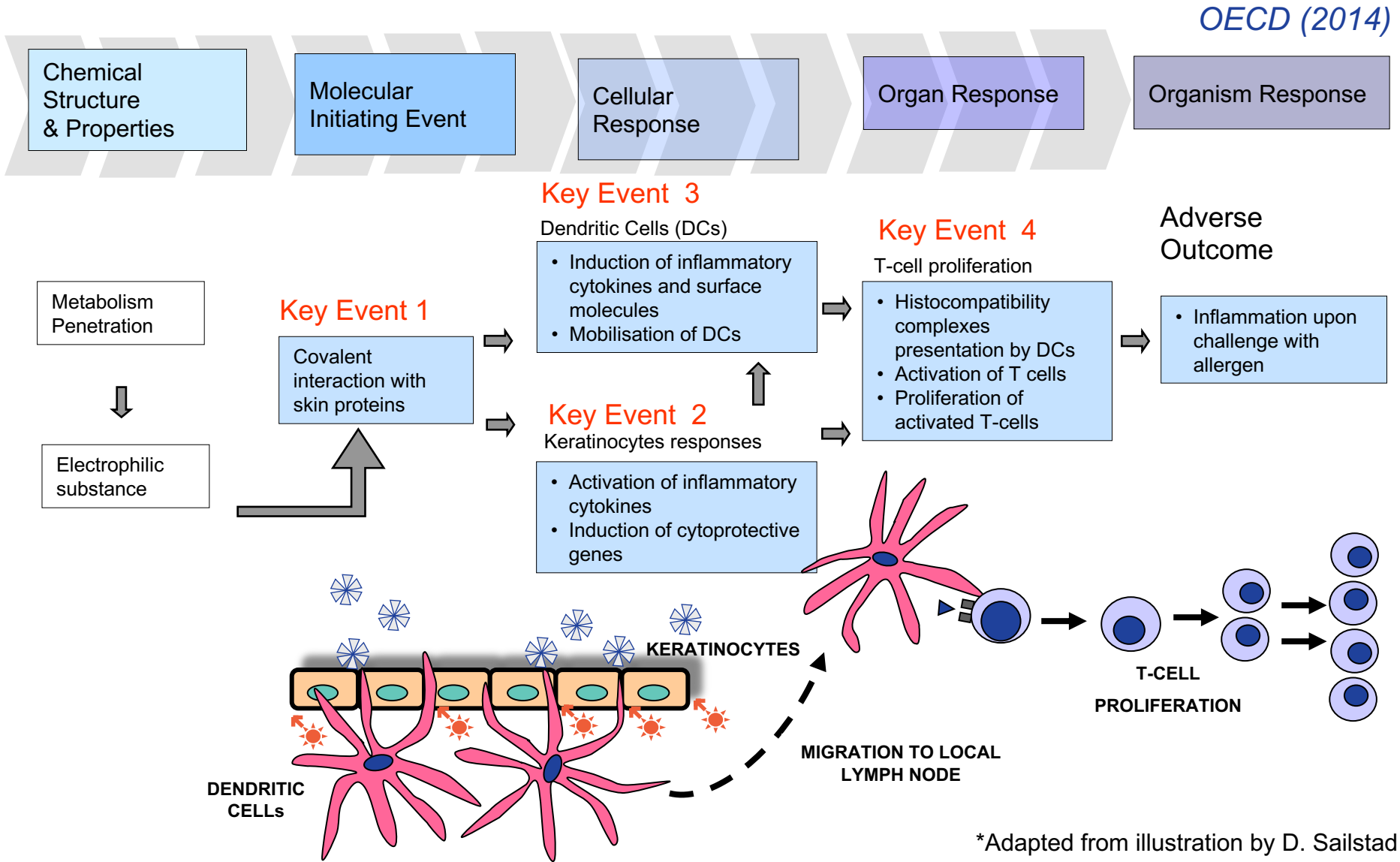


- Rat in vitro studies would still have utility if human in vitro data were not available and in vitro rat data provide estimates of dermal absorption that are at least as protective as in vivo rat data, and thus could also be considered adequate for use in establishing dermal absorption factors.

$$triple\ pack\ DAF = rat\ in\ vivo \times (human\ in\ vitro \div rat\ in\ vitro)$$

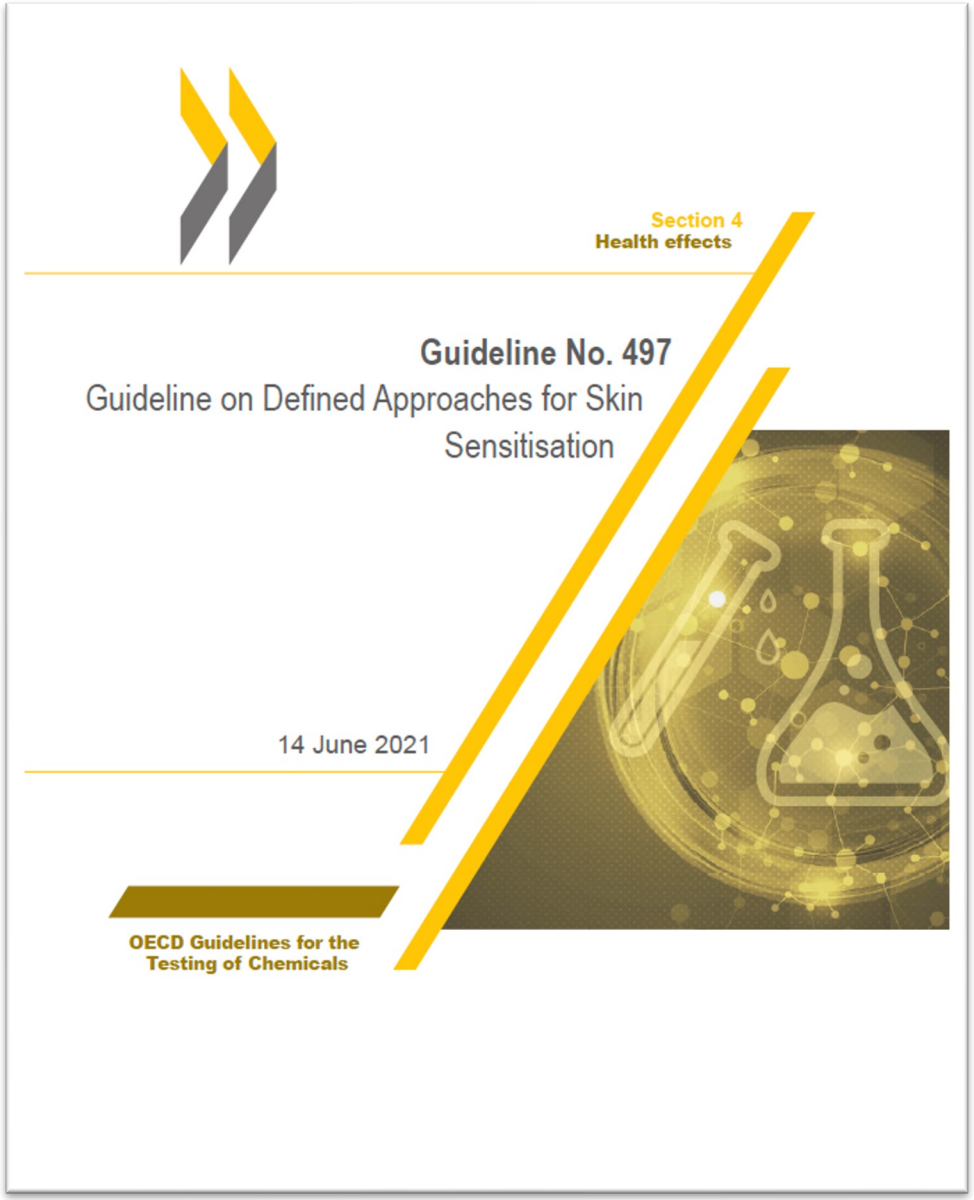
Skin Sensitization Adverse Outcome Pathway

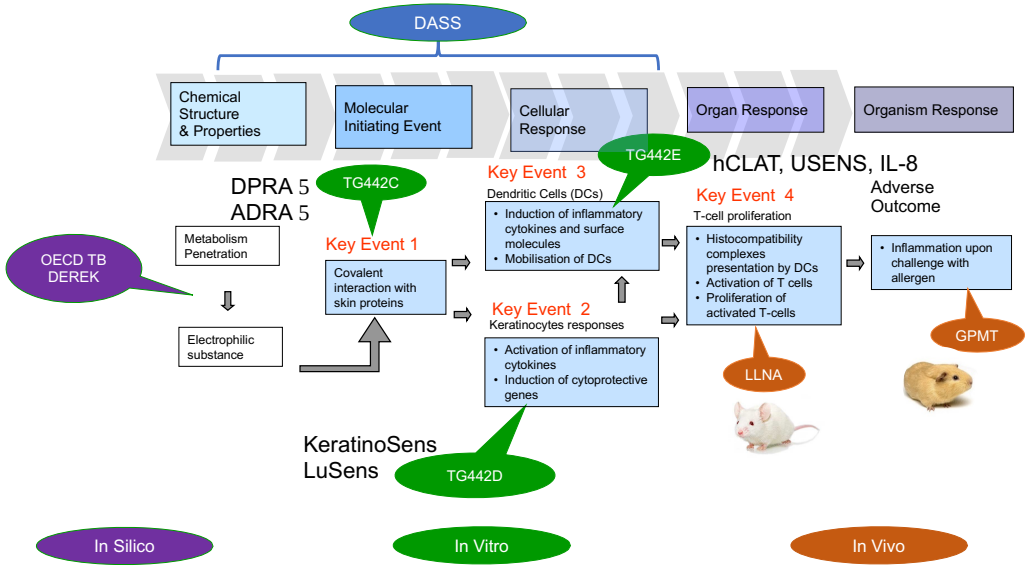
OECD (2014)



*Adapted from illustration by D. Sailstad

Defined Approaches for Skin Sensitization Guideline



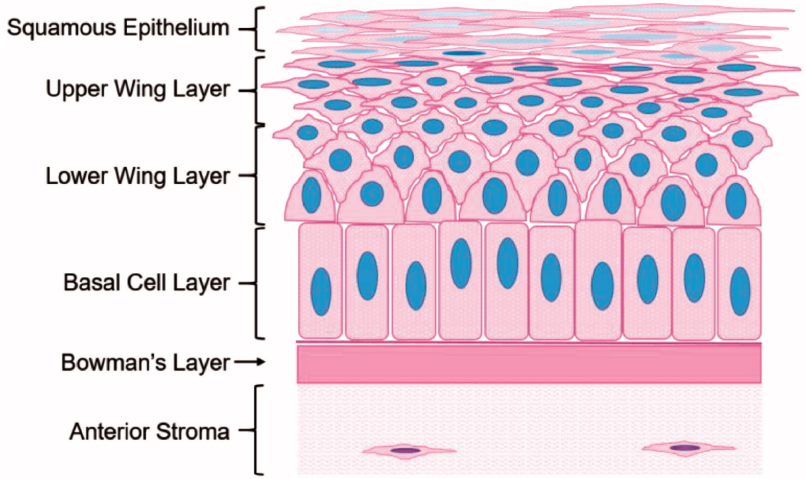


DA/Method	Information : Sources :	Capability (Hazard and/or Potency)	Hazard Performance vs. LLNA : N~168 :	Hazard Performance vs. Human N~63	GHS Potency Performance vs. LLNA (Accuracy)	GHS Potency Performance vs. Human (Accuracy)
2o3 DA	DPRA, KeratinoSens™, h-CLAT &	Hazard	84% BA, 82% Sens, 85% Spec	88% BA, 89% Sens, 88% Spec	-	-
ITSv1 DA	DPRA, h-CLAT, DEREK & Nexus v6.1.0 &	Hazard, Potency (GHS)	81% BA, & 92% Sens, & 70% Spec &	69% BA, 93% Sens, 44% Spec	70% NC, 71% 1B, 74% 1A	44% NC, 77% 1B, 65% 1A
ITSv2 DA	DPRA, h-CLAT, OECD & QSAR Toolbox v4.5 &	Hazard, Potency (GHS)	80% BA, & 93% Sens, & 67% Spec &	69% BA, 94% Sens, 44% Spec	67% NC, 72% 1B, 72% 1A	44% NC, 80% 1B, 67% 1A
LLNA (provided for comparison)	*in vivo*	Hazard, Potency	-	58% BA, 94% Sens, 22% Spec &	-	25% NC, 74% 1B, 56% 1A

Human-relevant approaches for eye corrosion/irritation potential

Clippinger et al. 2021 Cut Ocu Tox

(a)



Superficial Conjunctival or Corneal Epithelium (Figure 2c)
3D Reconstructed Human Cornea-like Epithelial Tissue
EYEIRR-IS
Vitrigel-Eye Irritancy
Bovine Corneal Opacity and Permeability
Isolated Chicken Eye
Isolated Rabbit Eye
Porcine Cornea Opacity Reversibility Assay
Ex Vivo Eye Irritation Test (EVEIT)
Fluorescein Leakage
Short Time Exposure
Neutral Red Release
Cytosensor Microphysiometer
Ocular Irritection
OptiSafe

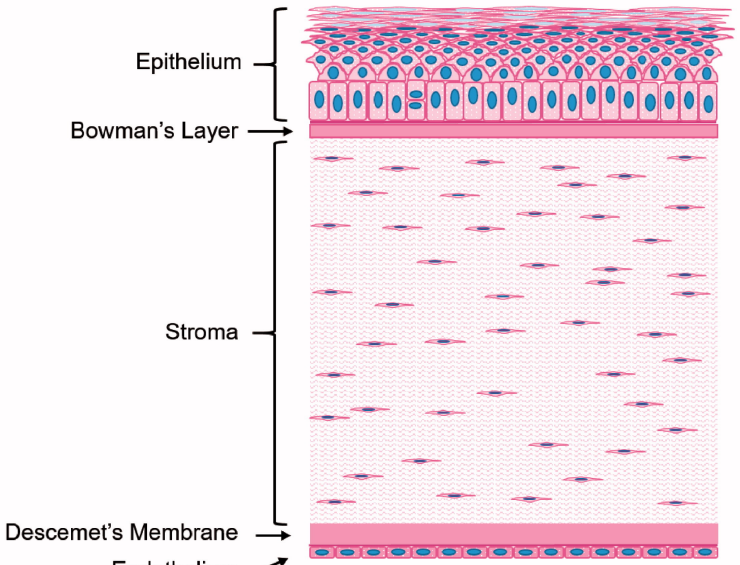
Wing Cell Layer of the Epithelium (Figure 2d)
3D Reconstructed Human Cornea-like Epithelial Tissue
EYEIRR-IS
Vitrigel-Eye Irritancy
Bovine Corneal Opacity and Permeability
Isolated Chicken Eye
Isolated Rabbit Eye
Porcine Cornea Opacity Reversibility Assay
Ex Vivo Eye Irritation Test (EVEIT)
Ocular Irritection
OptiSafe

Lower Wing Cell and Basal Cell Layers of the Epithelium (Figure 2e)
3D Reconstructed Human Cornea-like Epithelial Tissue
EYEIRR-IS
Bovine Corneal Opacity and Permeability
Porcine Cornea Opacity Reversibility Assay
Isolated Chicken Eye
Isolated Rabbit Eye
Ex Vivo Eye Irritation Test (EVEIT)
Ocular Irritection
OptiSafe

Consider strengths and limitations of all available methods with respect to:

- their relevance to human ocular anatomy
- the mechanisms of eye irritation/corrosion in humans

(b)



Corneal Stroma (Figure 2f)
Bovine Corneal Opacity and Permeability
Isolated Chicken Eye
Isolated Rabbit Eye
Ex Vivo Eye Irritation Test (EVEIT)
Ocular Irritection
OptiSafe

Corneal Endothelium (Figure 2g)
Bovine Corneal Opacity and Permeability
Isolated Chicken Eye
Isolated Rabbit Eye
Ex Vivo Eye Irritation Test (EVEIT)

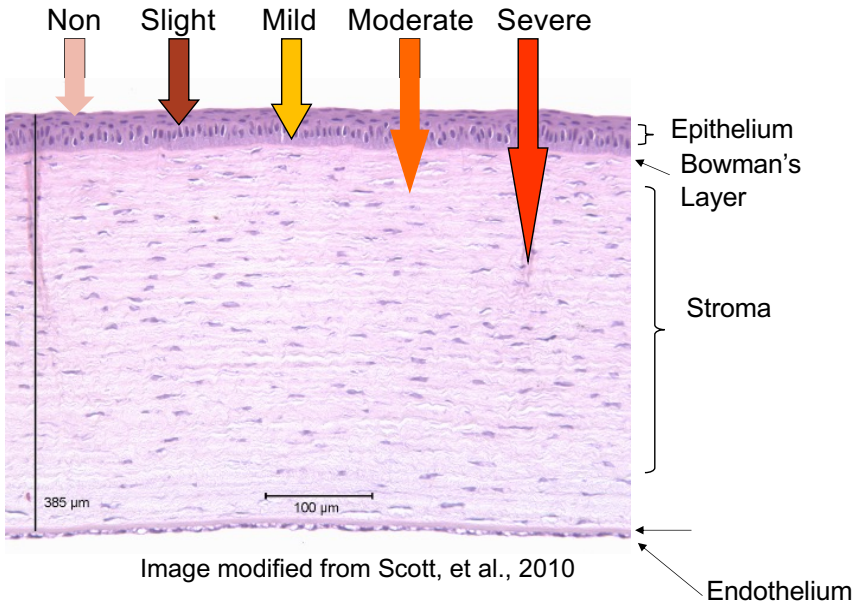
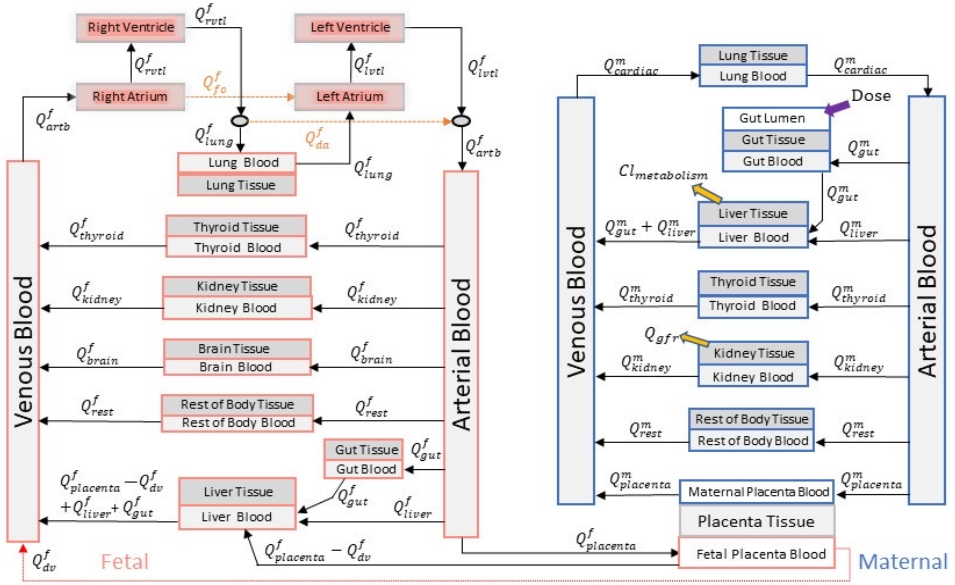


Image modified from Scott, et al., 2010

DevTox Screening: Human Stem Cell Assay + IVIVE

- >A biomarker-based human pluripotent stem cell assay was combined with IVIVE using a pregnancy PBPK model to predict equivalent administered doses (EAD) that would result in internal concentrations identified as potentially developmentally toxic.
- >The EAD derived from the human iPS cell-based devTOX^{qP} assay was a better predictor of the human teratogenic clinical dose for VPA than the LELs from the rat developmental toxicity study.

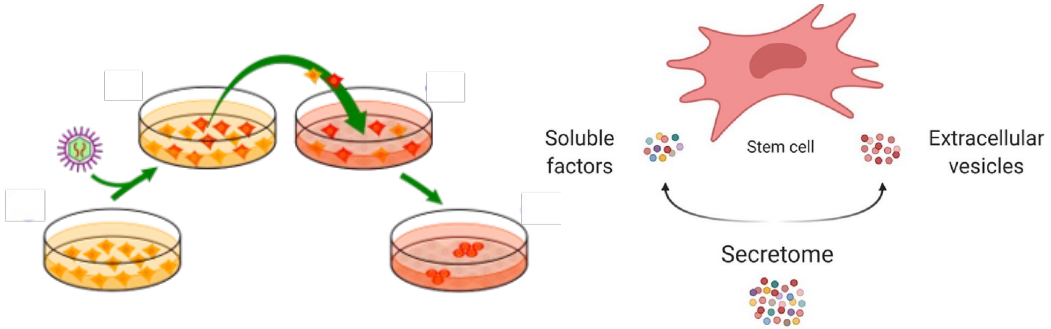


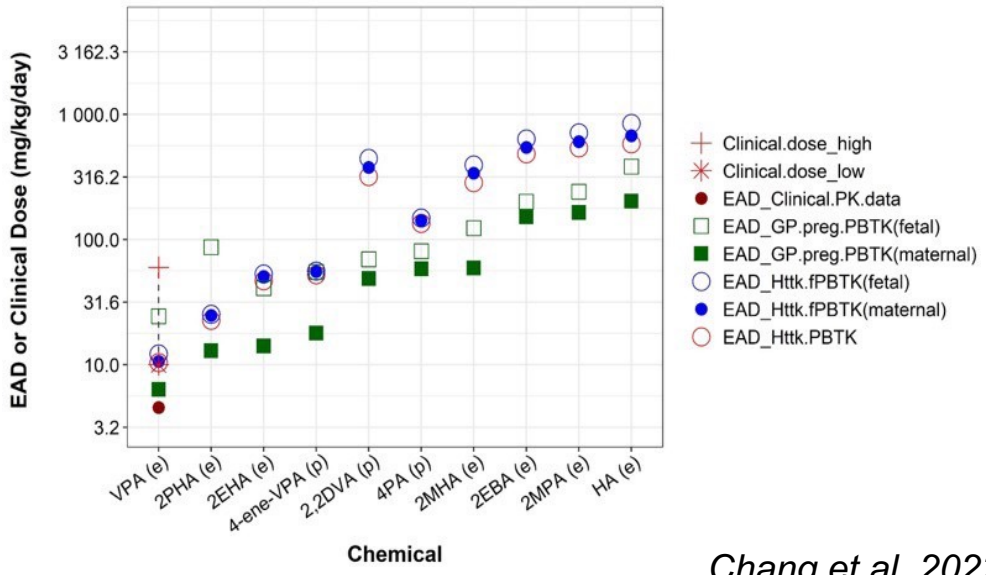
RESEARCH ARTICLE

Birth Defects Research | WILEY

Quantitative in vitro to in vivo extrapolation for developmental toxicity potency of valproic acid analogues

Xiaoqing Chang[1] | Jessica Palmer[2] | Annie Lumen[3] | Un Jung Lee[3] | Patricia Ceger[1] | Kamel Mansouri[4] | Catherine Sprankle[1] | Elizabeth Donley[2] | Shannon Bell[1] | Thomas B. Knudsen[5] | John Wambaugh[5] | Bethany Cook[1] | David Allen[1] | Nicole Kleinstreuer[4]





Chang et al. 2022

Test Readiness Criteria of NAMs for DNT

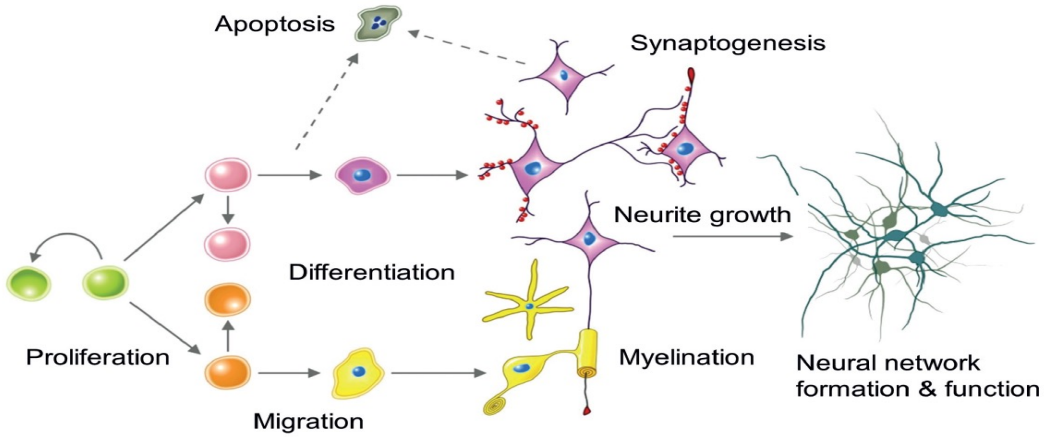
Human Relevance Consideration

*OECD IATA Case Study Published Sept. 2022

Phase I	Max. score	UKN2 cMINC	Phase II	Max. score	UKN2 cMINC	Phase III (optional)	max. score	UKN2 cMINC
1 Test system	10	9	8 Testing strategy	4	3	*13 Screening hits*	*4*	*4*
2 Exposure scheme	3	3	9 Robustness	4	3	Score 0 = D Score 1 = C Score 2 = B Score 3 - 4 = A		
3 Documentation/SOP	5	5	10 Test benchmarks	4	4			
4 Main endpoints	4	4	11 Prediction model	4	3			
5 Cytotoxicity	5	5	12 Applicability domain	3	1			
6 Test method controls	4	4						
7 Data evaluation	4	4						
Sum	**35**	**34**	**Sum**	**19**	**14**	**Sum**		**4**

The scores of the different phases are evaluated and result in the ranks of readiness

Phase I		Phase II			
Score	Grading	Score	Grading		Explanation of grading
< 7	D	< 4	D	D	Not ready at all
8 - 17	C	5 - 9	C	C	Substantial improvements required to be ready
18 - 28	B	10 - 14	B	B	Improvements required to be ready
29 - 35	A	15 - 19	A	A	Test method is close to ready or ready

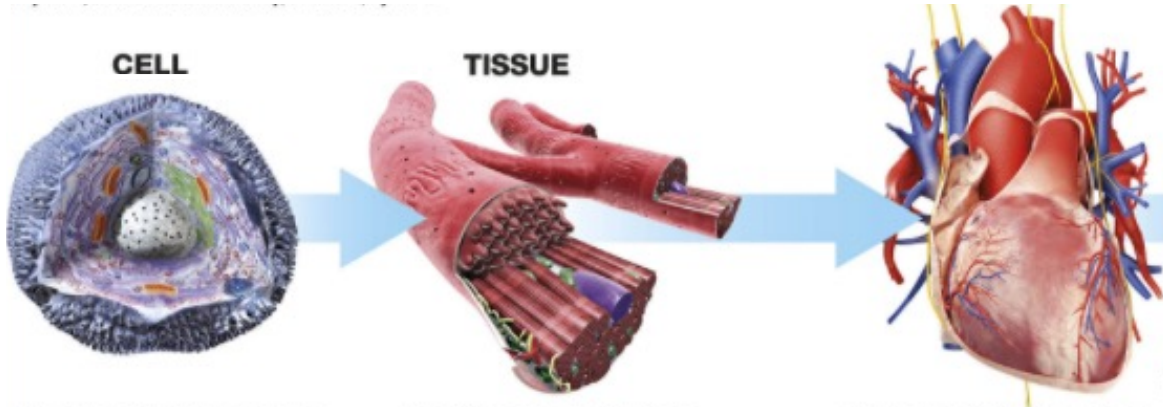


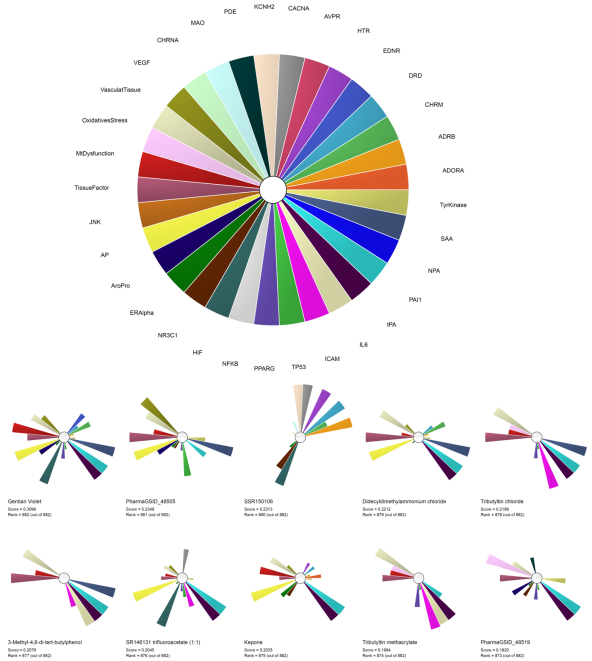
Criteria	Description
1 Test system	
1a What is modelled	Is there a clear rationale given for what target organ/tissue relevant for human poisoning/pathology the test systems should reflect
1b Relevance	Is the chosen test system known to be a key component in pathogenesis, or why is it thought to reflect a key component, mechanism or tissue
1c System uncertainties and human correlate (HC)	(i) Is there a discussion on where the test system differs from the mimicked human tissue, and which gaps of analogy need to be considered? (ii) Do toxicant-altered genes (or other biomarkers) correspond to changes in mimicked human tissue (after poisoning or in relevant pathologies)

Is the target organ/tissue relevant for human poisoning/pathology?

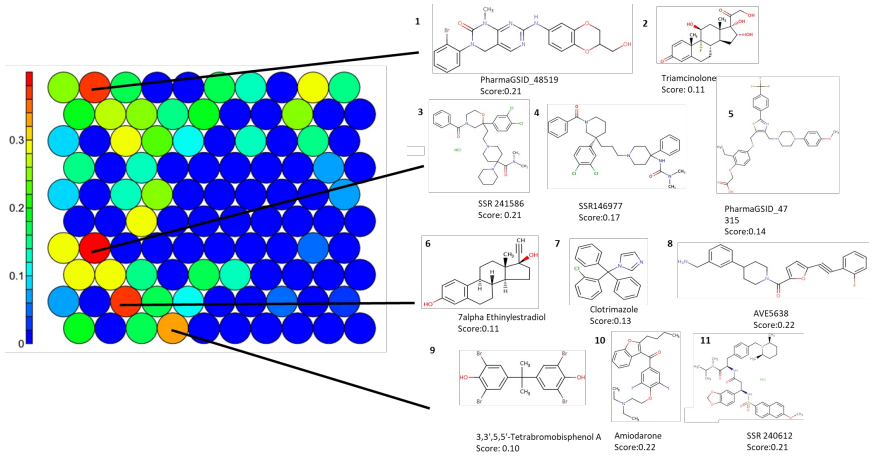
Are correlation/differences to human tissue discussed?

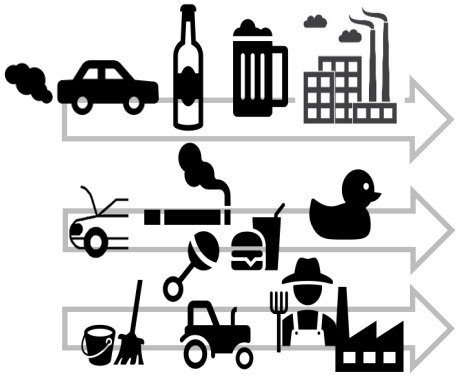
CardioToxPi: HTS Data Augmenting Expert Intelligence

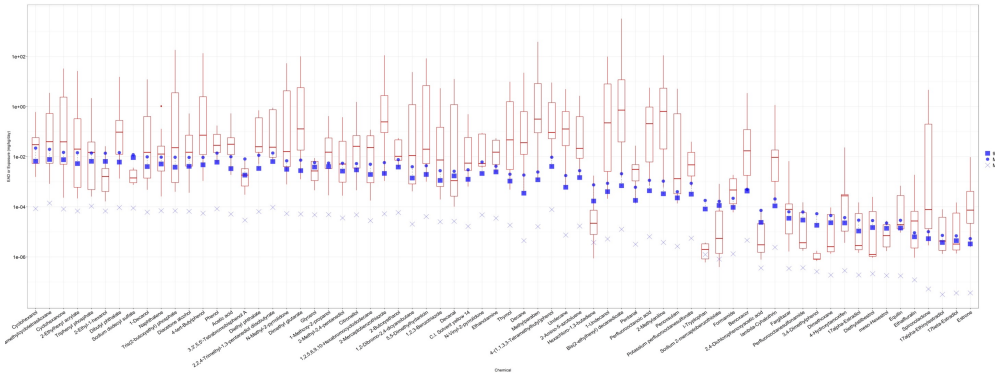




Using human cell-based data mapped to cardiovascular failure modes to predict environmental chemical contributions to cardiovascular diseases.



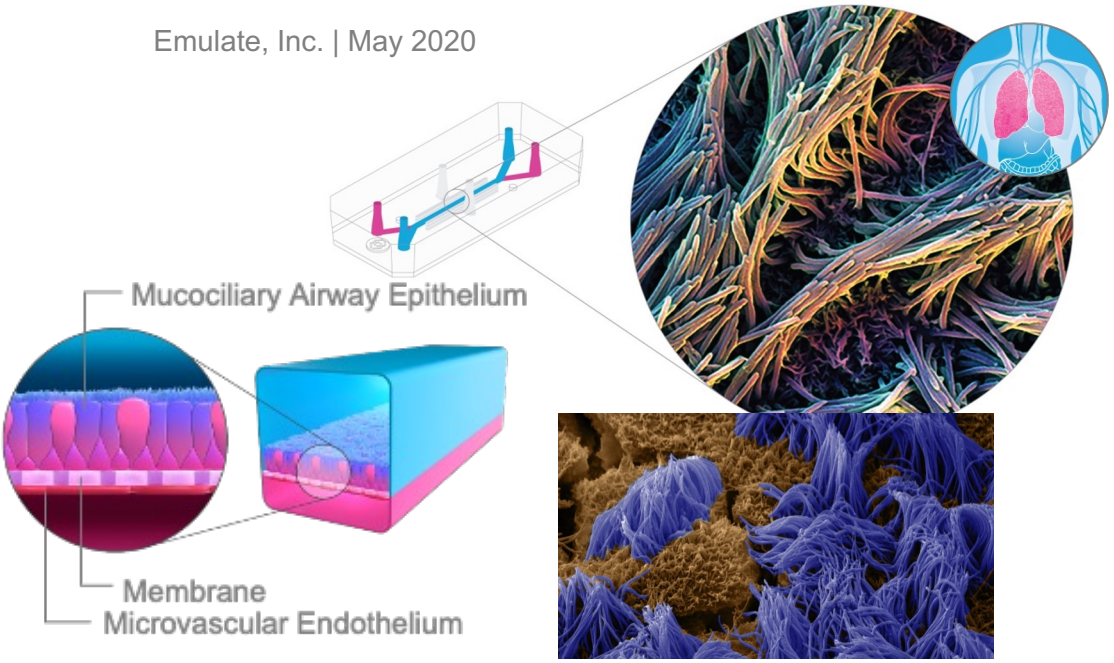




Krishna et al. 2021, Chem Res Tox; Krishna et al. 2022 in prep

MPSCoRe: Microphysiological Systems for COVID-19 Research

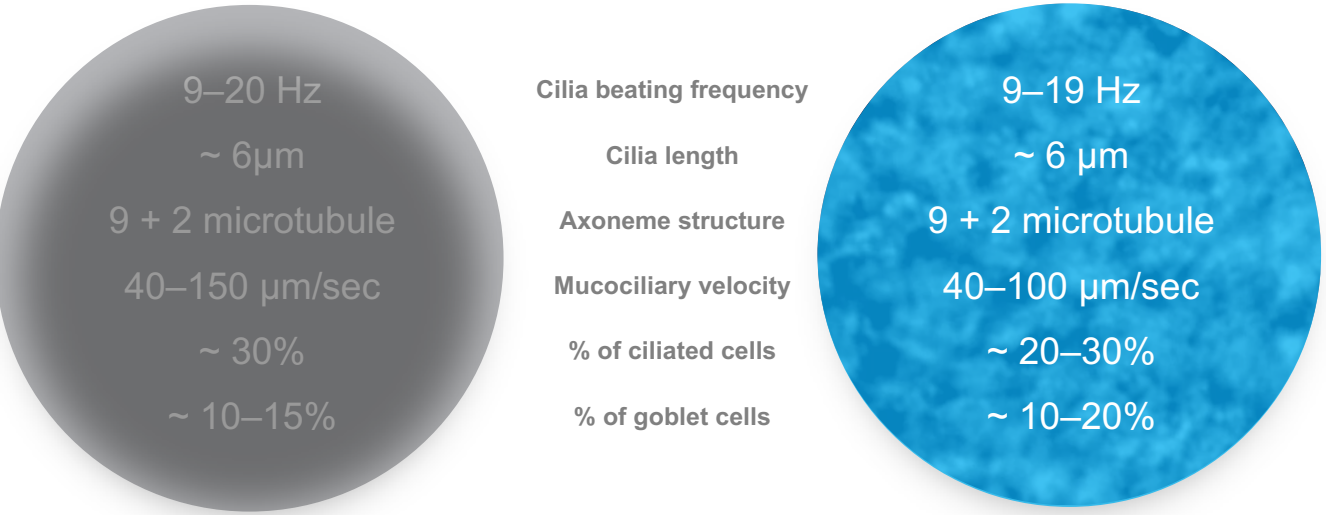
Emulate, Inc. | May 2020

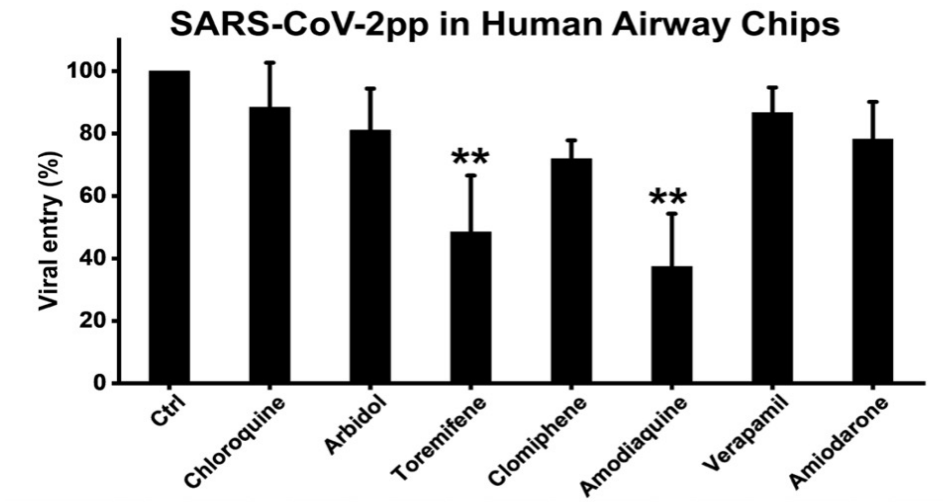


Joint working group to support global COVID-19 tissue chip research activities
Partnership with NC3Rs, DoD, NIAID, NCATS, others.

https://ntp.niehs.nih.gov/go/mps

Kleinstreuer & Holmes (2021) Drug Discovery Today

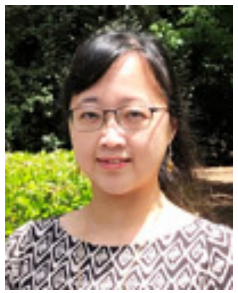




Li et al. 2021 Nat Biomed Eng

Acknowledgments

The NICEATM Group



- ICCVAM Agency Partners
- OECD Secretariat
- OECD DASS EG
- NGO Collaborators
- Cosmetics Europe
- MPSCoRe Leadership and Members
- DTT CV HEI Group

QUESTIONS?