May 16-17, 2018
IDEA workshop

Proposal of the artificial neural network(ANN) model predicting LLNA EC3 for supporting skin sensitization risk assessment.

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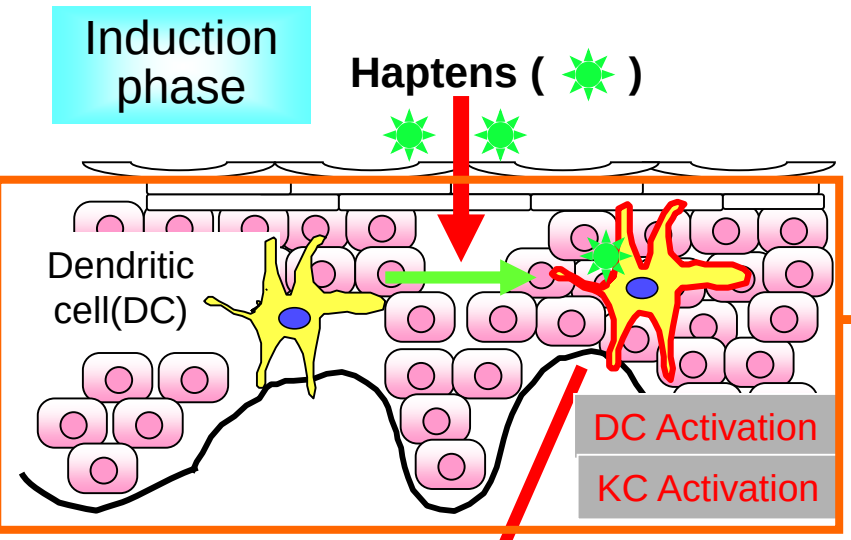
Agenda

1. About ANN model consisting of in vitro sensitization tests and in silico/structure alert model.

2. Case study of skin sensitization assessment of some fragrance ingredients.

Mechanism of induction phases of allergic contact dermatitis and AOP(Adverse Outcome Pathway)



Chemical Structure & Properties

Metabolism Penetration

Electrophilic substance(in silico)

Molecular initiating Event

KE1; Covalent interaction with cells protein (**protein-binding→DPRA**)

Event in skin

Cellular Response

KE2: Keratinocyte signal activation→ **KeratinoSens**

KE3: Dendritic cell activation→h-CLAT

Organ Response

Antigen presentation

T cell proliferation

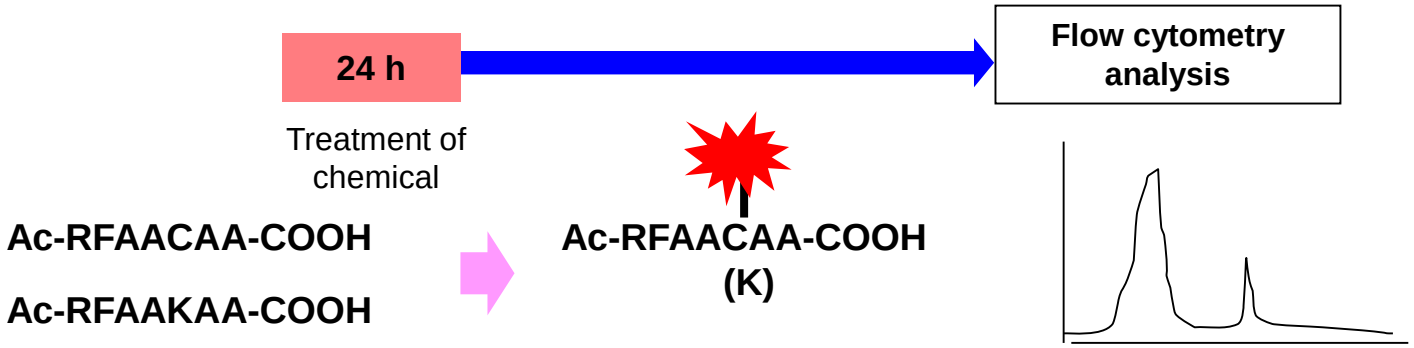
Event in Lymph Node

Organism Response

OECD, 2012

DPRA for hapten-protein binding

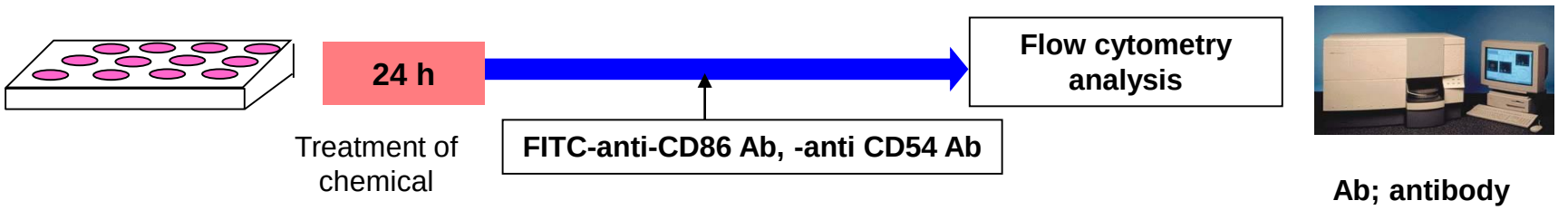
- ✓ DPRA (Direct Peptide Reactivity Assay) detects binding among chemicals and model peptides (Cys-peptide/Lys-peptide) using HPLC.



- ✓ Each of the % remaining peptide of Cys and Lys was used as a descriptor.

h-CLAT for dendritic cell activation

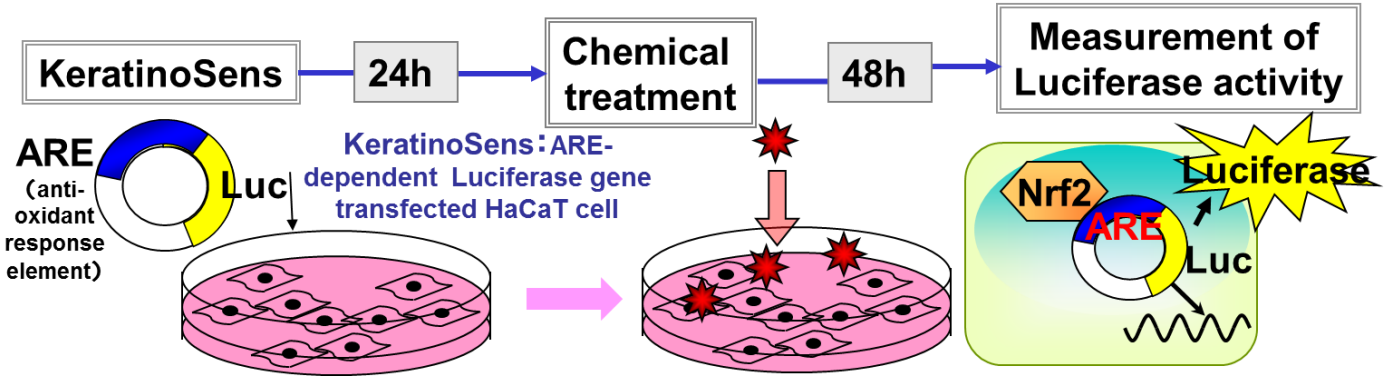
- ✓ h-CLAT (human Cell Line Activation Test) detects augmentation of CD86 and CD54 expression on chemical-treated THP-1 cells using flow cytometry.



- ✓ Minimum of threshold concentration of CD86/CD54 expression and CV75 (cell toxicity) was used as a descriptor.

KeratinoSens™ for ketatinocyte signaling

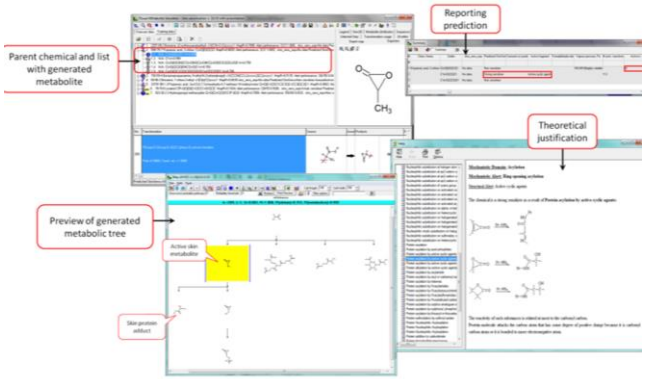
- ✓ KeratinoSens™ detects induction of ARE(anti-oxidant response element) dependent luciferase activity in HaCaT.



- ✓ EC1.5 (threshold concentration of ARE-dependent luciferase induction) was used as a descriptor.

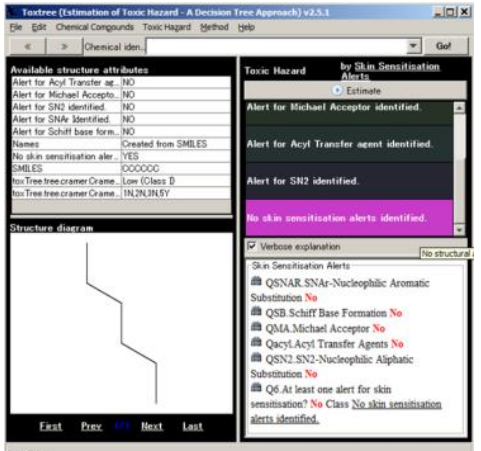
In silico structure alert for chemical structure & properties

TIMES-M



http://oasis-lmc.org/products/models/human-health-endpoints/skin-sensitization.aspx

Toxtree



Aim of the study

Development of the prediction model for LLNA threshold (e.g. EC3 values) using non-animal assays (***in vitro*** or/and ***in silico***).

LLNA: Local lymph node assay (OECD TG 429)

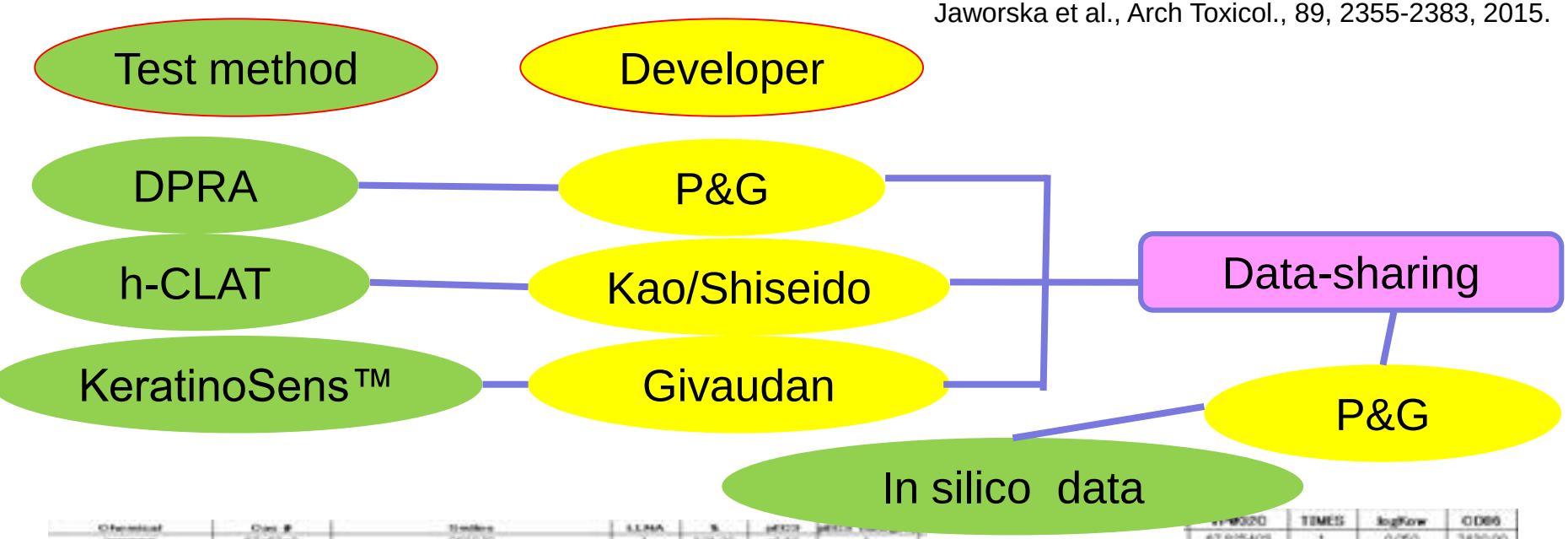
Our aim:

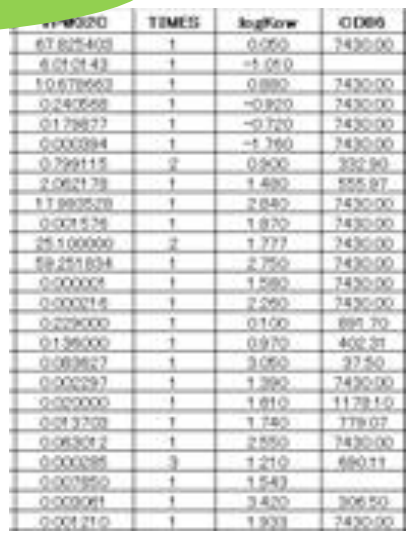
QRA(Quantitative Risk Assessment) of cosmetic ingredients for skin sensitization using non-animal assays (***in vitro***/***in silico***).

Database of DPRA, h-CLAT, KeratinoSens™ and TIMES

Database (134 chemicals) consisting of LLNA EC3, h-CLAT, DPRA, KeratinoSens™ and TIMES-M was shared in collaboration with P&G/Givaudan/Kao in 2013 (now published)..

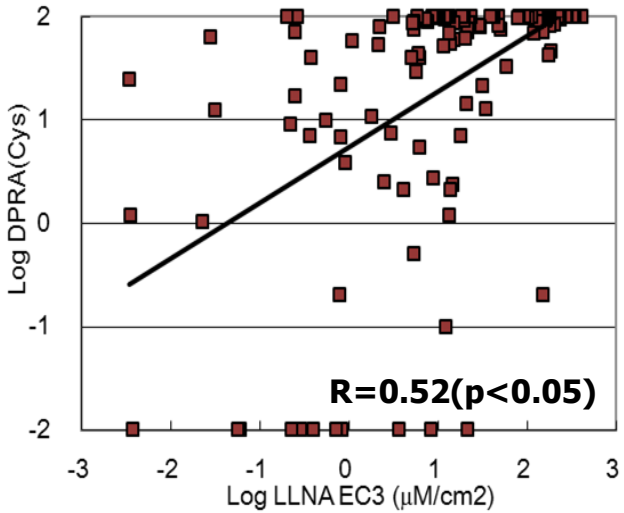
Jaworska et al., Arch Toxicol., 89, 2355-2383, 2015.



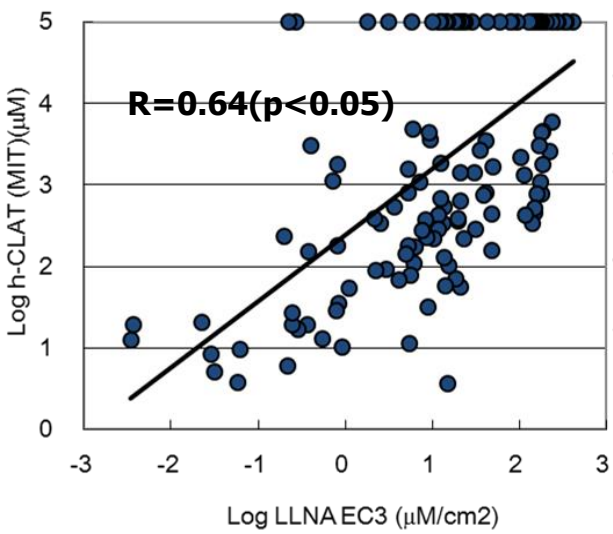


Correlation between LLNA, h-CLAT, DPRA and KeratinoSens™

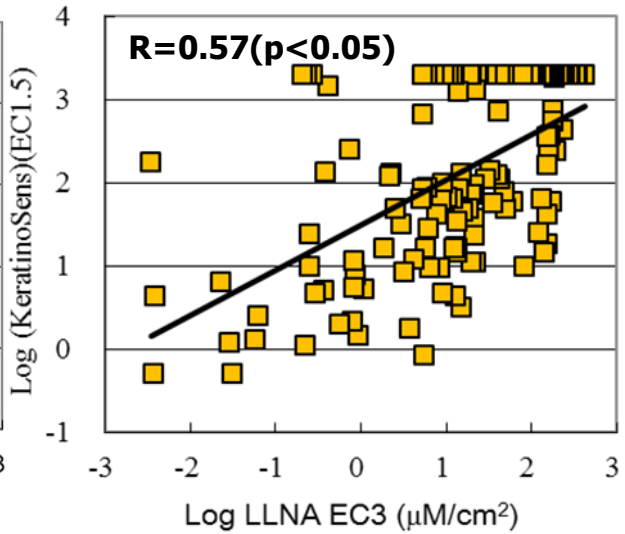
LLNA vs DPRA(Cys)



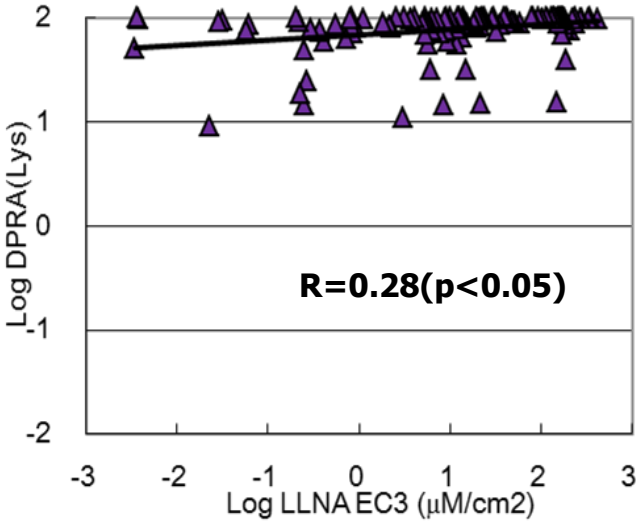
LLNA vs h-CLAT(MIT)



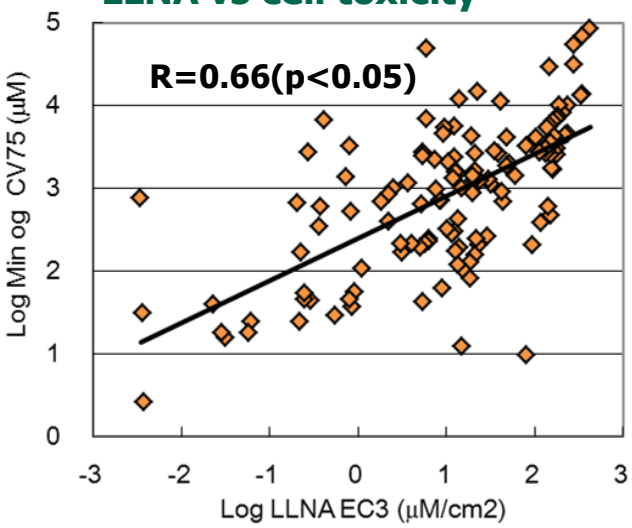
LLNA vs KetatinoSens™(EC1.5)



LLNA vs DPRA(Lys)

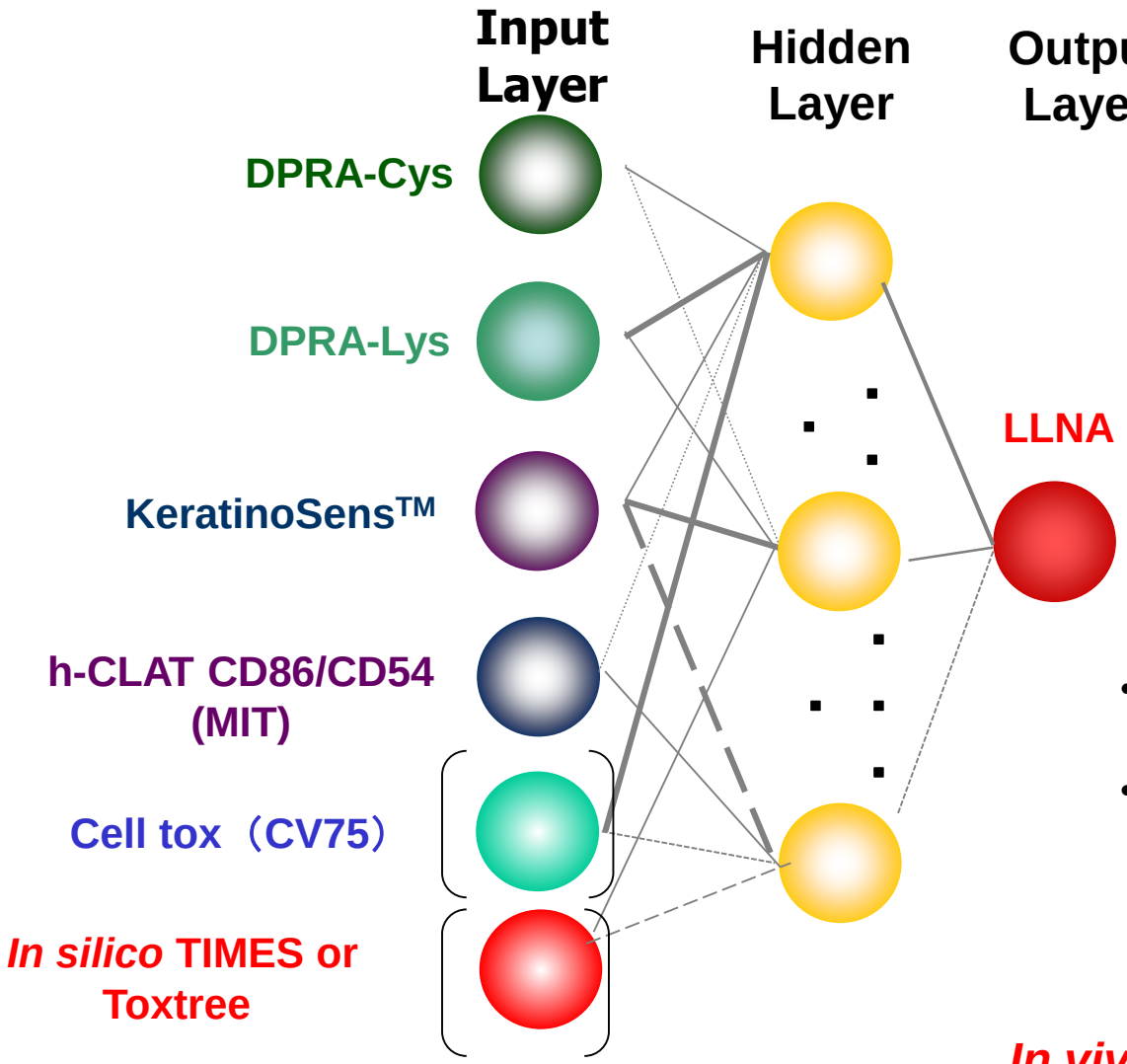


LLNA vs cell toxicity



- **Each descriptor derived from h-CLAT, DPRA (Cys), DPRA (Lys) and KeratinoSens™ correlated with LLNA EC3, significantly.**
- **However, each indicator is not enough for risk assessment.**

Artificial Neural Network (ANN) analysis



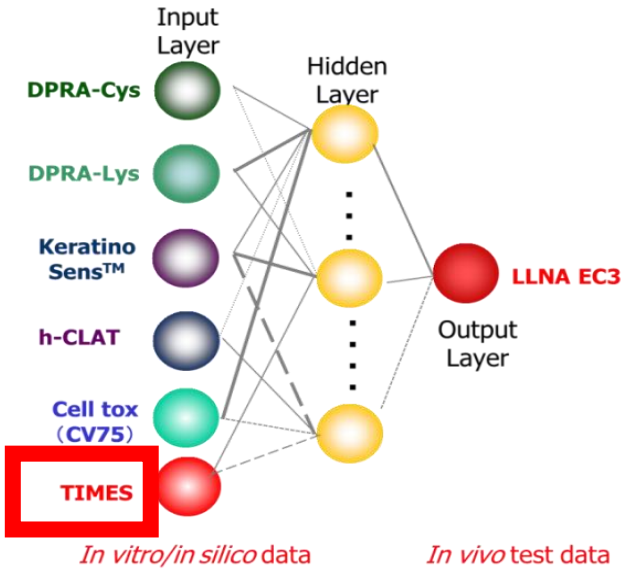
LLNA EC3

- EC3 values for LLNA positive chemicals
- For LLNA negative chemicals, 101% was used as EC3 value (converted to μM/cm^2 unit).

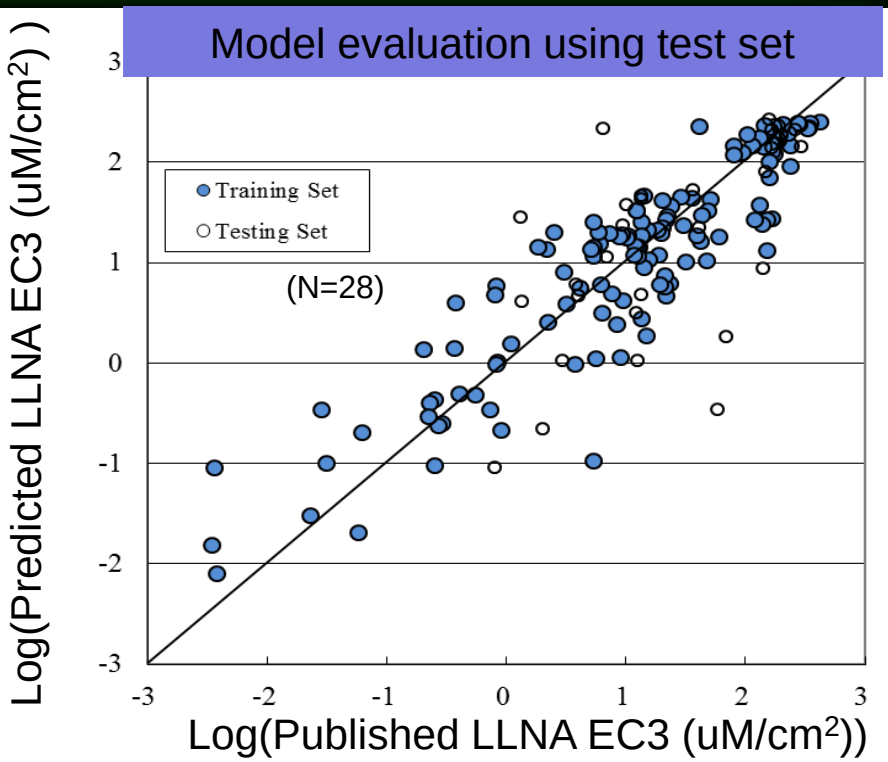
In this study, the 6-5-2-1 ANN analysis was performed.

MIT; Minimum induction threshold of CD86/CD54 in h-CLAT

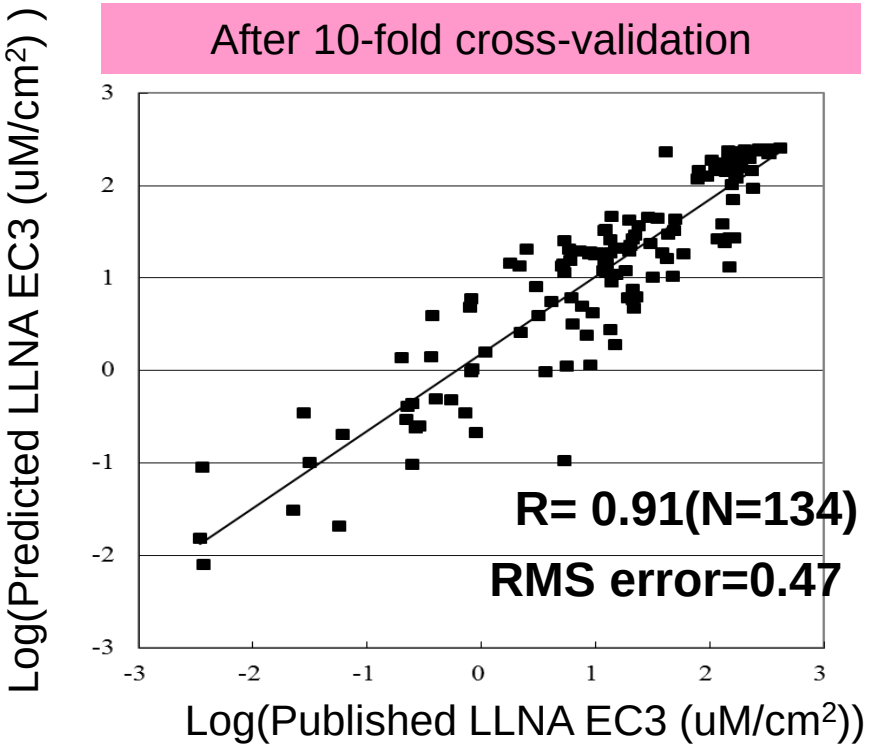
Prediction of LLNA EC3s using ANN models-1



Model evaluation using test set



After 10-fold cross-validation



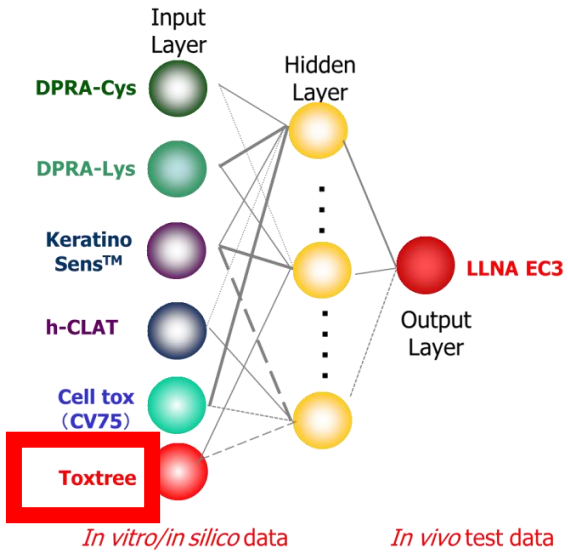
This ANN model (in silico descriptor; TIMES-M) showed good correlation between predicted values and tested values.

*Root mean square (RMS) error =

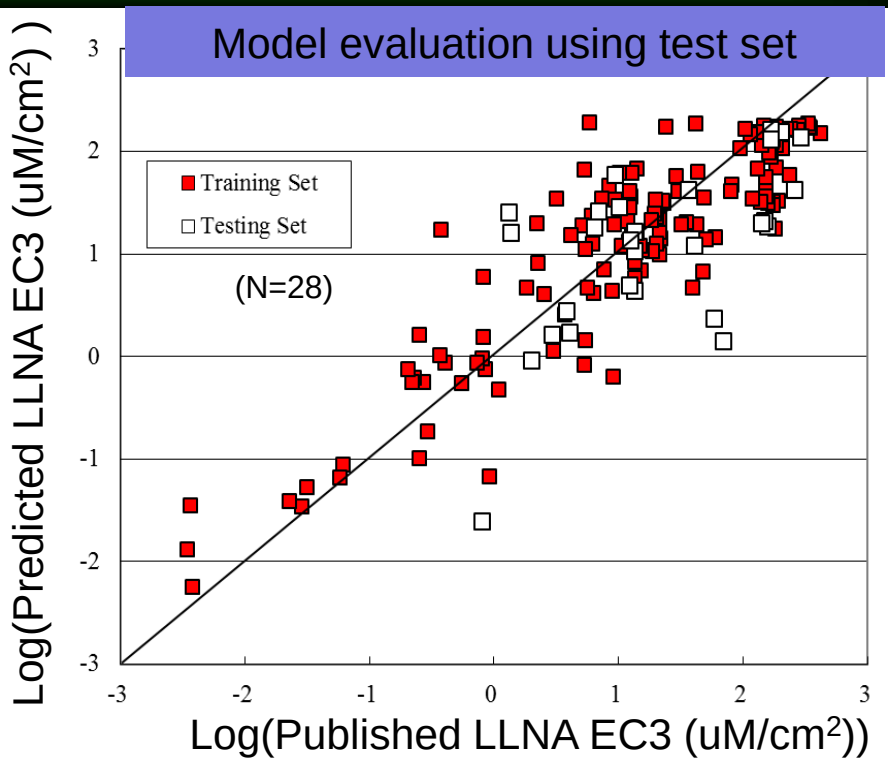
$$\sqrt{\sum((\text{measured value} - \text{predicted value})^2) / \text{number of data}}$$

Hirota et al., J. Appl. Toxicol., 38 (2017) 514-526

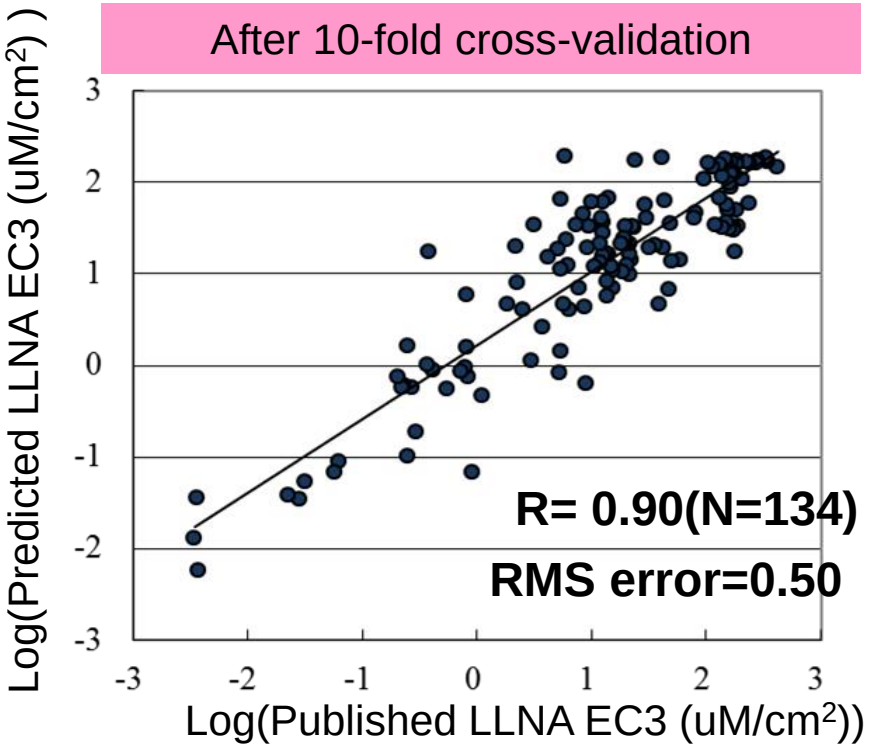
Prediction of LLNA EC3s using ANN models-2



Model evaluation using test set



After 10-fold cross-validation



This ANN model (in silico descriptor; Toxtree) also showed good correlation between predicted values and tested values.

*Root mean square (RMS) error =

$$\sqrt{\sum((\text{measured value} - \text{predicted value})^2) / \text{number of data}}$$

Hirota et al., J. Appl. Toxicol., 38 (2017) 514-526

Decision tree for category prediction

(1) DPRA
(2) KeratinoSens™
(3) h-CLAT (CD86, CD54)

One or more positive

All of (1), (2) and (3) are negative.

(Bottom up 3 out of 3)

Predicted EC3 from ANN model (a)

Predicted EC3	GHS Category
< 2(%)	GHS 1A
2 ≤ (a) < 100(%)	GHS 1B
(a) ≥100(%)	Negative

Predicted EC3	Category
< 1(%)	Extreme or Strong
1 ≤ (a) < 10(%)	Moderate
10 ≤ (a) < 100(%)	Weak
(a) ≥100(%)	Negative

GHS Category

Negative

Category
Negative

ANN analysis does not performed

Category prediction of the ANN(GHS category)

ANN model (h-CLAT/DPRA/KeratinoSens™/cell toxicity/TIMES-M)

	Potency Category	LLNA GHS classification (*in vivo*) (Learning Set(N=134))			LLNA GHS classification (*in vivo*) (Test Set (N=28))		
		1A (34)	1B (60)	NS (40)	1A (6)	1B (14)	NS (8)
ANN model (h-CLAT/DPRA/KeratinoSens™/cell toxicity/TIMES-M)	1A	24	8	0	3	3	0
	1B	10	49	15	3	10	4
	Negative	0	3	25	0	1	4
	Accuracy (%)	73.1			60.7		
	Over predicted (%)	17.2			25.0		
	Under predicted (%)	9.7			14.3		

ANN model (h-CLAT/DPRA/KeratinoSens™/cell toxicity/Toxtree)

	Potency Category	LLNA GHS classification (*in vivo*) (Learning Set(N=134))			LLNA GHS classification (*in vivo*) (Test Set (N=28))		
		1A (34)	1B (60)	NS (40)	1A (6)	1B (14)	NS (8)
ANN model (h-CLAT/DPRA/KeratinoSens™/cell toxicity/Toxtree)	1A	25	4	0	4	3	0
	1B	9	53	21	2	11	4
	Negative	0	3	19	0	0	4
	Accuracy (%)	72.4			67.9		
	Over predicted (%)	18.7			25.0		
	Under predicted (%)	9.0			7.1		

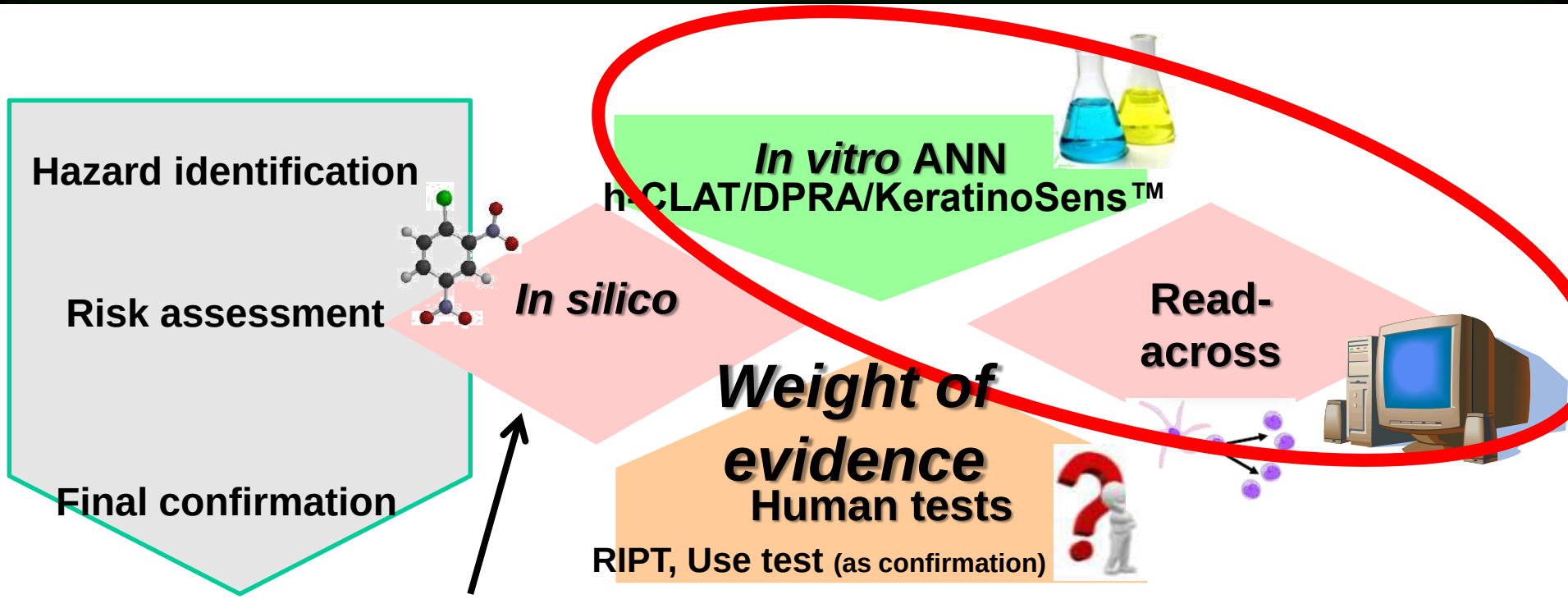
Predicted EC3<2%→ 1A
Predicted EC3>2%→ 1B
Negative result (all of 3 in vitro tests)→NS(no sensitizer)

Agenda

1. About ANN model consisting of in vitro sensitization test and in silico/structure alert model.

2. Case study of skin sensitization assessment of some fragrance ingredients.

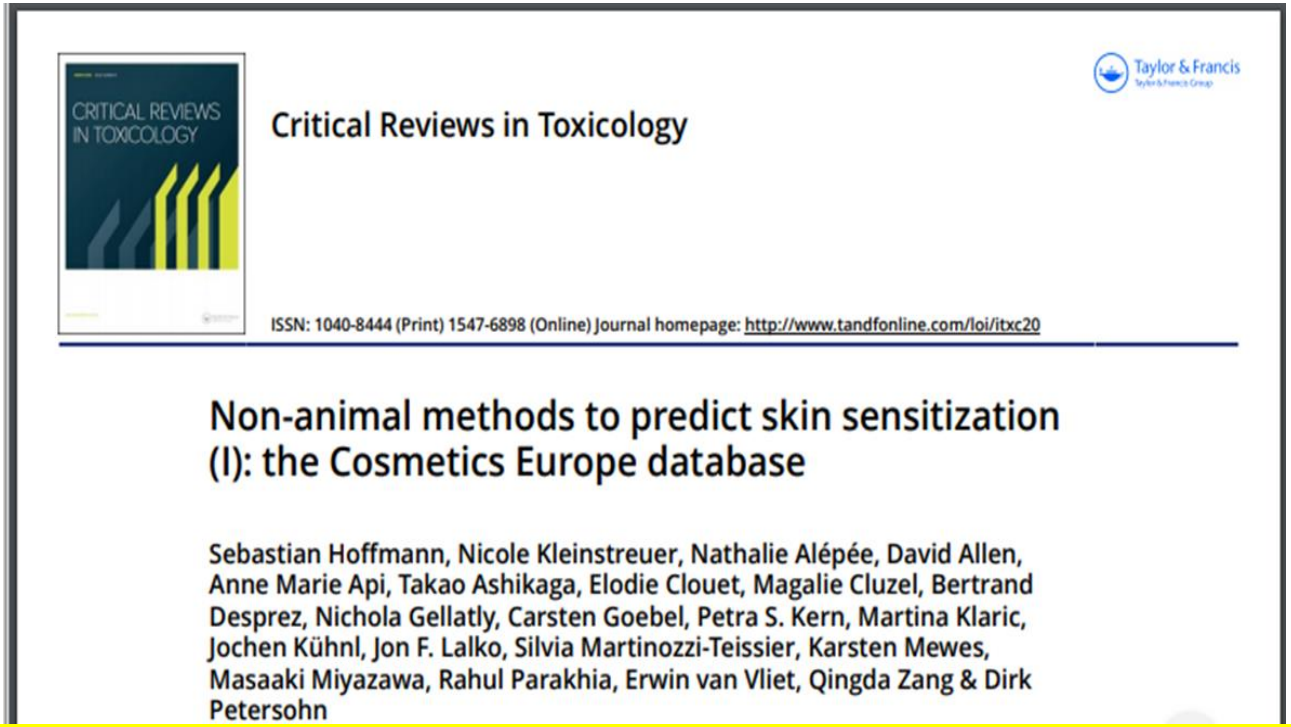
Basic strategy of safety assurance for skin sensitization



Original QSAR model(Tsujita-Inoue et al., *J. Toxicol. Sci*., 40 (2015) 193-209)

- In our company, we usually assess cosmetic ingredient using in vitro, in silico(QSAR) , read across and human confirmatory test based on ‘weight of evidence’ concept.
- In this case study, we introduce the example using in vitro ANN model and read across.

Chemical selection of this case study



Taylor & Francis

Critical Reviews in Toxicology

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

Sebastian Hoffmann, Nicole Kleinstreuer, Nathalie Alépée, David Allen, Anne Marie Api, Takao Ashikaga, Elodie Clouet, Magalie Cluzel, Bertrand Desprez, Nichola Gellatly, Carsten Goebel, Petra S. Kern, Martina Klaric, Jochen Kühnl, Jon F. Lalko, Silvia Martinozzi-Teissier, Karsten Mewes, Masaaki Miyazawa, Rahul Parakhia, Erwin van Vliet, Qingda Zang & Dirk Petersohn

- ✓ **Recently, the database of 128 substances with h-CLAT/DPRA/ KeratinoSens™, U-SENS, LLNA and human skin sensitization potency was published.**
- ✓ **In this database, the 58 fragrance materials were included.**
- ✓ **In the simple chemicals (not mixture) with h-CLAT, DPRA and KeratinoSens™, the fragrances with TIMES-M data (*) or Toxtree data were selected (48 chemicals with TIMES-M data, 51 chemicals with Toxtree data.)**

- **TIMES-M data of fragrances were taken from Jaworska et al., (Arch Toxicol., 89, 2355-2383, 2015)**

51 fragrance ingredients with in vitro/in silico data

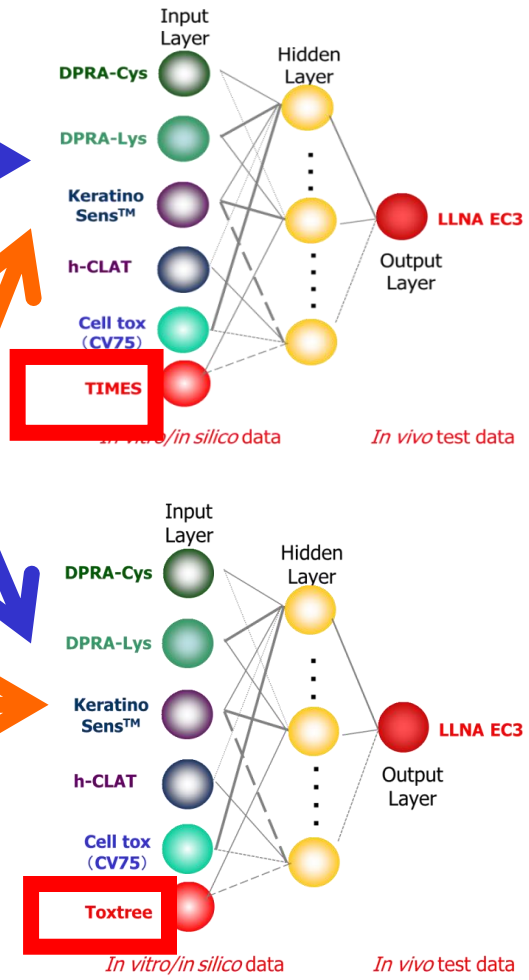
Name	CAS no	use class	In the learning set ? Yes/No	MW	h-CLAT	DPRA	KeratinoSe	ToxTree	TIMES-M
Cinnamic aldehyde	104-55-2	fragrance	Yes	132.2	↙	↙	↙	↙	↙
Methyl heptine carbonate	111-12-6	fragrance	No	154.2	↙	↙	↙	↙	↙
Isoeugenol	97-54-1	fragrance	Yes	164.2	↙	↙	↙	↙	↙
Coumarin	91-64-5	fragrance	Yes	146.1	↙	↙	↙	↙	↙
Farnesol	4602-84-0	fragrance	No	222.4	↙	↙	↙	↙	↙
Amyl cinnamic aldehyde	122-40-7	fragrance	Yes	202.3	↙	↙	↙	↙	↙
Hexyl cinnamic aldehyde	101-86-0	fragrance	Yes	216.3	↙	↙	↙	↙	↙
Citral	5392-40-5	fragrance	Yes	152.2	↙	↙	↙	↙	↙
Eugenol	97-53-0	fragrance	Yes	164.2	↙	↙	↙	↙	↙
Lillial	80-54-6	fragrance	Yes	204.3	↙	↙	↙	↙	↙
Cinnamic alcohol	104-54-1	fragrance	Yes	134.2	↙	↙	↙	↙	↙
Geraniol	106-24-1	fragrance	Yes	154.2	↙	↙	↙	↙	↙
Linalool	78-70-6	fragrance	Yes	154.2	↙	↙	↙	↙	↙
Hydroxycitronellal	107-75-5	fragrance	Yes	172.3	↙	↙	↙	↙	↙
Amylcinnamyl alcohol	101-85-9	fragrance	No	204.3	↙	↙	↙	↙	↙
Carvone	6485-40-1	fragrance	Yes	150.2	↙	↙	↙	↙	↙
Hexyl salicylate	6259-76-3	fragrance	Yes	222.3	↙	↙	↙	↙	↙
Diethyl phthalate	84-66-2	fragrance	Yes	222.2	↙	↙	↙	↙	↙
Vanillin	121-33-5	fragrance	Yes	152.1	↙	↙	↙	↙	↙
Anethole	104-46-1	fragrance	No	148.2	↙	↙	↙	↙	
Anisyl alcohol	105-13-5	fragrance	No	138.2	↙	↙	↙	↙	↙
Benzyl benzoate	120-51-4	fragrance	Yes	212.2	↙	↙	↙	↙	↙
Benzyl salicylate	118-58-1	fragrance	Yes	228.2	↙	↙	↙	↙	↙
Citronellol	106-22-9	fragrance	No	156.3	↙	↙	↙	↙	↙
(R)-(+)-Limonene	5989-27-5	fragrance	No	136.2	↙	↙	↙	↙	
Benzaldehyde	100-52-7	fragrance	Yes	106.1	↙	↙	↙	↙	↙
5-Methyl-2,3-hexanedione	13706-86-0	fragrance	Yes	128.2	↙	↙	↙	↙	↙
Lyral	31906-04-4	fragrance	Yes	210.3	↙	↙	↙	↙	↙
Allyl phenoxyacetate	7493-74-5	fragrance	No	192.2	↙	↙	↙	↙	↙
p-t-Butyl-dihydrocinnamaldehyde (Bourgenol)	18127-01-0	fragrance	No	190.3	↙	↙	↙	↙	↙
Cinnamyl nitrile	1885-38-7	fragrance	No	129.2	↙	↙	↙	↙	↙
Dibenzyl ether	103-50-4	fragrance	No	198.3	↙	↙	↙	↙	↙
Isocyclocitral	1335-66-6	fragrance	No	152.2	↙	↙	↙	↙	↙
Isocyclogeraniol	68527-77-5	fragrance	No	154.3	↙	↙	↙	↙	↙
β, β 3-Trimethyl benzenepropanol	103694-68-4	fragrance	No	178.3	↙	↙	↙	↙	↙
Menthadiene-7-methyl formate	68683-20-5	fragrance	No	194.3	↙	↙	↙	↙	↙
4-Methoxy-α-methyl benzenpropanal	5462-06-6	fragrance	No	178.2	↙	↙	↙	↙	
α-Methyl-1,3-benzodioxole- 5-propionaldehyde	1205-17-0	fragrance	No	192.2	↙	↙	↙	↙	↙
6-Methyl-3,5-heptadien-2-one	1604-28-0	fragrance	No	124.2	↙	↙	↙	↙	↙
α-iso-Methylionone	127-51-5	fragrance	No	206.3	↙	↙	↙	↙	↙
OTNE	54464-57-2	fragrance	No	234.4	↙	↙	↙	↙	↙
Benzyl Alcohol	100-51-6	fragrance	No	108.1	↙	↙	↙	↙	↙
Benzyl Cinnamate	103-41-3	fragrance	Yes	238.3	↙	↙	↙	↙	↙
trans -2-Hexenal	6728-26-3	fragrance	Yes	98.1	↙	↙	↙	↙	↙
p-Isobutyl-α-methyl hydrocinnamaldehdye	6658-48-6	fragrance	No	204.3	↙	↙	↙	↙	↙
p-Mentha-1,8-dien-7-al	2111-75-3	fragrance	Yes	150.2	↙	↙	↙	↙	↙
α-Methyl cinnamic aldehyde	101-39-3	fragrance	Yes	146.2	↙	↙	↙	↙	↙
Methyl 2-nonynoate (Methyl octine carbonate)	111-80-8	fragrance	Yes	168.2	↙	↙	↙	↙	↙
2-Methoxy-4-methylphenol	93-51-6	fragrance	Yes	138.2	↙	↙	↙	↙	↙
Phenylacetaldehyde	122-78-1	fragrance	Yes	120.1	↙	↙	↙	↙	↙
3-Propylidenephthalide	17369-59-4	fragrance	Yes	174.2	↙	↙	↙	↙	↙

ANN evaluation using fragrance ingredients

ANN models(Learning Set(N=134))

Data set (in vitro, Times, Toxtree) of 29 fragrances, which is included in learning set

Data set (in vitro, Times, Toxtree) of 19 or 22 fragrances, which is not included in learning set (test set)

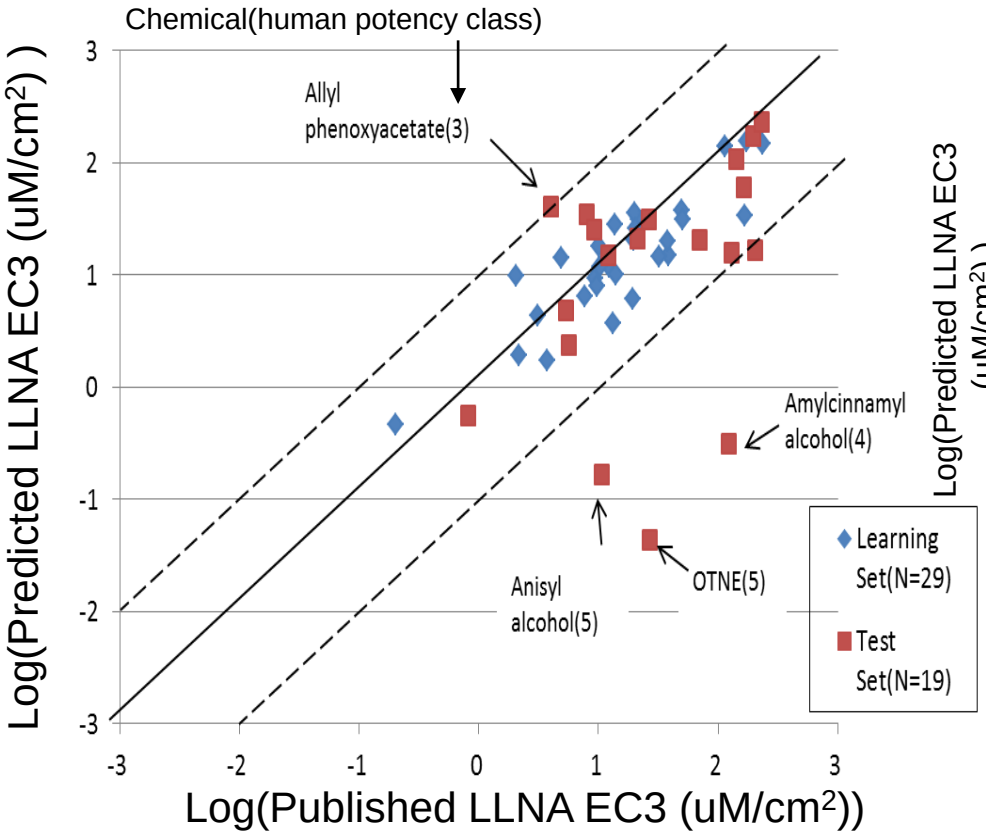


Predicted EC3s

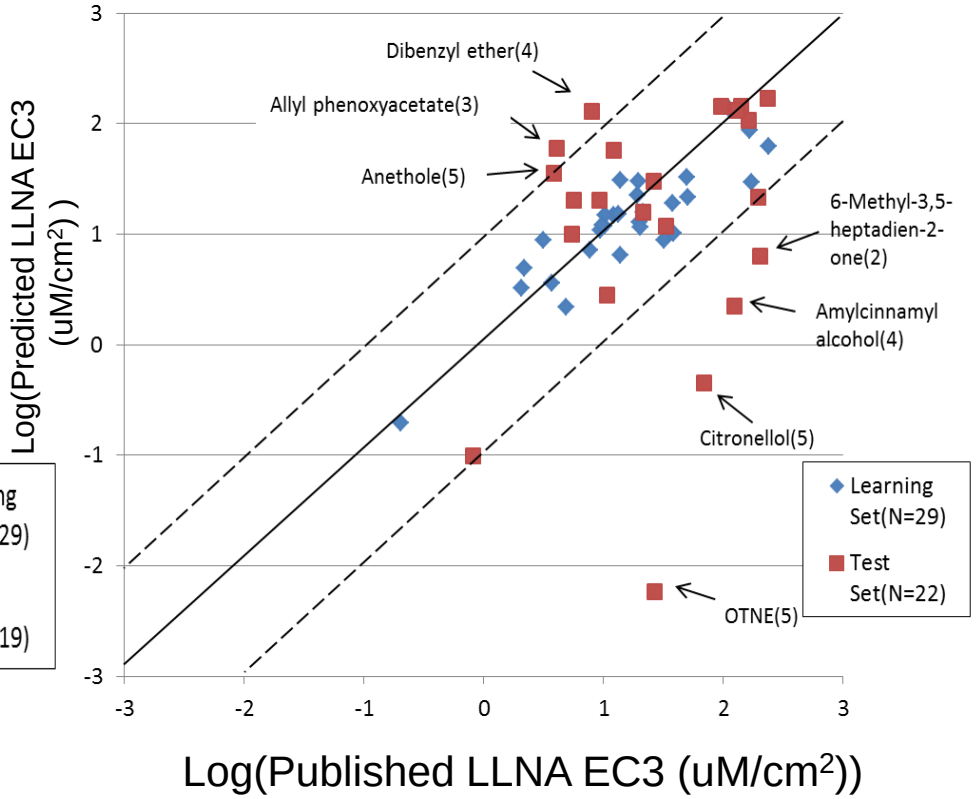
Comparison with published EC3 values

Prediction results of fragrance ingredients

ANN model (h-CLAT/DPRA/KeratinoSens™ /cell toxicity/TIMES-M)



ANN model (h-CLAT/DPRA/KeratinoSens™/ cell toxicity/Toxtree)



✓ About the fragrance ingredients not to be included in learning set, the predicted EC3 values derived from ANN model with TIMES-M were correlated with published LLNA EC3 better than those from ANN model with Toxtree.

Category prediction of fragrance ingredients using ANN models-1

ANN model (h-CLAT/DPRA/KeratinoSens™/cell toxicity/TIMES-M)

Learning Set(BP 3 out of 3)		LLNA		
		1A(3)	1B(22)	NS(4)
ANN model (h-CLAT/DPRA /KeratinoSens™/cell toxicity/TIMES-M)	1A	2	1	0
	1B	1	21	2
	NS	0	0	2

Accuracy 86.2 %
Over predicted 10.3 %
Under predicted 3.4 %

Test Set (BP 3 out of 3)		LLNA		
		1A(1)	1B(11)	NS(7)
ANN model (h-CLAT/DPRA /KeratinoSens™/cell toxicity/TMES-M)	1A	1	3	1
	1B	0	8	6
	NS	0	0	0

Accuracy 47.4%
Over predicted 52.6%
Under predicted 0.0%

It is possible to consider that accuracy is about 60% (if 3 of 6 FP (in red circle) chemicals is judged as NS)

Name	CAS no.	Predicted EC3 (%) (in Test set)
Amylcinnamyl alcohol	101-85-9	0.25
Cinnamyl nitrile	1885-38-7	88.68
Isocyclogeraniol	68527-77-5	36.91
β, β 3-Trimethyl benzenepro	103694-68-4	77.51
Menthadiene-7-methyl formate	68683-20-5	12.29
6-Methyl-3,5-heptadien-2-one	1604-28-0	8.17
Benzyl Alcohol	100-51-6	98.47

Almost 100%

- ✓ **Accuracy of category prediction(test sets) was about 60%.**
- ✓ **More safe site prediction was performed (but high over predicted result).**

NS: not sensitizing in LLNA

Category prediction of fragrance ingredients using ANN models-2

ANN model (h-CLAT/DPRA/KeratinoSens™/cell toxicity/Toxtree)

Learning Set(BP 3 out of 3)		LLNA		
		1A(3)	1B(22)	NS(4)
ANN model (h-CLAT/DPRA /KeratinoSens™/cell toxicity/Toxtree)	1A	1	1	0
	1B	2	21	2
	NS	0	0	2

Accuracy 82.8%
Over predicted 10.3%
Under predicted 6.9%

Test Set (BP 3 out of 3)		LLNA		
		1A(1)	1B(14)	NS(7)
ANN model (h-CLAT/DPRA /KeratinoSens™/cell toxicity/Toxtree)	1A	1	3	1
	1B	0	10	4
	NS	0	1	2

Accuracy 59.1%
Over predicted 36.4%
Under predicted 4.8%

- Dibenzyl ether
  - ANN(predicted EC3 > 100%)
  - DPRA(-) KeratinoSens™(+), h-CLAT(+)
  - In vivo LLNA EC3 6.3%(moderate)
  - Human class; category 4

- ✓ **Accuracy of category prediction(test sets) was about 60%.**
- ✓ **More safe site prediction was performed (but high over predicted result).**
- ✓ **Dibenzyl ether was false negative**

NS: not sensitizing in LLNA

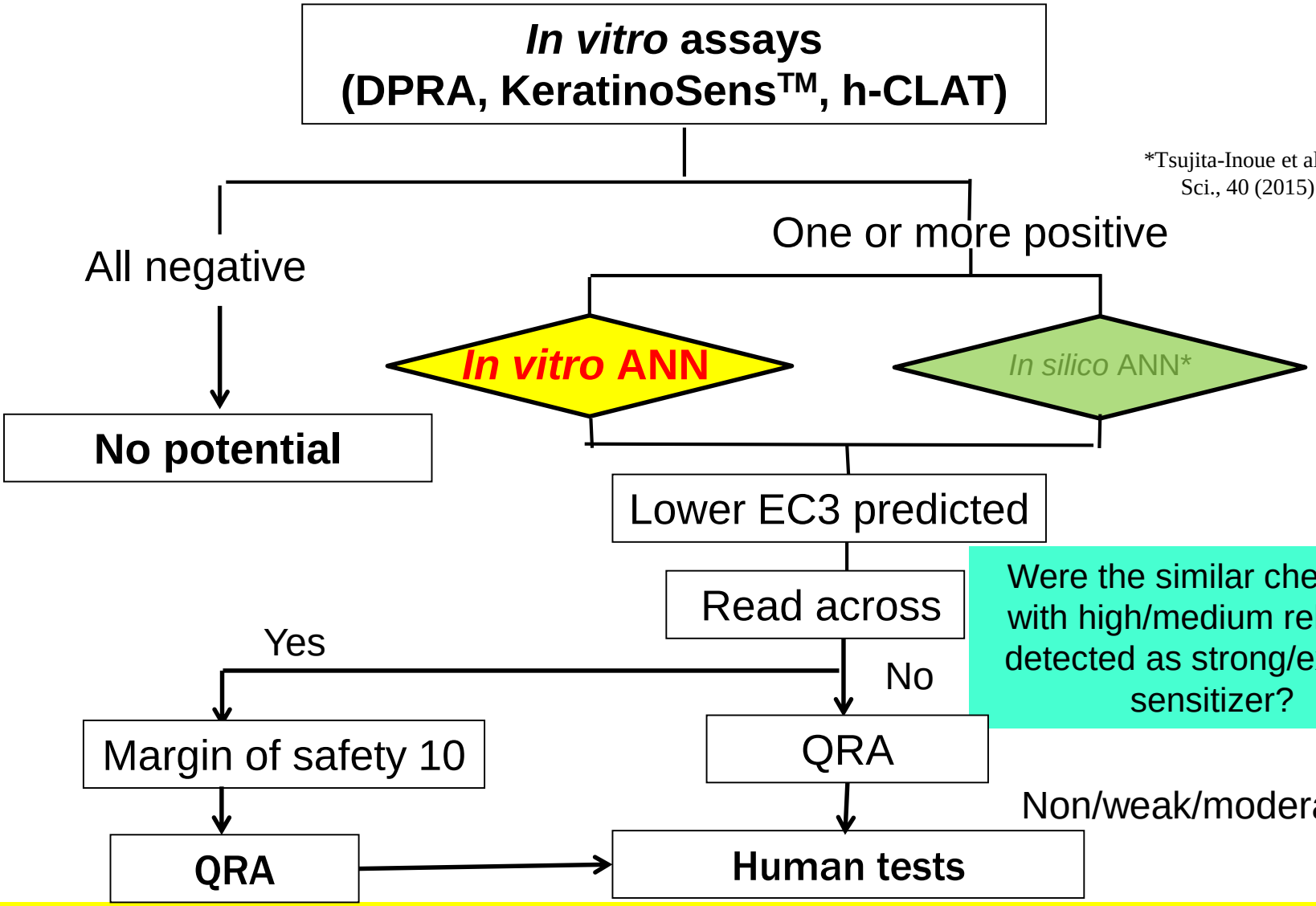
Chemical Selection of the case study

Chemicals	Published LLNA EC3(%)	Human potency	Predicted EC3(%) (by ANN model(TIMES))	Predicted EC3(%) (by ANN model(Toxtree))
Allyl phenoxyacetate	3.1	3	31	46.3
Dibenzyl ether	6.3	4	27.6	103%(=100%)
OTNE	25.14	5	0.04	0.005

- ✓ ANN model(TIMES): ANN model (h-CLAT/DPRA/KeratinoSens™/cell toxicity/TIMES)
- ✓ ANN model(Toxtree): ANN model (h-CLAT/DPRA/KeratinoSens™/cell toxicity/Toxtree)

- Allyl phenoxyacetate, Dibenzyl ether; these chemicals were under-predicted in both ANN models.
- OTNE; this chemical was over-predicted.

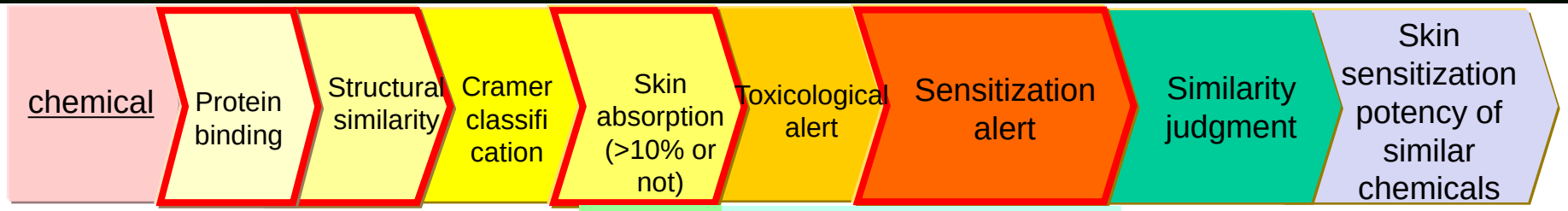
Application of read across analysis



*Tsujita-Inoue et al., J. Toxicol. Sci., 40 (2015) 193-209

To avoid under-prediction of hazardous compounds, chemicals which were predicted strong or extreme sensitizer in this process were imposed 10 times safety margin to the predicted EC3 value.

Extraction of similar chemicals by read across

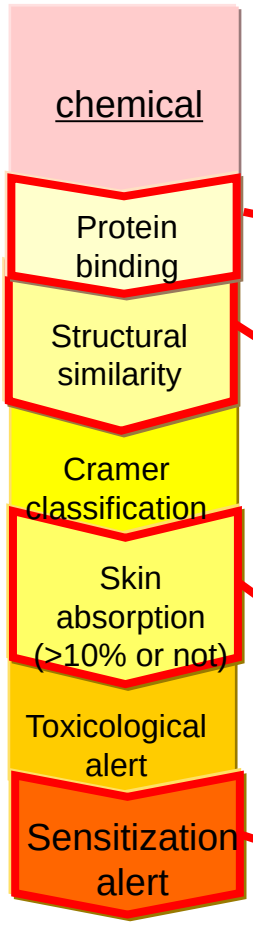


* : Atobe et al., J. Toxicol. Sci., 40 (2015) 277-294

	Protein binding	Structural similarity (%)	Cramer classification	Skin absorption (%)	Toxicological alert	Sensitization alert	Certain similarity	sensitization potency (Reported data)	Predicted Potential
Target chemical	No alert	100	Low	25	No alert	No alert			Moderate
Chemical A	No alert	> 85	Low	30	No alert	No alert	High	Moderate	
Chemical B	No alert	> 85	Low	18	Catechol	No alert	Medium	Moderate	
Chemical C	No alert	> 85	Low	8	No alert	Conjugated diene	Medium	Non	
Chemical D	No alert	> 85	Intermediate	20	No alert	No alert	Medium	Moderate	
Chemical E	No alert	> 80	High	5	No alert	1,2-Dihydroxybenzene	Low	No data	
Chemical F	No alert	> 75	Low	0.1	Alkyl alcohol	Conjugated diene	Low	Extream	
Chemical G	Alert	> 85	Intermediate	35	No alert	No alert	Medium	No data	
Chemical H	Alert	> 85	Low	20	No alert	No alert	Medium	Non	
Chemical I	Alert	> 80	Intermediate	5	Alkyl alcohol	Conjugated diene	Very low	Strong	
Chemical J	Alert	> 80	High	0.1	Peracid	Conjugated diene	Very low	No data	
Chemical K	Alert	> 80	High	0.5	Organic peroxide	1,2-Dihydroxybenzene	Very low	Non	
Chemical L	Alert	> 75	Low	4	No alert	No alert	Low	Strong	

When the similar chemicals with strong (extreme) sensitization potential (not low reliability) were detected, the safety margin(x 10) was needed.

Tentative criteria for matching up with the target chemicals



Chemicals

Protein binding alert (OECD tool box) is detected, and category is same with target chemical

Structural similarity with target chemical is > 75%

In silico skin absorption rate is similar (both target and candidate are high (>10%) or low(<10%))

Sensitization alert (DEREK) is detected, and category is same with target chemical

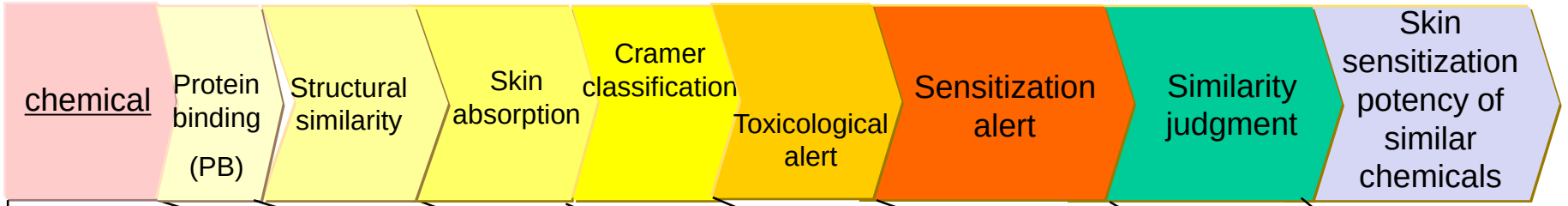
Tentative criteria for matching up with the target chemicals

All parameters matches up → high reliability

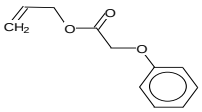
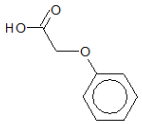
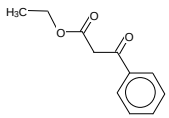
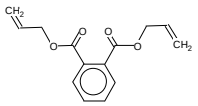
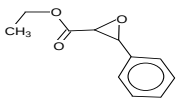
2 or 3 of 4 parameters matches up → medium reliability

0 or 1 of 4 parameters matches up → low reliability

Read Across of 3 fragrance materials-1

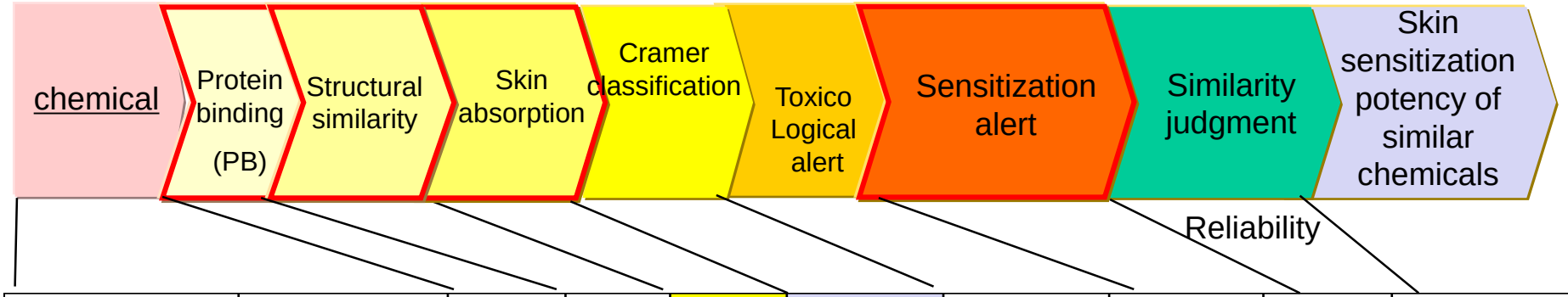


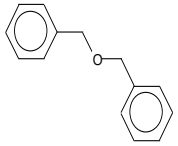
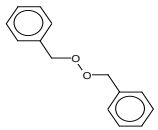
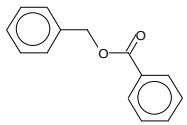
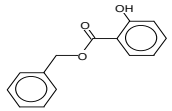
Reliability

Allyl phenoxyacetate (Target)		SN2	100%	15.9%	High	✓ O-allyl compound ✓ phenoxyacetic acid	No alert	-	
Phenoxyacetic acid		No alert	>65%	35.9%	High	phenoxyacetic acid	No alert	Low	LLNA 50% negative
Ethyl benzoylacetate		No alert	>50%	0.01%	Low	beta-keto carboxylic acid	No alert	Low	LLNA negative
Diallylphthalate		SN2	>50%	71.4%	Intermediate	✓ O-allyl compound ✓ phthalate	No alert	Medium	LLNA(moderate)
3-Phenylglycidic acid, ethyl ester		SN2	>50%	17.3%	High	Epoxide	Epoxide	Medium	**Strong Sensitizer(GPMT)**

Allyl phenoxyacetate: similar to Strong sensitizer (Additional safety margin 10)

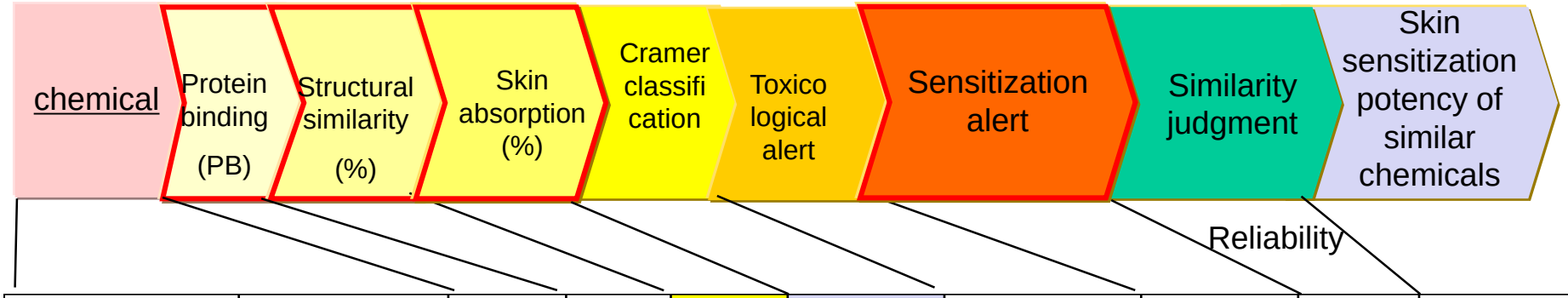
Read Across of 3 fragrance materials-2

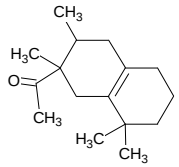
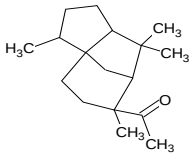
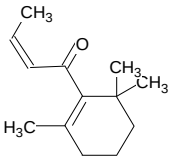


Dibenzyl ether (Target)		No alert	100%	80.1%	High	No alert	No alert	-	
Benzyl peroxide		No alert	>75%	35.7%	High	Organic peroxide	No alert	Medium	**Strong Sensitizer(LLNA)**
Benzyl benzoate		SN2	>75%	70.6%	Intermediate	Benzyl ester	Benzyl ester	Medium	LLNA EC3 17%(Weak)
Benzyl salicylate		SN2	>50%	25.0%	Intermediate	Salicylic acid or analogue	ubstituted phenol precurser	Low	LLNA EC3 2.9%(Moderate)

Dibenzyl ether: similar to Strong sensitizer (Additional safety margin 10)

Read Across of 3 fragrance materials-3



OTNE (Target)		No alert	100%	4.4%	Intermediate	No alert	Terpenoid	-	
Methyl cedryl ketone		No alert	>50%	10.2%	Intermediate	Alpha-2-mu-globulin nephropathy Isopentyl group	No alert	Low	Non-sensitizer
2-Buten-1-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, (2z)-		Ketones	>50%	28.1%	Low	alpha,beta-unsaturated aldehyde, ketone	alpha,beta-unsaturated ester and precursor	Low	Strong

OTNE: the similar chemicals with strong/extreme skin sensitization potential (with medium/high reliability) were not detected (No additional margin)

Result of read across

Chemicals	Published LLNA EC3(%)	Human potency	Predicted EC3(%) (by ANN model(TIMES))	Predicted EC3(%) (by ANN model(Toxtree))
Allyl phenoxyacetate	3.1	3	31	46.3
Dibenzyl ether	6.3	4	27.6	103%(=100%)
OTNE	25.14	5	0.04	0.005

- ✓ ANN model(TIMES): ANN model (h-CLAT/DPRA/KeratinoSens™/cell toxicity/TIMES)
- ✓ ANN model(Toxtree): ANN model (h-CLAT/DPRA/KeratinoSens™/cell toxicity/Toxtree)

Chemicals	Additional safety margin by read across	Predicted EC3(%) (by ANN model(TIMES)) (adjusted)	Predicted EC3(%) (by ANN model(Toxtree)) (adjusted)
Allyl phenoxyacetate	needed	3.1	4.63
Dibenzyl ether	needed	2.76	10
OTNE	Not needed	0.04	0.005

QRA2

Refinements to Dermal Sensitization Quantitative Risk Assessment 2 (QRA 2) For Fragrance Ingredients and Systemic Toxicity

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RIFM Quarterly We
Oct. 17, 2017

QRA2 Upper Use Levels Product Categories with Adjustment Factors: Citral

Cate-gory	Product Type	Max QRA 2 use level by category (%)	QRA 2 aggregate adjustment factor	QRA 2 aggregate exposure adjusted upper use levels (%)
1	Lip Products	0.119	0.91	0.11
2	Deodorants & Antiperspirants of all types including fragranced body sprays	0.051	0.62	0.03
3	Eye Products	0.645	1.00	0.65
4	Fine Fragrance (eau de toilette, parfum etc.)	0.63	0.95	0.60
5	Insect repellent (intended to be applied to the skin)	0.46	0.33	0.15
6	Toothpaste	1.102	0.32	0.35
7	Hair sprays	2.121	0.50	1.23
8	Baby wipes	0.063		0.06
9	Bar soap	2.333	0.50	1.17
10	Hand dishwashing detergent	7.000	0.60	4.21
11	Feminine liners	2.333		2.33
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted		

QRA2: Allyl phenoxyacetate

Predicted EC3(%)(by ANN model(**TIMES**))(adjusted)
3.1(775μg/cm^2/d*)

Automatically calculated

* EC3(%) x 250

This data is simulation when published animal data/human data is not used in WoE approach.

Category	Product Type	SAF	Exposure mg/cm2/day	Max QRA2 use level by category (%)	QRA2 aggregate adjustment factor	QRA2 aggregate exposure adjusted upper use levels (%)
1	Lip Products	100	11.8	0.066	0.91	0.06
2	Deodorants & Antiperspirants of all types including fragranced body sprays	300	9.1	0.028	0.62	0.02
3	Eye Products	100	2.17	0.357	1.00	0.36
4	Fine Fragrance (eaude toilette, parfum etc.)	100	2.21	0.351	0.95	0.33
5	Insect repellent (intended to be applied to the skin)	100	3.02	0.257	0.33	0.08
6	Toothpaste	100	1.27	0.610	0.32	0.20
7	Hair sprays	30	2.2	1.174	0.50	0.59
8	Baby wipes	300	7.4	0.035		0.03
9	Bar soap	300	0.2	1.292	0.50	0.65
10	Hand dishwashing detergent	100	0.2	3.875	0.60	2.33
11	Feminine liners	300	0.2	1.292		1.29
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted				

Predicted EC3(%)(by ANN model(**Toxtree**))(adjusted)
4.63(1158μg/cm^2/d*)

Automatically calculated

Category	Product Type	SAF	Exposure mg/cm2/day	Max QRA2 use level by category (%)	QRA2 aggregate adjustment factor	QRA2 aggregate exposure adjusted upper use levels (%)
1	Lip Products	100	11.8	0.098	0.91	0.09
2	Deodorants & Antiperspirants of all types including fragranced body sprays	300	9.1	0.042	0.62	0.03
3	Eye Products	100	2.17	0.533	1.00	0.53
4	Fine Fragrance (eaude toilette, parfum etc.)	100	2.21	0.524	0.95	0.50
5	Insect repellent (intended to be applied to the skin)	100	3.02	0.383	0.33	0.13
6	Toothpaste	100	1.27	0.911	0.32	0.29
7	Hair sprays	30	2.2	1.754	0.50	0.88
8	Baby wipes	300	7.4	0.052		0.05
9	Bar soap	300	0.2	1.929	0.50	0.96
10	Hand dishwashing detergent	100	0.2	5.788	0.60	3.47
11	Feminine liners	300	0.2	1.929		1.93
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted				

QRA2 : Dibenzyl ether

Predicted EC3(%)(by ANN model(**TIMES**))(adjusted)
2.76 (690μg/cm^2/d*)

Automatically calculated
*EC3(%) x 250

This data is simulation when published animal data/human data is not used in WoE approach.

Category	Product Type	SAF	Exposure mg/cm2/day	Max QRA2 use level by category (%)	QRA2 aggregate adjustment factor	QRA2 aggregate exposure adjusted upper use levels (%)
1	Lip Products	100	11.8	0.058	0.91	0.05
2	Deodorants & Antiperspirants of all types including fragranced body sprays	300	9.1	0.025	0.62	0.02
3	Eye Products	100	2.17	0.318	1.00	0.32
4	Fine Fragrance (eaude toilette, parfum etc.)	100	2.21	0.312	0.95	0.30
5	Insect repellent (intended to be applied to the skin)	100	3.02	0.228	0.33	0.08
6	Toothpaste	100	1.27	0.543	0.32	0.17
7	Hair sprays	30	2.2	1.045	0.50	0.52
8	Baby wipes	300	7.4	0.031		0.03
9	Bar soap	300	0.2	1.150	0.50	0.58
10	Hand dishwashing detergent	100	0.2	3.450	0.60	2.07
11	Feminine liners	300	0.2	1.150		1.15
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted				

Predicted EC3(%)(by ANN model(**Toxtree**))(adjusted)
10(2500μg/cm^2/d*)

Automatically calculated
*EC3(%) x 250

Category	Product Type	SAF	Exposure mg/cm2/day	Max QRA2 use level by category (%)	QRA2 aggregate adjustment factor	QRA2 aggregate exposure adjusted upper use levels (%)
1	Lip Products	100	11.8	0.212	0.91	0.19
2	Deodorants & Antiperspirants of all types including fragranced body sprays	300	9.1	0.092	0.62	0.06
3	Eye Products	100	2.17	1.152	1.00	1.15
4	Fine Fragrance (eaude toilette, parfum etc.)	100	2.21	1.131	0.95	1.07
5	Insect repellent (intended to be applied to the skin)	100	3.02	0.828	0.33	0.27
6	Toothpaste	100	1.27	1.969	0.32	0.63
7	Hair sprays	30	2.2	3.788	0.50	1.89
8	Baby wipes	300	7.4	0.113		0.11
9	Bar soap	300	0.2	4.167	0.50	2.08
10	Hand dishwashing detergent	100	0.2	12.500	0.60	7.50
11	Feminine liners	300	0.2	4.167		4.17
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted				

QRA2: OTNE

Predicted EC3(%)(by ANN model(**TIMES**))(adjusted)
0.04 (10μg/cm^2/d*)

Automatically calculated
*EC3(%) x 250

This data is simulation when published animal data/human data is not used in WoE approach.

Category	Product Type	SAF	Exposure mg/cm2/day	Max QRA2 use level by category (%)	QRA2 aggregate adjustment factor	QRA2 aggregate exposure adjusted upper use levels (%)
1	Lip Products	100	11.8	0.0001	0.91	0.0001
2	Deodorants & Antiperspirants of all types including fragranced body sprays	300	9.1	0.00005	0.62	0.0000
3	Eye Products	100	2.17	0.001	1.00	0.0006
4	Fine Fragrance (eaude toilette, parfum etc.)	100	2.21	0.001	0.95	0.0005
5	Insect repellent (intended to be applied to the skin)	100	3.02	0.000	0.33	0.0001
6	Toothpaste	100	1.27	0.001	0.32	0.0003
7	Hair sprays	30	2.2	0.002	0.50	0.0009
8	Baby wipes	300	7.4	0.00006		0.0001
9	Bar soap	300	0.2	0.002	0.50	0.0010
10	Hand dishwashing detergent	100	0.2	0.006	0.60	0.0038
11	Feminine liners	300	0.2	0.002		0.0021
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted				

Predicted EC3(%)(by ANN model(**Toxtree**))(adjusted)
0.005(1.25μg/cm^2/d*)

Automatically calculated
*EC3(%) x 250

Category	Product Type	SAF	Exposure mg/cm2/day	Max QRA2 use level by category (%)	QRA2 aggregate adjustment factor	QRA2 aggregate exposure adjusted upper use levels (%)
1	Lip Products	100	11.8	0.001	0.91	0.0008
2	Deodorants & Antiperspirants of all types including fragranced body sprays	300	9.1	0.0004	0.62	0.0002
3	Eye Products	100	2.17	0.005	1.00	0.0046
4	Fine Fragrance (eaude toilette, parfum etc.)	100	2.21	0.005	0.95	0.0043
5	Insect repellent (intended to be applied to the skin)	100	3.02	0.003	0.33	0.0011
6	Toothpaste	100	1.27	0.008	0.32	0.0025
7	Hair sprays	30	2.2	0.015	0.50	0.01
8	Baby wipes	300	7.4	0.0005		0.0005
9	Bar soap	300	0.2	0.017	0.50	0.008
10	Hand dishwashing detergent	100	0.2	0.050	0.60	0.03
11	Feminine liners	300	0.2	0.017		0.02
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted				

About ANN models proposed in this presentation-1

	ANN model (h-CLAT/DPRA/ KeratinoSens™/cell toxicity/ TIMES)	ANN model (h-CLAT/DPRA/ KeratinoSens™/cell toxicity/ Toxtree)
(1)-1 Inputs (in vitro)	◆ h-CLAT Minimum induction threshold (MIT) (μM) ◆ DPRA ✓ The percentage remaining cysteine peptide value ✓ The percentage remaining lysine peptide value ◆ KeratinoSens™ EC1.5(μM)	
(1) -2 Inputs (in silico structure alert)	TIMES-M (non-sensitizer, weak or moderate sensitizer, strong or extreme sensitizer)	Toxtree (no alert. SN2, Acyl transfer agent, Michael acceptor, Schiff base formation, SNAr, >2 alerts)
(2) Calculation	ANN(artificial neural network), Software; QwikNet	
(3)methodology of calculation	✓ Non public (QwikNet is the commercial software (but cheap)) However, it is possible to remake using non commercial software (e.g. Python, R) (Kleinstreuer et al., Crit Rev Toxicol., 48, 344-358, 2018.) ✓ We do not have any patents about our ANN model. ✓ KeratinoSens™, TIMES and DEREK need the license fees. ✓ Toxtree and OECD tool box is non commercial.	
(4) an interpretation of the value derived	Our ANN models predict LLNA EC3 directly. As we know, EC3 can be used in QRA assessment.	
(5) measure of the uncertainty involved	Our ANN models were evaluated by 10-fold cross validation and outer validation using the test sets which is not included in the learning set. In the WoE approach including read across study, uncertainty of in vitro ANN models are complemented.	

About ANN models proposed in this presentation-2

	ANN model (h-CLAT/DPRA/ KeratinoSens™/cell toxicity/ TIMES)	ANN model (h-CLAT/DPRA/ KeratinoSens™/cell toxicity/ Toxtree)
(6)Basis for dose selection and reason for benchmark selection.	◆ Our model can calculated EC3, directly. By comparing with predicted EC3 by these ANN models of the rational similar chemicals, evaluation by comparing with benchmark can be done.	
(7) Limitation	✓ The limitation of each in vitro tests might affect this model. ✓ Ingredients with human exposure history and its similar ingredients are applicable as evaluation target in our models.	
(8) How would you measure the error in each prediction?	✓ In ANN model, there is no indicator which can measure the error in each prediction. ✓ So, in order to avid under-prediction result, we incorporate read across evaluation in our safety assessment flow.	
(9) Area of further study	✓ The number of chemicals in the learning set should be increased in ANN models. ✓ Re-making ANN models based on non-commercial algorithm (R-code, Python) ✓ Development of risk assessment model for mixture (e.g. botanical oil) (ref. Hirota et al, JAT, ;35, 1333-1347.2015)	

Conclusions & Discussion

- The ANN models using h-CLAT, DPRA, KeratinoSens™ and in silico structure alert detecting models(TIMES/Toxtree) had a capacity to predict LLNA EC3.
- Each event in the AOP of skin sensitization (protein-binding, dendritic cell activation and keratinocyte signaling) might affect skin sensitization potency level.
- EC3 predicting tool like ANN model might make it easy to perform QRA of fragrance ingredients.
- The combination of in vitro ANN model(s) and read across study (a kind of WoE approach) is useful for avoiding under-predicted results.
- **It will be possible to perform QRA assessment by using our ANN model.**

Finally,・・・

Thank you for your attention.