NTP
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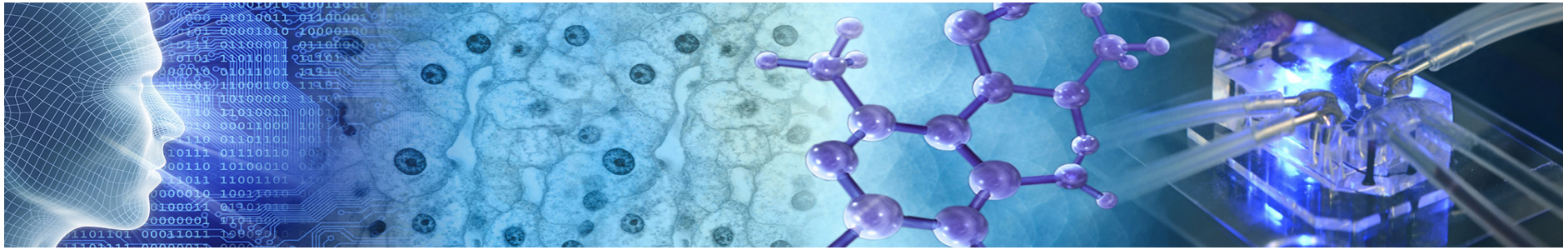

NTP Approaches to *In Vitro* Assessment of Dermal Hypersensitivity: Using Alternative Methods to Predict Skin Sensitization

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Presentation Outline

- Background
- *In vivo* and *in vitro* methods to evaluate potential sensitizers
- Developing confidence and expanding chemical space
- A quick success story
- Summary and Conclusions

Skin Sensitization

"Allergic Contact Dermatitis"

Accounts for 10-15% of all occupational disease *(Anderson et al. 2011)*

Major safety issue for cosmetics, pesticides, industrial chemicals, etc.

U.S. Regulatory Requirements/Considerations

		Reference Animal Method	Classification Criteria	Alternatives Accepted
	Pesticides, Industrial chemicals	 LLNA	NS S Hazard	Beginning in 2018 two specific non-animal integrated strategies are accepted by the USEPA, OPP/ OPPT for pesticide actives and inert materials
	Household Products	 LLNA	NS S SS Potency	Case by case basis
	Dermatological Products	 GPMT	Potency*	As an alternative to accepted guinea pig tests, FDA will consider a battery of in silico, in chemico, and in vitro studies that have been shown to adequately predict human skin sensitization with an accuracy similar to existing in vivo methods

*preference

Adapted from Strickland et al 2019

Skin Sensitization Process

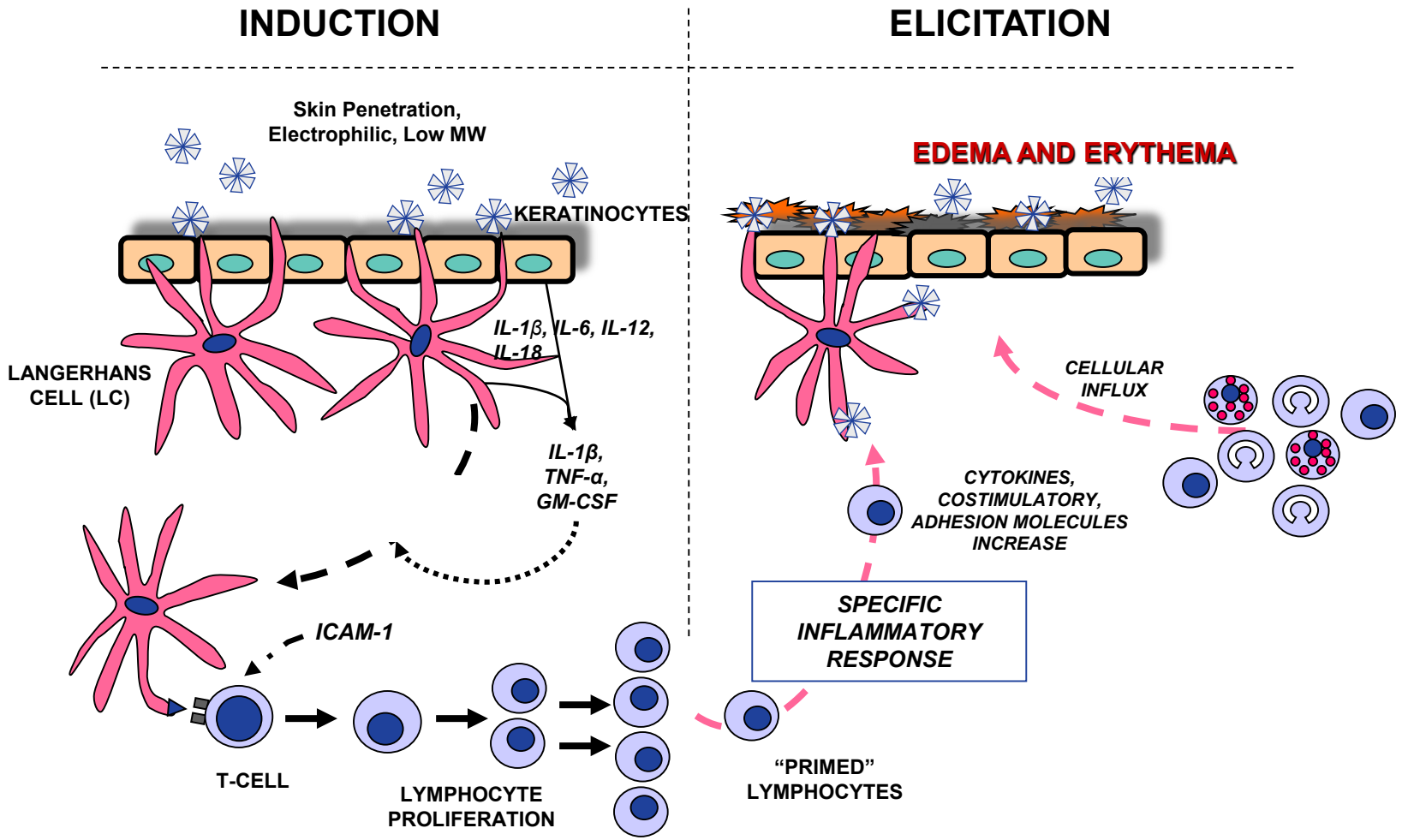


*Illustration by D. Sailstad

In Vivo Tests for Assessment of Dermal Sensitization

Guinea Pig Maximization Test

- Intradermal and topical sensitization
- Topical challenge
- Measure erythema response 24 - 48 hours post challenge

Buehler

- Topical sensitization with closed patch
- Topical challenge distal to sensitization with closed patch
- Measure erythema response following removal of patch

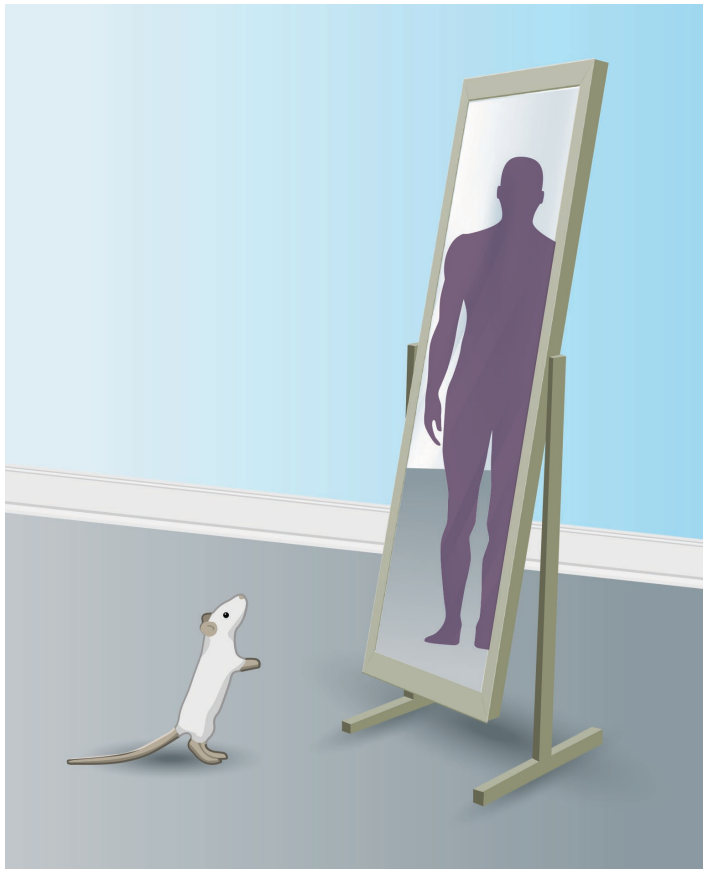
Local Lymph Node Assay

- Topical treatment on dorsal surface of the ear
- Inject with radiolabel or fluorochrome
- Measure cell proliferation in the lymph nodes associated with the site of application

Comparison of LLNA and Human Data

Accuracy:

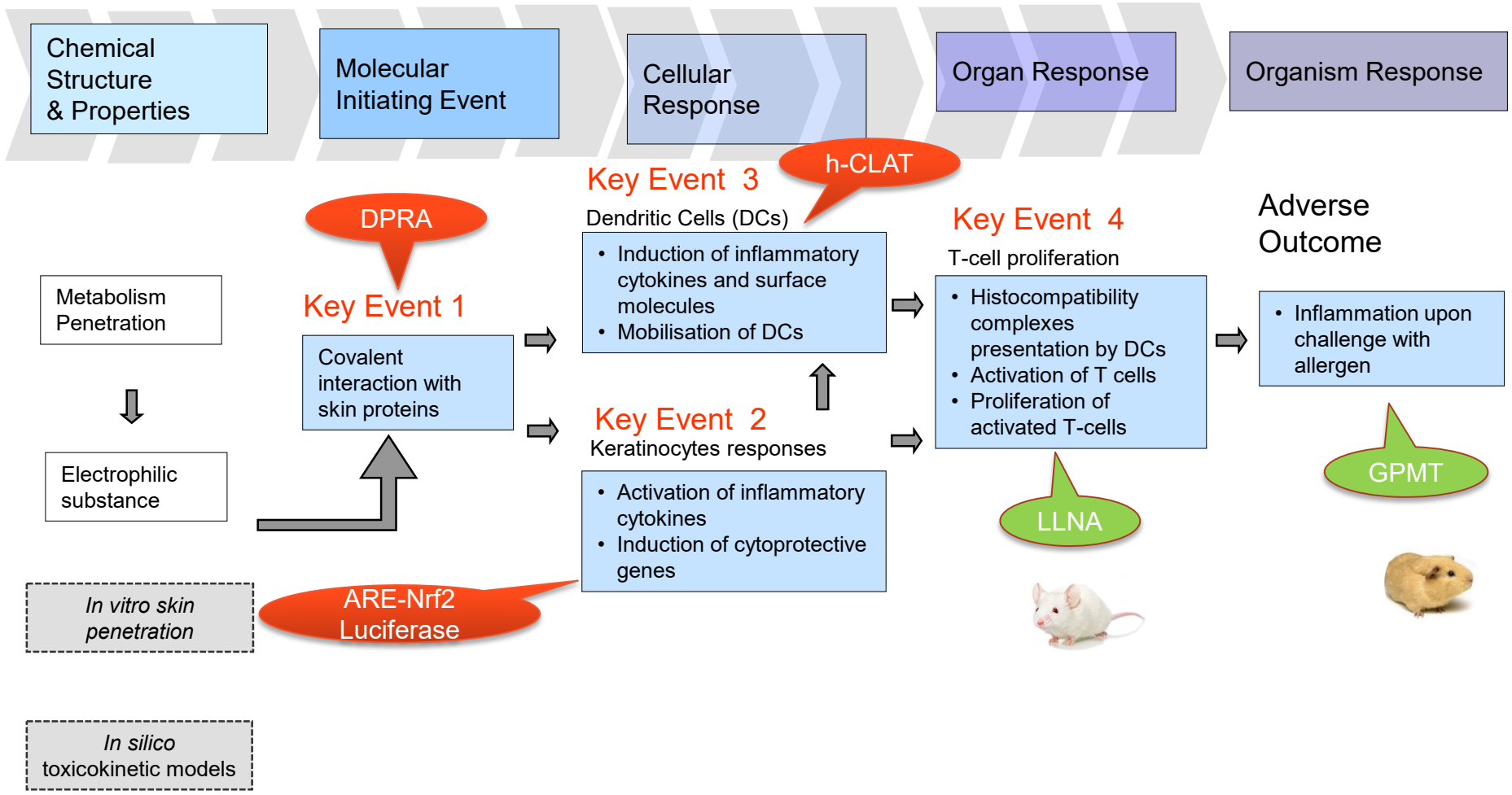
- **74 %** for Hazard (NS/S)
- **59 %** for Potency 3-class (NS, Weak/ Moderate, Strong/ Extreme)
- **45 %** for Potency 5-class (NS, Weak, Moderate, Strong, Extreme)



Provides a benchmark for comparison with new approaches

Adapted from Kleinstreuer et al. 2018

OECD AOP for Skin Sensitization



In Vitro Models Used by the NTP for Assessing Dermal Sensitization

- **Direct peptide reactivity assay**
  - Assesses the ability of a substance to form a hapten-protein complex
- **KeratinoSens**
  - Assesses the ability of a substance to activate cytokines and induce cytoprotective genes in keratinocytes
- **h-CLAT**
  - Assesses the ability of a substance to activate and mobilize dendritic cells in the skin

Individual Assays Compared to Human

	hCLAT vs Human	DPRA vs Human	Keratino vs Human
	NEG POS	NEG POS	NEG POS
NEG	20 11	29 25	31 22
POS	19 77	10 63	8 67
Sensitivity %:	**89.7**	**72.9**	**75.0**
Specificity %:	**52.5**	**74.4**	**77.5**
Accuracy %:	**78.0**	**73.4**	**75.8**
	≥ LLNA		**≥ LLNA**
	n=127	n=124	n=128

Hoffmann et al. 2018 Crit Rev Tox
Kleinstreuer et al. 2018 Crit Rev Tox

Defined Approach Evaluation

- Most non-animal testing strategies evaluated so far perform **better** than the LLNA at predicting human skin sensitization **hazard and potency**
- Combining multiple *in vitro* assays and *in silico* methods or physico chemical properties increases the ability to predict sensitizers
- Specific Defined Approaches for data analysis, hazard and potency prediction have been published

Expanding Coverage of Chemical Space

- **A significant number of chemicals used in the validation of non-animal test methods have been cosmetics ingredients**
- NTP is supporting testing of other types of chemicals in three alternative test methods: DPRA, KeratinoSens, hCLAT
  - Expanded chemical space includes: pesticides, agrochemical formulations, dermal excipients, personal care product ingredients, “challenge” chemicals
- Chemical nominations from multiple agencies
  - EPA: Office of Pesticides, Office of Pollution Prevention and Toxics, Office of Research and Development
  - Consumer Product Safety Commission
  - Food and Drug Administration
  - NTP
- Testing of >200 chemicals has been completed

IT Compounds

Common Name	Chemical Name	CAS #	Product Name	Donor
BBIT	1,2-benzisothiazolin-3-one, 2-butyl	4299-07-4	Vanquish 100	Lonza
BIT	1,2-Benzisothiazolin-3-one	2634-33-5	Mergal BIT Technical	Troy
CMIT/MIT	Mixture	55965-84-9	Mergal MITZ	Troy
DCOIT	4,5-Dichloro-2-octyl-3(2h)-isothiazolone	64359-81-5	KATHON 287T industrial Microbicide	Dow
MIT	2-Methyl-4-isothiazolin-3-one	2682-20-4	KORDEK 573F BIOCIDE	Dow
OIT	2-n-Octyl-4-isothiazolin-3-one	26530-20-1	ACTICIDE OIT	Thor

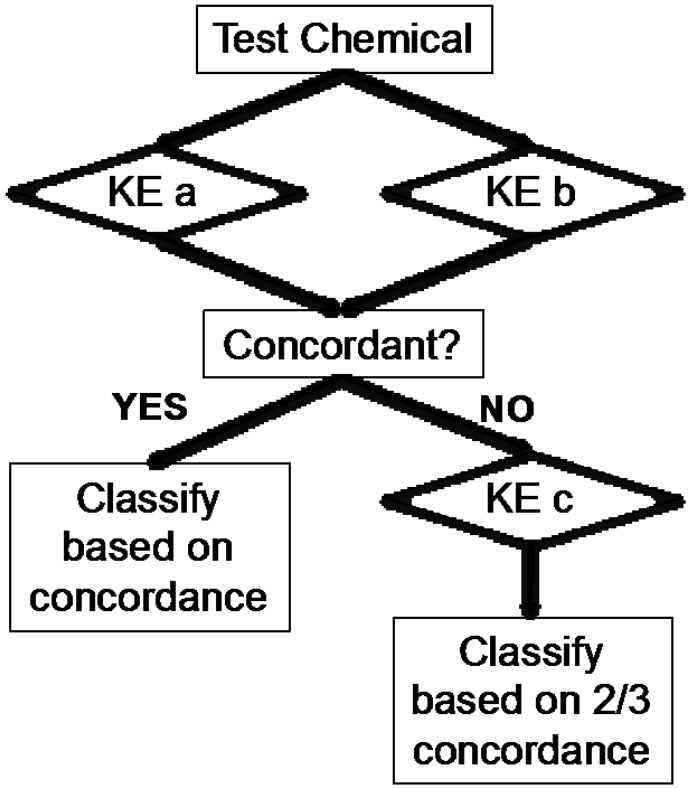
CMIT = 5-Chloro-2-methyl-4-isothiazolin-3-one

- Partnership between EPA OPP, ACC Isothiazolinone Task Force, and NTP/NICEATM
- Test 6 isothiazolinones donated by Task Force member companies in NTP in vitro assays
- Collect and analyze all available in vivo data
- Consider methods for using in vitro data for risk assessment and compare to results using in vivo methods

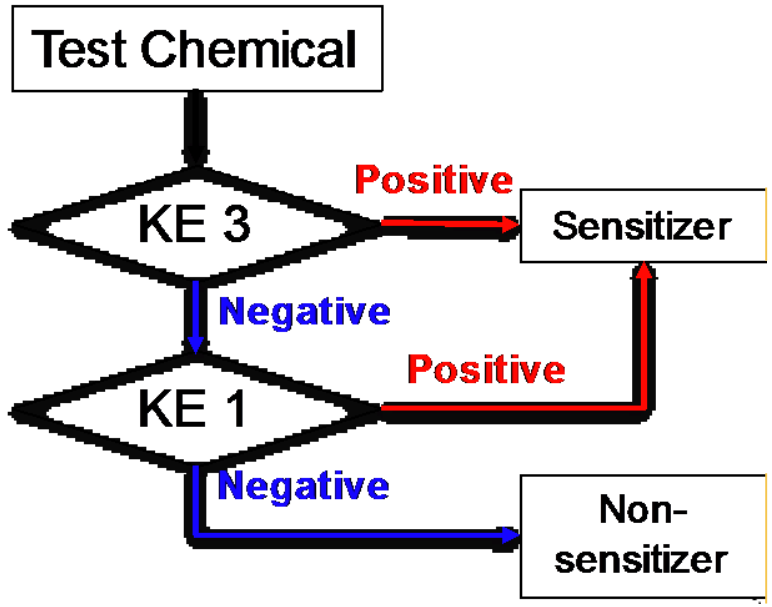
Available Data

- *In chemico* and *in vitro* data from testing at Burleson Research Technologies (NTP CRO for Immunotoxicology Studies)
  - Direct Peptide Reactivity Assay (DPRA); OECD TG 442C
  - KeratinoSens™; OECD TG 442D
  - Human cell line activation test (h-CLAT); OECD TG 442E
- *In silico* predictions from OECD QSAR Toolbox v4.3
- Physicochemical properties from EPA OPP and OPERA v2.3 (OPEn structure-activity Relationship App) at https://github.com/NIEHS/OPERA
- LLNA data from Dow Chemical and scientific literature

Defined Approaches Accepted by EPA



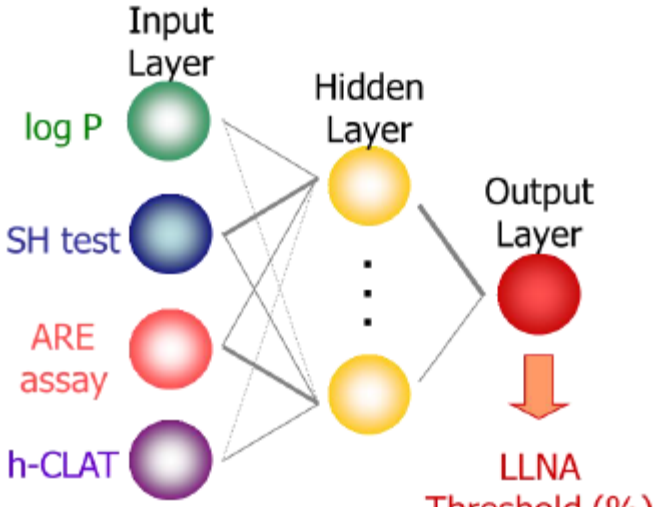
AOP 2 out of 3



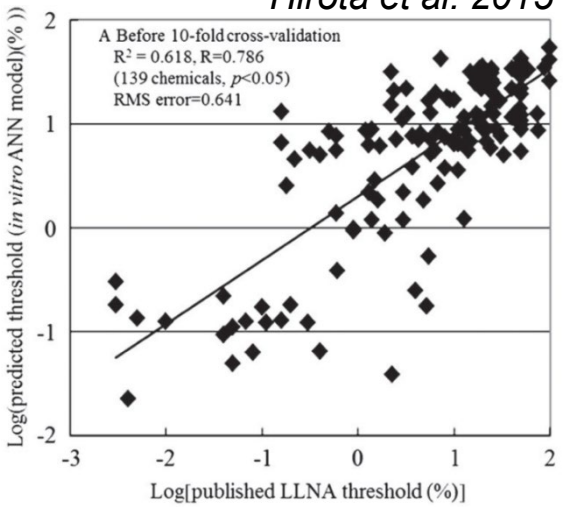
Key Event 3/1 Sequential Testing Strategy

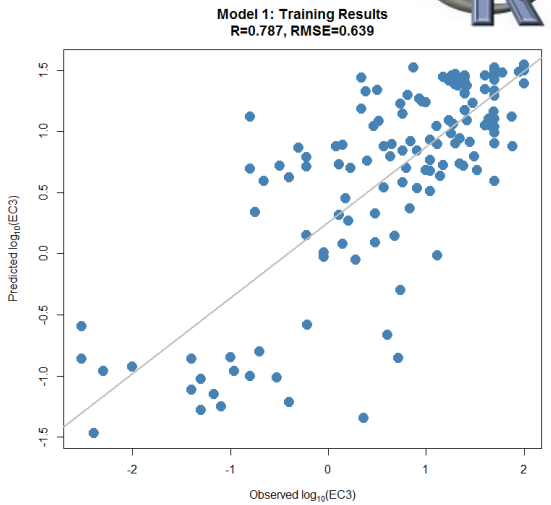
DAs for Quantitative EC3 Prediction

Future consideration for OECD GL



Hirota et al. 2015





Artificial Neural Network Models

- Continuous EC3 prediction
- Can be translated into potency classes:
  NS, Weak/Moderate, Strong/Extreme
- Built using proprietary software (QwikNet), reproduced in R
- Two models:
  DPRA, hCLAT
  DPRA, hCLAT, KeratinoSens
- Run over multiple iterations and averaged

IT Compounds

Skin Sensitization Measurement Endpoints for LLNA and Non-animal Methods

Chemical	Dow LLNA EC3 (%)	NICEATM EC3 (%)[a]	DPRA Mean Depletion (%)	Keratino-Sens EC1.5 (µM)[b]	Keratino-Sens Imax	h-CLAT Minimum Induction Threshold (µg/mL)[b]
DCOIT	0.004	0.008 (0-0.053)	55.2	1.32	4.37	0.92
CMIT/MIT	0.002	0.018 (0.0011-0.034)	55.3	3.41	5.61	2.63
OIT	0.2-0.25	0.361 (0.029-0.69)	50	2.19	3.70	0.95
MIT	0.863	1.154 (0-3.476)	50	9.54	15.84	11.6
BIT	1.54	10.57 (0-23.36)	NA	3.14	17.64	7.63
BBIT	NA	NA	50	3.84	19.61	3.01

Abbreviations: NA = not available

[a] Numbers in parentheses are the 95% confidence limits for the mean EC3

[b] Results corrected for % active ingredient

Potency Rank by Test Method

Chemical	Dow LLNA	NICEATM LLNA	KeratinoSens	h-CLAT
DCOIT	2	1	1	1
CMIT/MIT	1	2	4	3
OIT	3	3	2	2
MIT	4	4	6	6
BIT	5	5	3	5
BBIT	NA	NA	5	4

NA = not available (no LLNA data for BBIT)

- Used by EPA for Draft Risk Assessment published in the FR in May, 2020
- The use of in vitro and in chemico assays and neural network-based defined approaches (DAs) is the first use of such information in regulatory risk assessment
- EPA is using the results of the ANN-EC3 DA to derive EC3 values to extrapolate dermal risk for the currently registered isothiazolinones as part of registration review

Summary and Conclusions

- Most non-animal testing strategies evaluated to date perform **better** than *in vivo* models at predicting human skin sensitization **hazard**
- Multiple models using different combinations of non-animal data demonstrate high accuracy in hazard classification predictions
  - This provides flexibility for investigators/companies to chose from among different test methods as their resources allow
- Global collaborations are addressing issues such as potency and further expanding the applicability domain
- The NTP has transitioned to using non-animal methods to screen for the potential to induce dermal sensitization
- *In vitro* data can (and has) be used for hazard identification and risk assessment

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