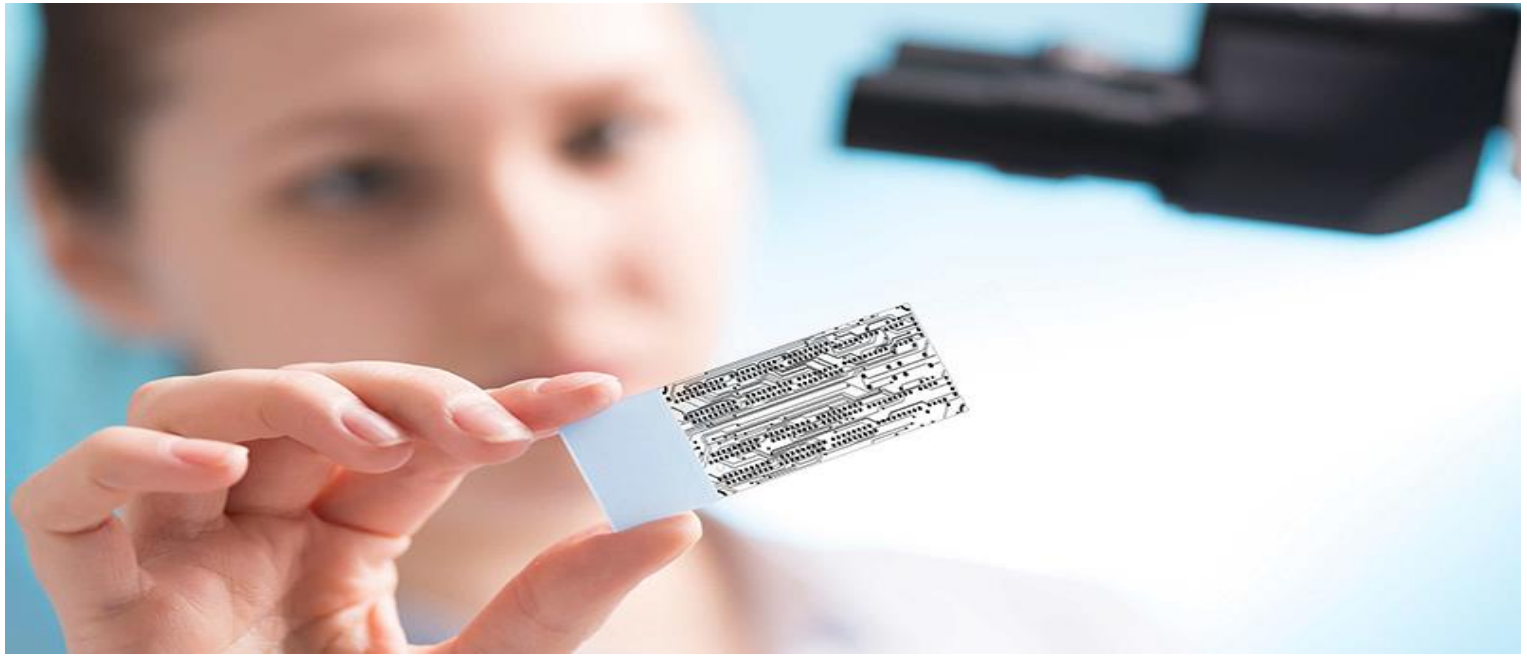
The RIFM sensitization research projects have focused on New Approach Methodologies (NAMs)



RIFM

RIFM ensures the safe use of fragrance materials through our safety assessment program.

Step 1: Data

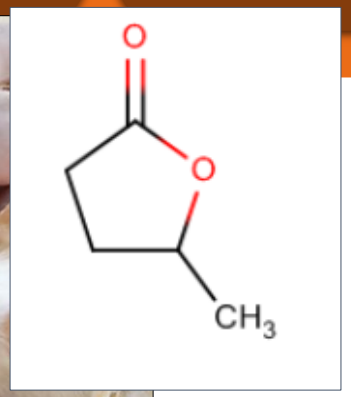
Step 2: Read-Across

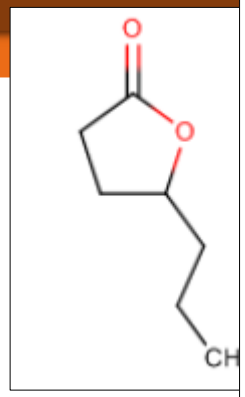
Step 3: Threshold for Toxicological Concern (TTC)

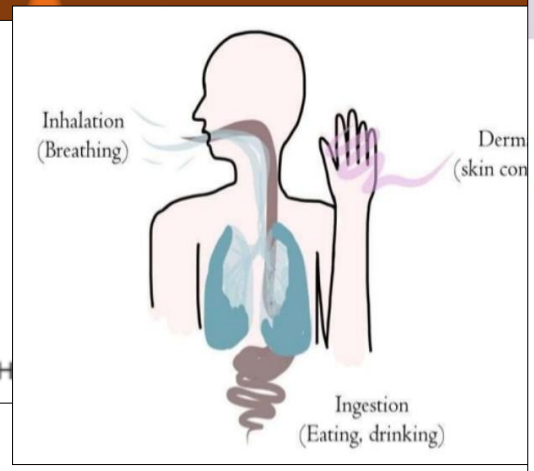
Step 4: Testing

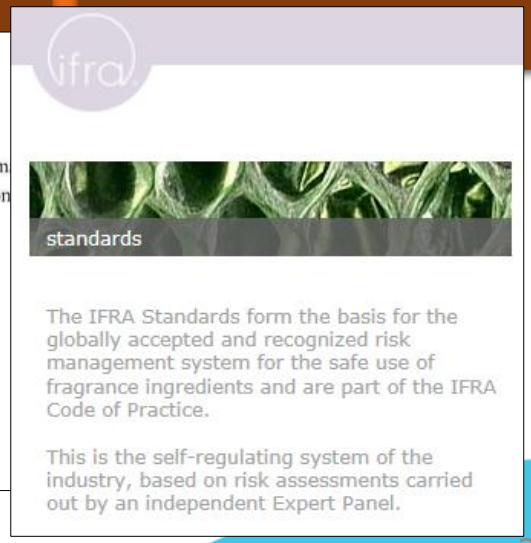
OR

Risk Management







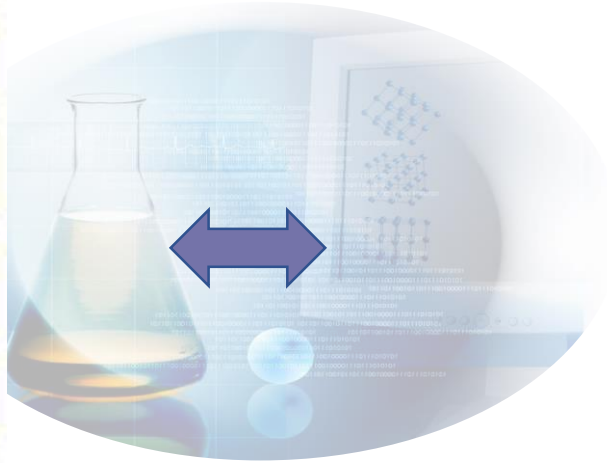


Our staff works on two complimentary scientific endeavors to bring the best science to fragrance material safety

Endpoint Assessments

Exposure

- Environmental
- Genotoxicity
- Photo Tox./Allergy
- Repeat Dose
- Reproduction
- Respiratory
- Sensitization



Research

- Exposure Methodologies
- Improved/Alternative Test Methodologies
- *In Silico* Models

The RIFM sensitization research projects have focused on NAMs to identify sensitization potency

Human Data →
- Human potency categorization (Api,2017)
- Publication of all RIFM conducted HRIPTs (in progress)

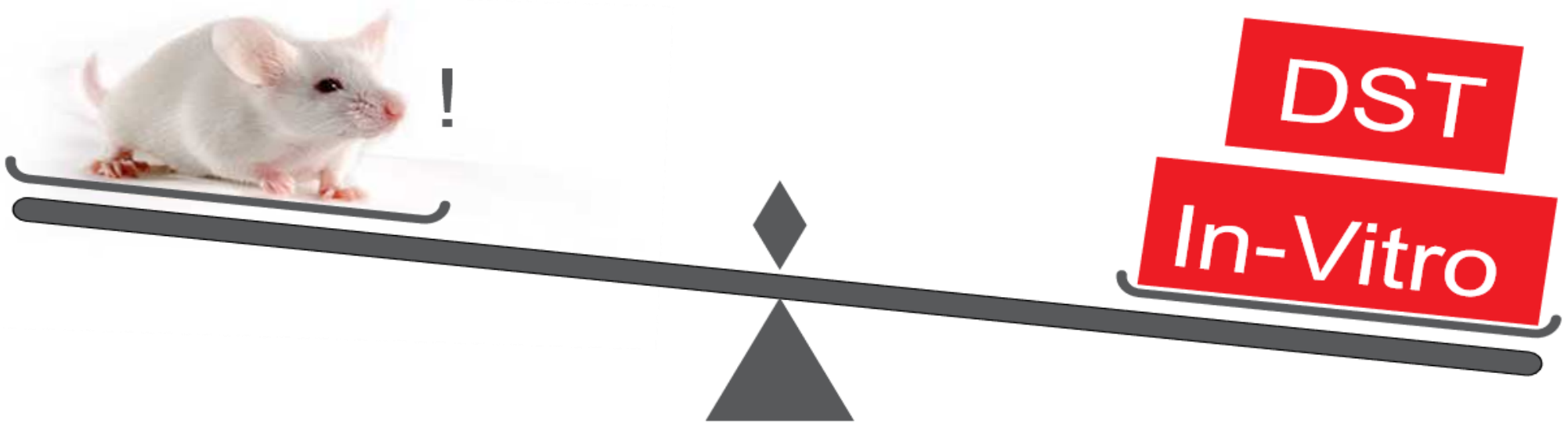
Human + LLNA Data →
- Correlation of human and LLNA induction thresholds (Api,2014; in progress)

Animal Data →
- Contribute to the development of **Lhasa's** EC3 and Negative Prediction Models (Canipa,2017 & Chilton,2018)

***In Chemico/In Vitro* Data** →
- **EDELWEISS CONNECT**
- Recategorization of human categories utilizing additional data

DST →
- Integration of high potency chemicals (Roberts,2015) into **OASIS TIMESS 2.28.1**
- Working w/ Bob Safford to strengthen the DST dataset
- Working w/ **Lhasa** and Frank Gerberick to predict NESILs using EC3 predictions and DST

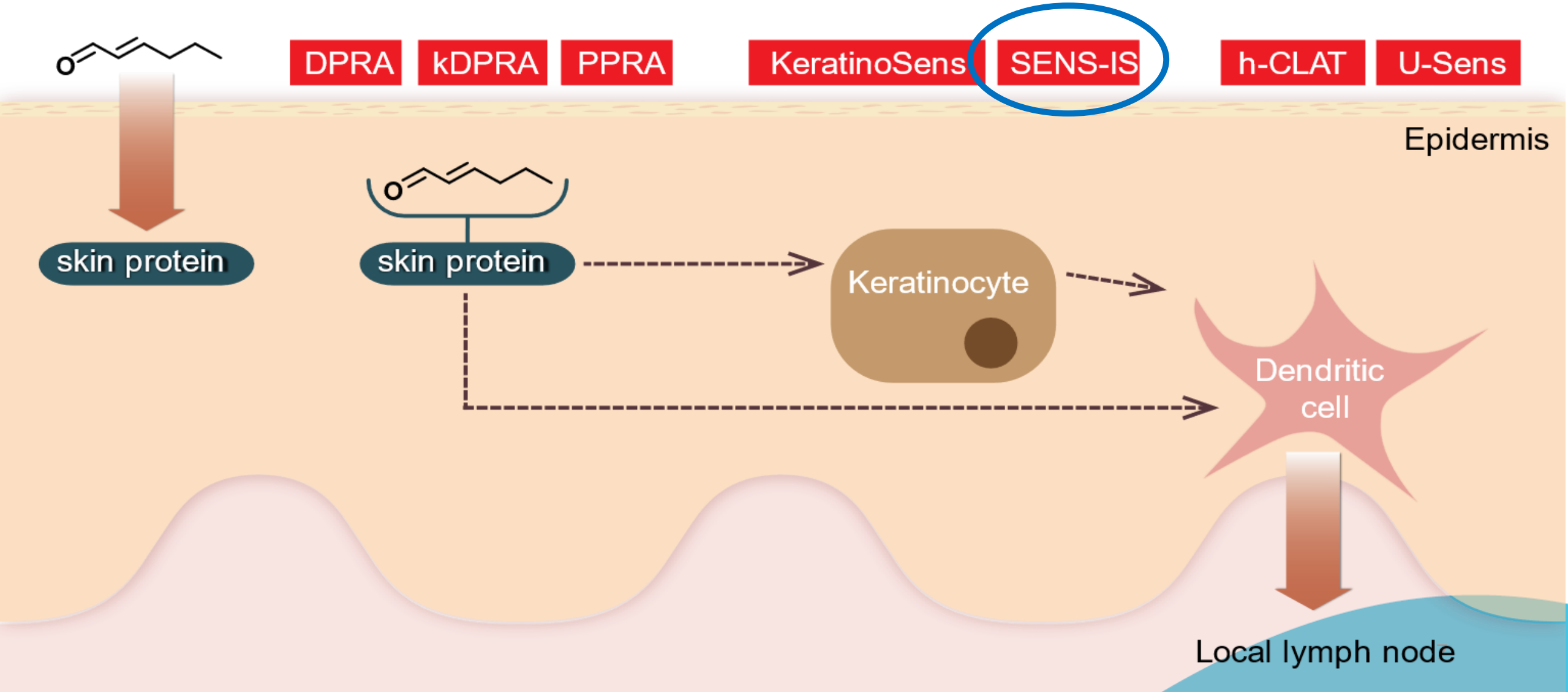
The main focus of the RIFM skin sensitization research is developing alternatives to animal testing and determining potency



Dermal Sensitization Threshold (DST) identifies an exposure below which there is no appreciable risk for the induction of skin sensitization for an untested chemical

- RIFM collaborated with R Safford and D Roberts to extend DST for reactive chemicals and identify high potency chemicals
  - Safford, R.J., Api, A.M., Roberts, D.W., Lalko, J.F., 2015. Extension of the Dermal Sensitisation Threshold (DST) approach to incorporate chemicals classified as reactive. Regulatory Toxicology and Pharmacology, 72, 694-701.
  - Roberts, D.W., Api, A.M., Safford, R.J., Lalko, J.F., 2015. Principles for identification of High Potency Category Chemicals for which the Dermal Sensitisation Threshold (DST) approach should not be applied. Regulatory Toxicology and Pharmacology, 72, 683-693
- RIFM currently collaborating with scientists at Kao to develop a DST for high potency chemicals

RIFM is leading the effort in combining multiple in vitro methods to predict skin sensitization potency



Reliable animal and human data are required for appropriate in vitro data evaluation and predictive models

Api, 2014	Correlation of human and LLNA induction thresholds	**In Progress**	Correlation of humand and LLNA induction thresholds **Part II**
Api, 2017	Skin sensitization potency categorization based on human data	**In Progress**	Compilation of RIFM conducted HRIPTs
		In Progress	Pilot study on the new HRIPT protocol
		In Progress	Skin sensitization potency **re-categorization**
		In Progress	Comparison of re-categorized skin sensitization potency and SENS-IS data

Skin sensitization potency re-categorization incorporates more than just human data

Re-Categorization

1 Test material id
- discrete chemical or mixture?
- stabilizer information

2 Human data
- LOELs where available
- the highest NESIL
- other HRIPT, hMAX LOEL

3 LLNA data
- EC3

4 Chemistry Predictions
- OECD Toolbox
- OASIS TIMESS

5 In vitro data
- DPRA, PPRA
- KeratinoSens, h-CLAT
- SENS-IS

6 Other in vivo data
- guinea pig studies

7 Exposure data
- Creme-RIFM aggregate exposure model

8 Diagnostic patch test

9

Ranges were modified for the re-categorization

Category Name	LLNA (µg/cm^2)	Basketter (µg/cm^2)	Modified Basketter (µg/cm^2)
Extreme	<25	<25	<25
Strong	25 - 250	25 - 500	25 - 500
Moderate	250 - 2,500	500 - 2,500	500 - 2,500
Weak	2,500 - 25,000	2,500 - 10,000	2,500 - 5,000
Very Weak	---	> 10,000	>5,000
Non Sensitizer	---	Negative	---

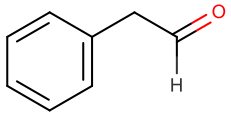
We went through this exercise for 75 materials and categories for 22 materials were changed

			HRIPT				HRIPT	HRIPT	LLNA	DPRA	PPRA	KeratinoSens	h-CLAT	SENS IS	ToolBox	TIMESS	TIMESS
	CAS Number	Chemical Name	Category Range (Api, 2017)	Category Name (modified Api,2017)	Category Range (µg/cm^2) (modified Api,2017)	Human Categorization Notes (2017 vs 2019 difference)	NESIL (µg/cm^2)	LOEL (µg/cm2)	LLNA	DPRA	PPRA	KeratinoSens	h-CLAT	SENS-IS	Protein binding alerts for skin sensitization by OASIS	Parent Prediction	Metabolite Prediction
1	6728-26-3	Hexen-2-al	25 - 500				24	236	1012 [2]	Strong	HR			rong	Michael Addition	Strong sensitiser	Non sensitiser
2	3658-77-3	4-Hydroxy-2,5-dimethyl-3(2H)-furanone	500 - 2,50				590	1181	450	Strong	HF			ak	No alert found	Non sensitiser	Weak sensitiser
3	122-03-2	Cuminic Aldehyde	2,500 - 10,000			erate - *NESIL no effect level ot THE no effect level (LLNA (SI = 2.0 @ 10%) and HMAX (2760))	1100*	n/a	>2500	Low				oderate	Schiff Base	Weak sensitiser	Weak sensitiser
4	68991-97-9	1,2,3,4,5,6,7,8-Octahydro-8,8-dimethyl-2-naphthaldehyde	500 - 2,500	Moderate	500 - 2,500		550	n/a	1050	Minimal	MR	Negative	Positive	Non-sensitizer	Schiff Base	Weak sensitiser	Weak sensitiser
5	1885-38-7	Cinnamyl nitrile	500 - 2,500	Moderate	500 - 2,500		1060	1938	>2500	Minimal	MR	Positive	Positive	Moderate	No alert found	Non sensitiser	Strong sensitiser
6	123-11-5	p-Methoxybenzaldehyde	2,500 - 10,000	Weak	2,500 - 5,000		3,500	4,700	>6250	Moderate	R	Negative	Positive	Weak	No alert found	Non sensitiser	Non sensitiser
7	18794-84-8	β-Farnesene	2,500 - 10,000	Weak	2,500 - 5,000	Mixture of isomers; with BHT	3,700	6350	>7500	Minimal	R	Positive	Positive	Non-sensitizer	No alert found	Non sensitiser	Weak sensitiser
8	100-51-6	Benzyl alcohol	2,500 - 10,000	Very weak*	>5,000	Top use categories: Facial scrubs, EDP/EDT	5,900	8,858	>12,500	Minimal	R	Positive/Negative	Positive	Weak	No alert found	Non sensitiser	Non sensitiser
9	54464-57-2	1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone (OTNE)	> 10,000	Very weak	> 5,000	Do we consider NS?	47,000	n/a	3783 [3]	Strong/Low	MR	Negative	Positive	Weak	Nucleophillic Addition	Weak sensitiser	Weak sensitiser

75 materials

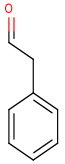
22 materials

Multiple sources of information are used when evaluating a fragrance material

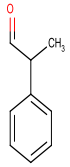


Phys-chem, Reactivity, Absorption & Metabolism, In silico tools	**In chemico In vitro studies**	**Mouse and Guinea pig Studies**	**Human data**
OECD QSAR Toolbox	DPRA (KE 1)	Local Lymph Node Assay (KE 4)	Human Repeated Insult Patch Test (HRIPT) (AO)
TIMES-SS DEREK NEXUS	KeratinoSens™ hCLAT (KE 2 + 3)	GP Maximization Test GP Buehler Test (AO)	
Toxtree, SAM	PPRA, kDPRA, U-Sens™, Sens-IS®	GP: OET, CET, FCAT, DT	H-Maximization test
		Mouse ear swelling test	Diagnostic Patch tests

In vitro data in Safety assessment



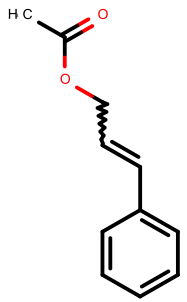
Phenylacetaldehyde
(CAS# 122-78-1)



2-Phenylpropionaldehyde
(CAS# 93-53-8)

KE-1	KE-2	KE-3	KE-4	Adverse Outcome
DPRA % depletion C= 60.7% K = 22.63% C = 26.59 K = 5.1	Keratino Sens™ µM EC1.5 = 28.5 EC1.5 = 111.6	h-CLAT µg/ml EC150 (CD86) and EC 200 (CD54) EC150 = 17.30; EC 200 = 13.00 EC150 = 38.20; EC 200 = 44.50	LLNA [# of studies] µg/cm^2 962 [2] 1575 [1]	HRIPT µg/cm^2 LOEL NOEL 1181 591 1938 388

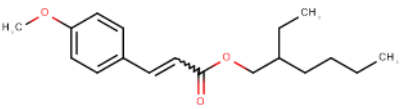
When the fragrance material cinnamyl acetate was being reviewed the first step in the process was to evaluate all the data on the material

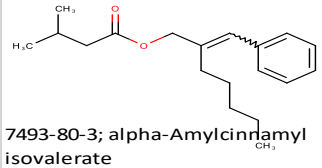
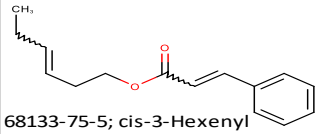
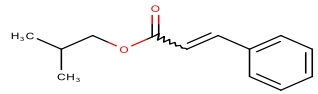
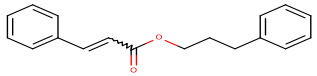
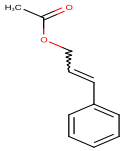
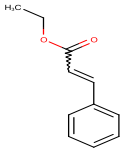
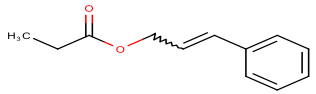
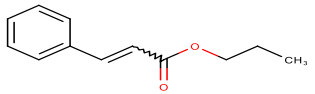
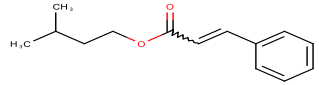
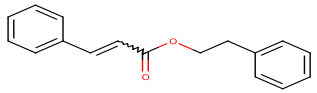
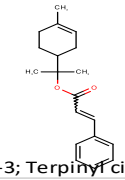
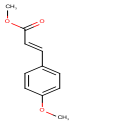
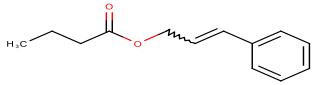
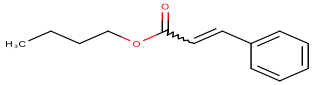
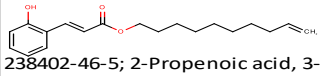
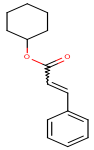
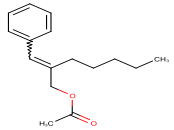
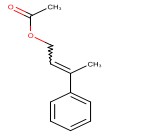
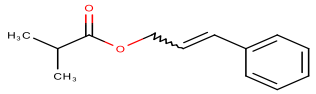
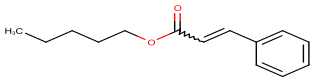
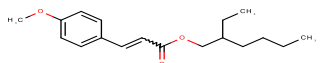
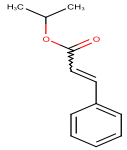
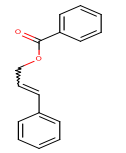
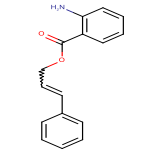
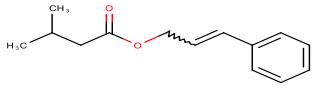
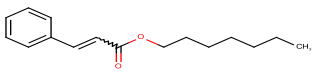
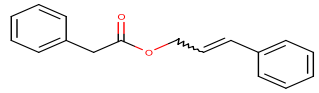
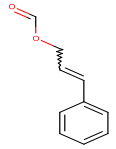
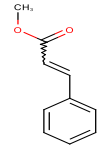
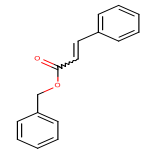
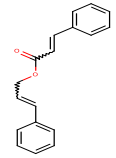
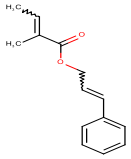
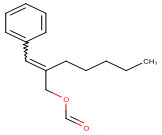
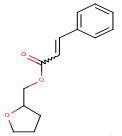
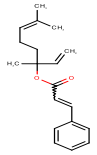


103-54-8 Cinnamyl acetate

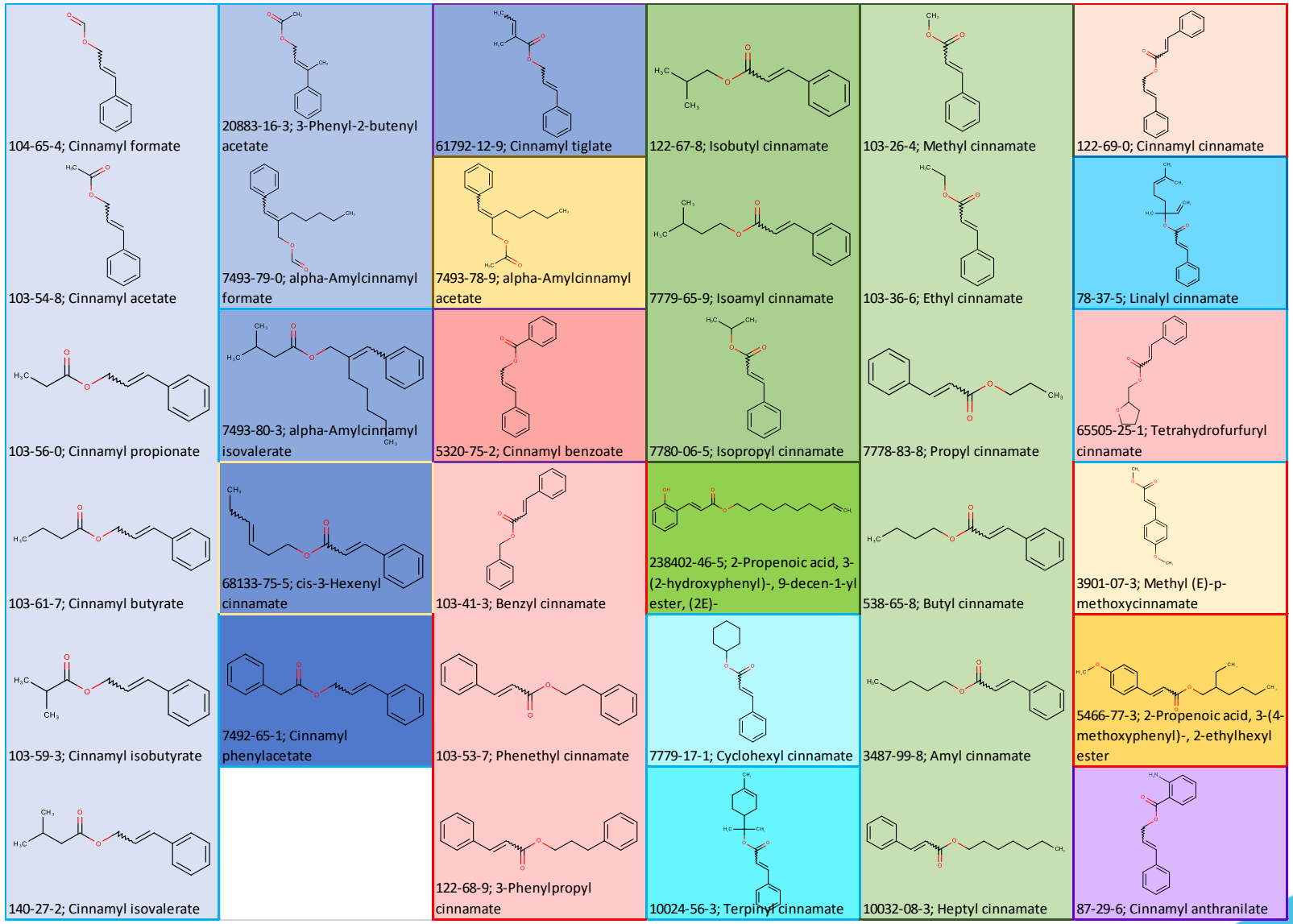
Animal Data	Human Data	In-vitro	In-silico prediction	Clears reactive DST?	details of the reactivity prediction-->	Protein binding alerts for skin sensitization by OASIS Toolbox 4.3.1	Protein binding alerts for skin sensitization by OASIS, with Autoxidation simulator Toolbox 4.3.1	Protein binding alerts for skin sensitization by OASIS, with Skin metabolism simulator Toolbox 4.3.1	Parent Predicted SkinSens (TIMES)	Metabolite Predicted SkinSens (TIMES)	Toxtree 3.1.0
No Data	Neg hMAX @5%	Neg DPRA, Neg h-CLAT	Reactive	No		SN2 >> SN2 Reaction at a sp3 carbon atom >> Activated alkyl esters and thioesters	SN2 >> SN2 Reaction at a sp3 carbon atom >> Activated alkyl esters and thioesters	SN2 >> SN2 Reaction at a sp3 carbon atom >> Activated alkyl esters and thioesters; No alert found; SN2 >> Ring opening SN2 reaction >> Epoxides, Aziridines and Sulfuranes	Weak sensitiser	Strong sensitiser	Acyl Transfer; Michael Acceptor; SN2

The next step was to evaluate all the structurally related materials

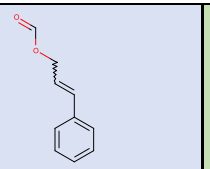
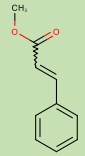
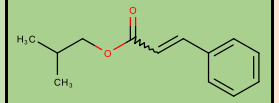
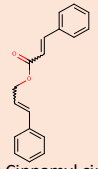
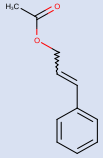
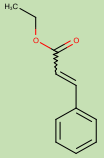
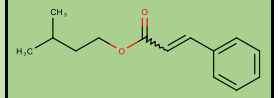
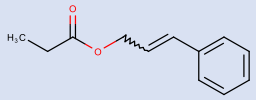
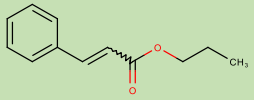
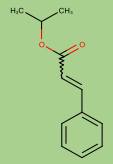
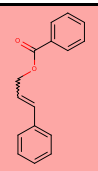
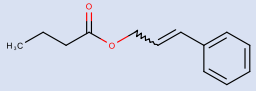
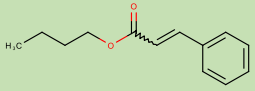
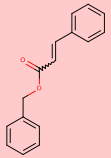
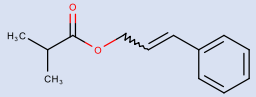
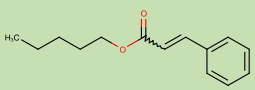
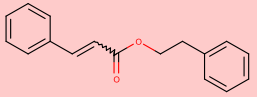
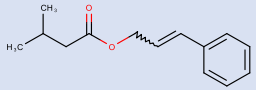
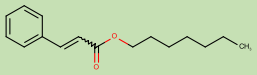
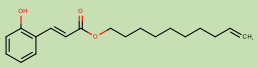
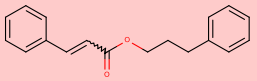


 7493-80-3; alpha-Amylcinnamyl isovalerate	 68133-75-5; cis-3-Hexenyl cinnamate	 122-67-8; Isobutyl cinnamate	 122-68-9; 3-Phenylpropyl cinnamate	 103-54-8; Cinnamyl acetate	 103-36-6; Ethyl cinnamate
 103-56-0; Cinnamyl propionate	 7778-83-8; Propyl cinnamate	 7779-65-9; Isoamyl cinnamate	 103-53-7; Phenethyl cinnamate	 10024-56-3; Terpinyl cinnamate	 3901-07-3; Methyl (E)-p-methoxycinnamate
 103-61-7; Cinnamyl butyrate	 538-65-8; Butyl cinnamate	 238402-46-5; 2-Propenoic acid, 3-(2-hydroxyphenyl)-, 9-decen-1-yl ester, (2E)-	 7779-17-1; Cyclohexyl cinnamate	 7493-78-9; alpha-Amylcinnamyl acetate	 20883-16-3; 3-Phenyl-2-butenyl acetate
 103-59-3; Cinnamyl isobutyrate	 3487-99-8; Amyl cinnamate	 5466-77-3; 2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	 7780-06-5; Isopropyl cinnamate	 5320-75-2; Cinnamyl benzoate	 87-29-6; Cinnamyl anthranilate
 140-27-2; Cinnamyl isovalerate	 10032-08-3; Heptyl cinnamate	 7492-65-1; Cinnamyl phenylacetate	 104-65-4; Cinnamyl formate	 103-26-4; Methyl cinnamate	 103-41-3; Benzyl cinnamate
 122-69-0; Cinnamyl cinnamate	 61792-12-9; Cinnamyl tiglate	 7493-79-0; alpha-Amylcinnamyl formate	 65505-25-1; Tetrahydrofurfuryl cinnamate	 78-37-5; Linalyl cinnamate	

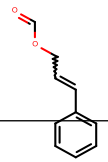
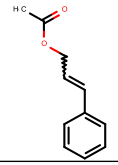
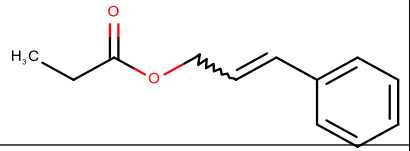
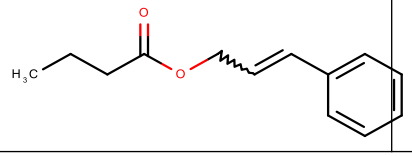
The structurally related materials needed to be refined



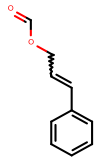
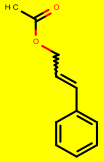
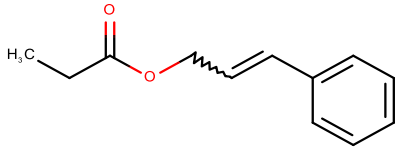
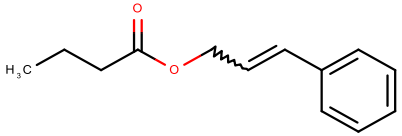
This was the final cluster of materials to be considered based on chemical predictivity and expert judgement

 104-65-4; Cinnamyl formate	 103-26-4; Methyl cinnamate	 122-67-8; Isobutyl cinnamate	 122-69-0; Cinnamyl cinnamate
 103-54-8; Cinnamyl acetate	 103-36-6; Ethyl cinnamate	 7779-65-9; Isoamyl cinnamate	
 103-56-0; Cinnamyl propionate	 7778-83-8; Propyl cinnamate	 7780-06-5; Isopropyl cinnamate	 5320-75-2; Cinnamyl benzoate
 103-61-7; Cinnamyl butyrate	 538-65-8; Butyl cinnamate		 103-41-3; Benzyl cinnamate
 103-59-3; Cinnamyl isobutyrate	 3487-99-8; Amyl cinnamate		 103-53-7; Phenethyl cinnamate
 140-27-2; Cinnamyl isovalerate	 10032-08-3; Heptyl cinnamate		 122-68-9; 3-Phenylpropyl cinnamate

The data on the structurally related materials confirmed this cluster can be used – no positive data

CAS	Name	Structure	Animal Data	Human Data	In-vitro	In-silico prediction	Clears reactive DST?
104-65-4	Cinnamyl formate			Neg hMAX @ 4%		Reactive	No
103-54-8	Cinnamyl acetate			Neg hMAX @5%	Neg DPRA, Neg h-CLAT	Reactive	No
103-56-0	Cinnamyl propionate			Neg hMAX @ 4%		Reactive	No
103-61-7	Cinnamyl butyrate			Neg hMAX @ 4%		Reactive	Yes

A confirmatory HRIPT was conducted on cinnamyl acetate because it was the most reactive in the cluster

CAS	Name	Structure	Animal Data	Human Data	In-vitro	In-silico prediction	Clears reactive DST?	Safety Assessment
104-65-4	Cinnamyl formate			Neg hMAX @ 4%		Reactive	No	Safe under the current use level
103-54-8	Cinnamyl acetate			Neg HRIPT @ 2.9% (3424); Neg hMAX @5%	Neg DPRA, Neg h-CLAT	Reactive	No	Safe under the current use level
103-56-0	Cinnamyl propionate			Neg hMAX @ 4%		Reactive	No	Safe under the current use level
103-61-7	Cinnamyl butyrate			Neg hMAX @ 4%		Reactive	Yes	Safe under the current use level