RIFM's in vitro research for dermal sensitization quantitative risk assessment
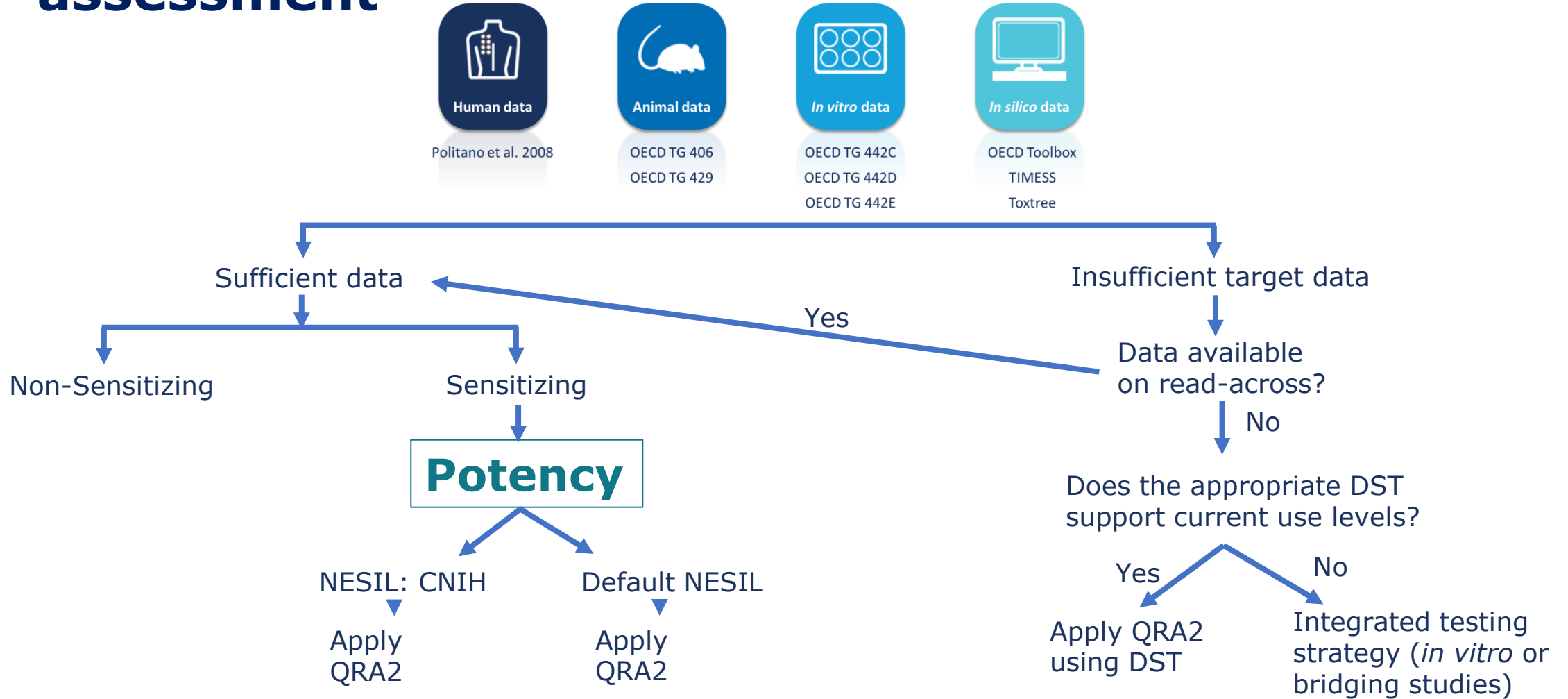
Isabelle Lee
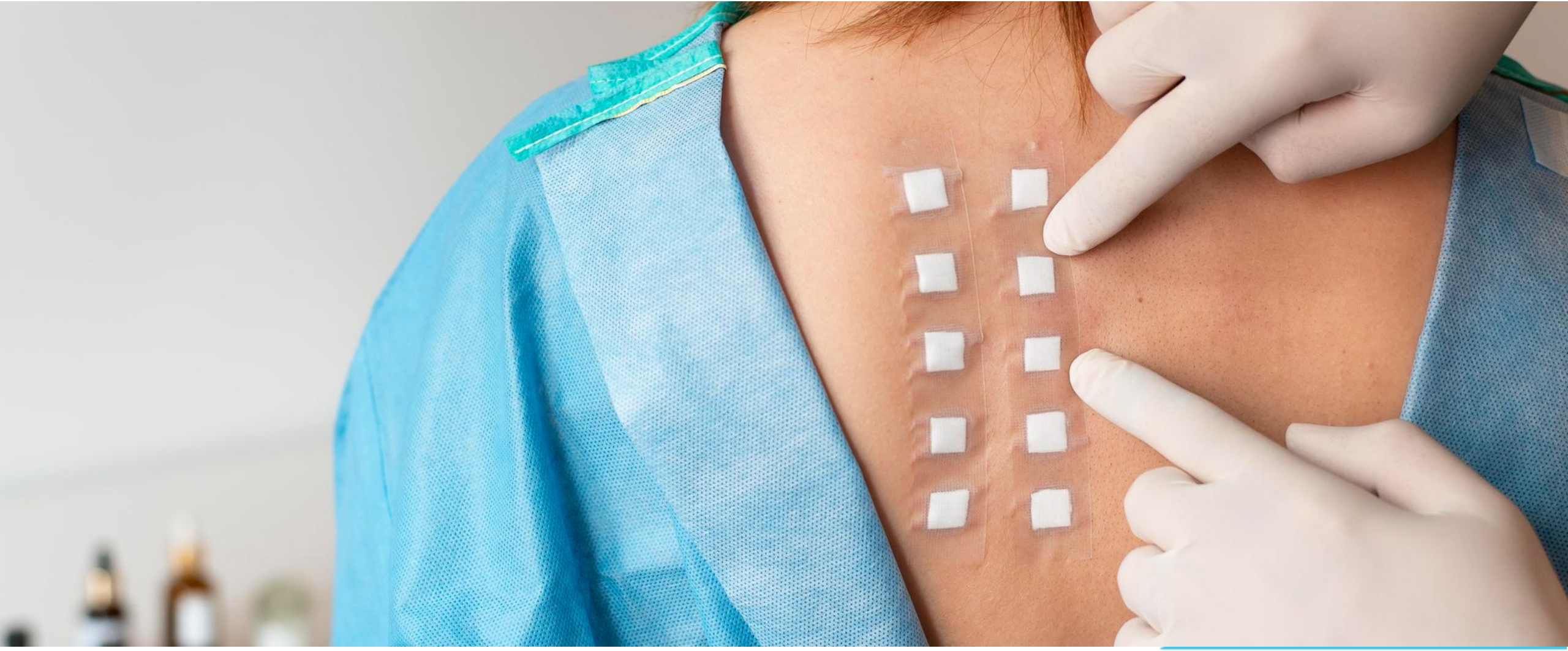
One area of RIFM's research focus is determining quantitative potency for dermal quantitative risk assessment

Human data
Politano et al. 2008

Animal data
OECD TG 406
OECD TG 429

In vitro data
OECD TG 442C
OECD TG 442D
OECD TG 442E

In silico data
OECD Toolbox
TIMESS
Toxtree

Sufficient data

Insufficient target data

Non-Sensitizing

Sensitizing

Yes

Data available on read-across?

No

Potency

NESIL: CNIH

Default NESIL

Apply QRA2

Apply QRA2

Does the appropriate DST support current use levels?

Yes

No

Apply QRA2 using DST

Integrated testing strategy (*in vitro* or bridging studies)
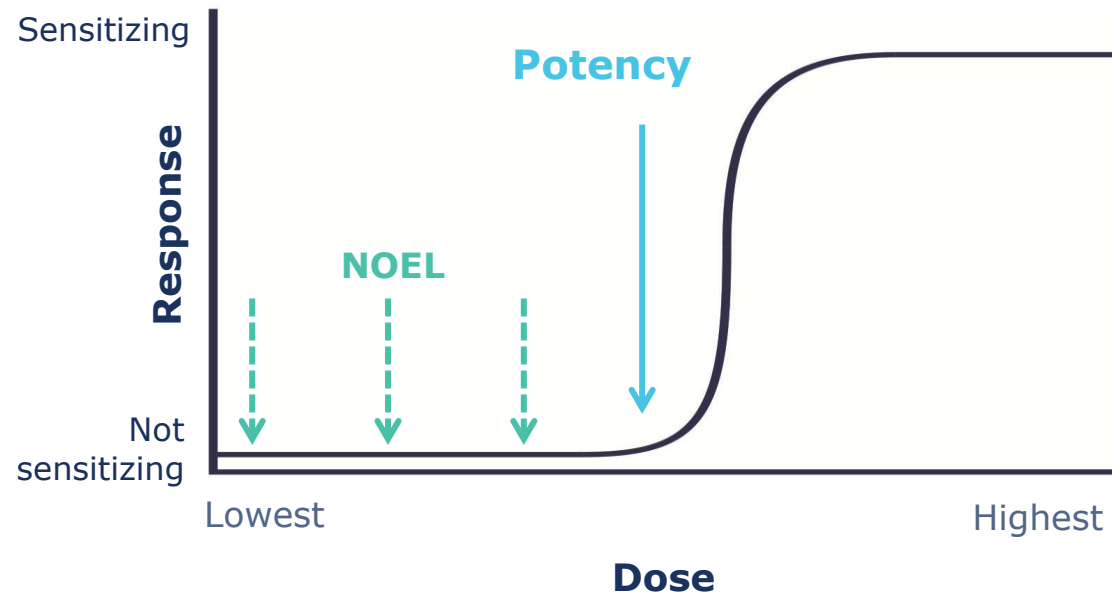
Research article by RIFM: Lee et al., 2022

The NESIL, currently confirmed through human testing, is the starting point for QRA2

Human data alone may not be sufficient to determine a material's quantitative potency

**NOEL** from human tests can be close to or far from the threshold dose that induces skin sensitization.

# RIFM has a developed reference database of fragrance ingredients for NAM testing

Category Name	Range ($\mu g/cm^2$)
Extreme	<25
Strong	>25 - 500
Moderate	>500 - 2,500
Weak	>2,500 - 10,000
Very Weak	> 10,000
Non-Sensitizer	Negative

~ 100 fragrance materials

Research article by RIFM: Na et al., 2022

These reference fragrance ingredients comprise a wide range of sensitization potency categories

Strong; 5
Non-sensitizers; 11
Moderate; 17
Very Weak; 28
Weak; 39
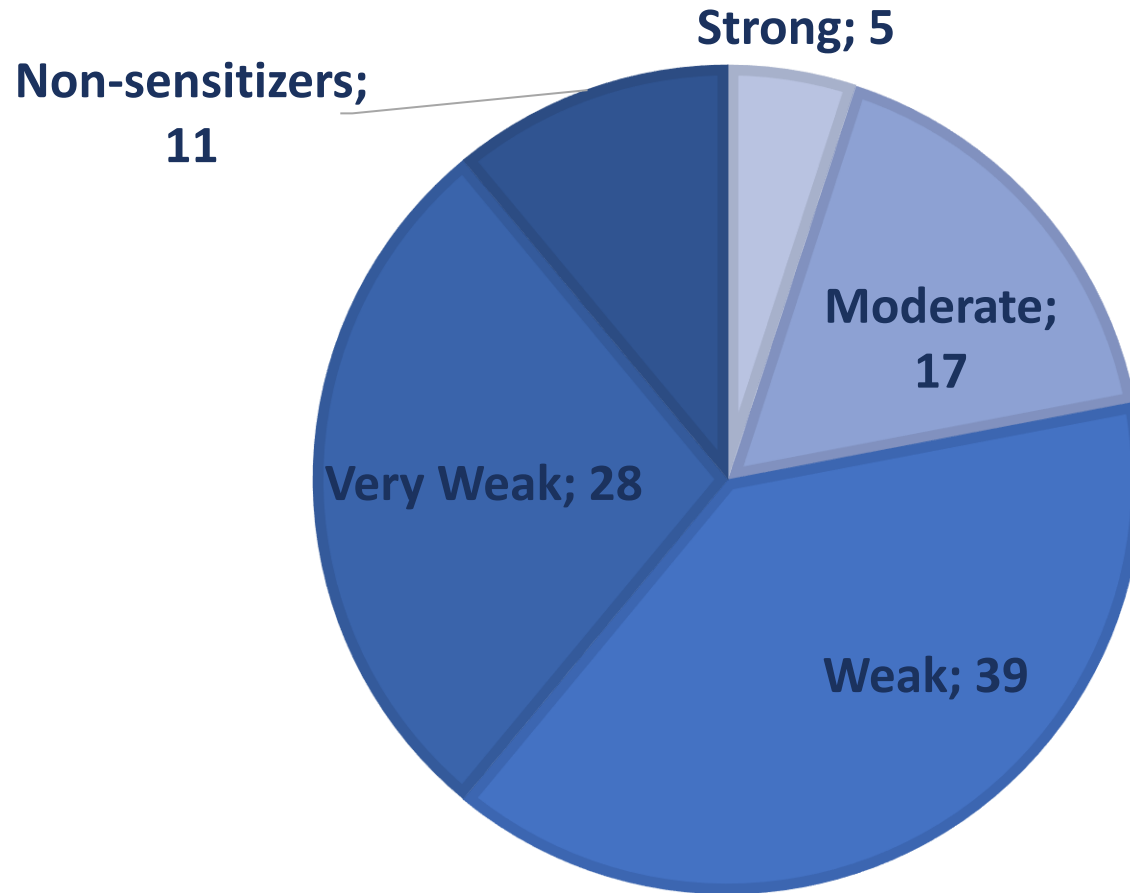
Reactivity alerts show the distribution of fragrance ingredient chemical domains represented
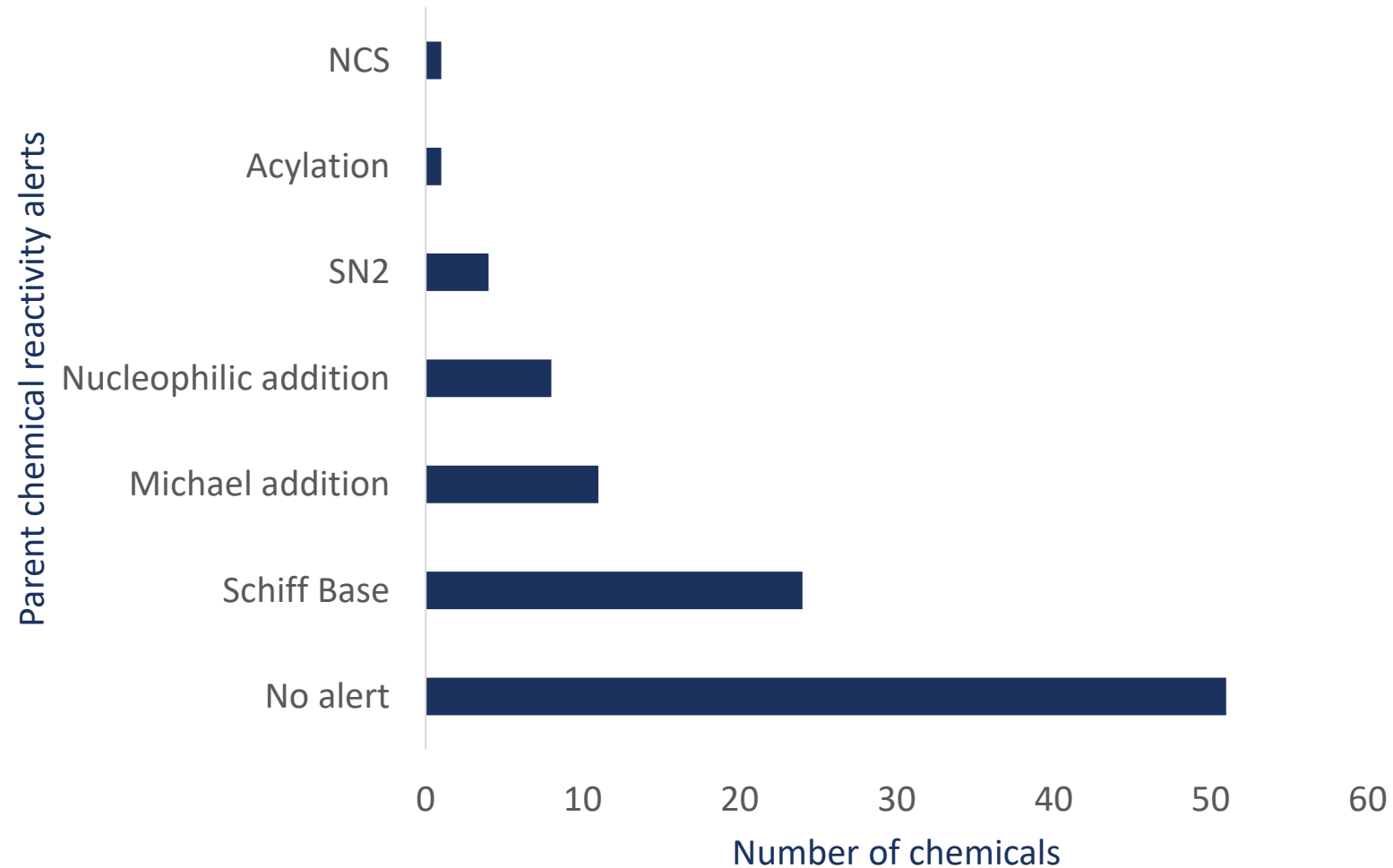
Point of departure (PoD) assessment using the integrated OECD *in vitro* models

# Regression models integrating *in vitro* methods to derive points of departure for QRA have been published

Regression Model	EC3 (LLNA)	PV (Human)
KS + kDPRA	Equation 1	Equation 1d
kDPRA + h-CLAT	Equation 4	Equation 4d
KS + kDPRA + h-CLAT	Equation 5	Equation 5d
KS (EC1.5) + h-CLAT	Equation 6	Equation 6d
KS (EC3) + h-CLAT/ KS + kDPRA + h-CLAT	Equation 7	Equation 5e

Method developed by Givaudan: Natsch et al., 2022 & 2023

Human potency values (PVs) and LLNA EC3 have been predicted for ~ 60 fragrance ingredients
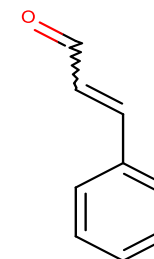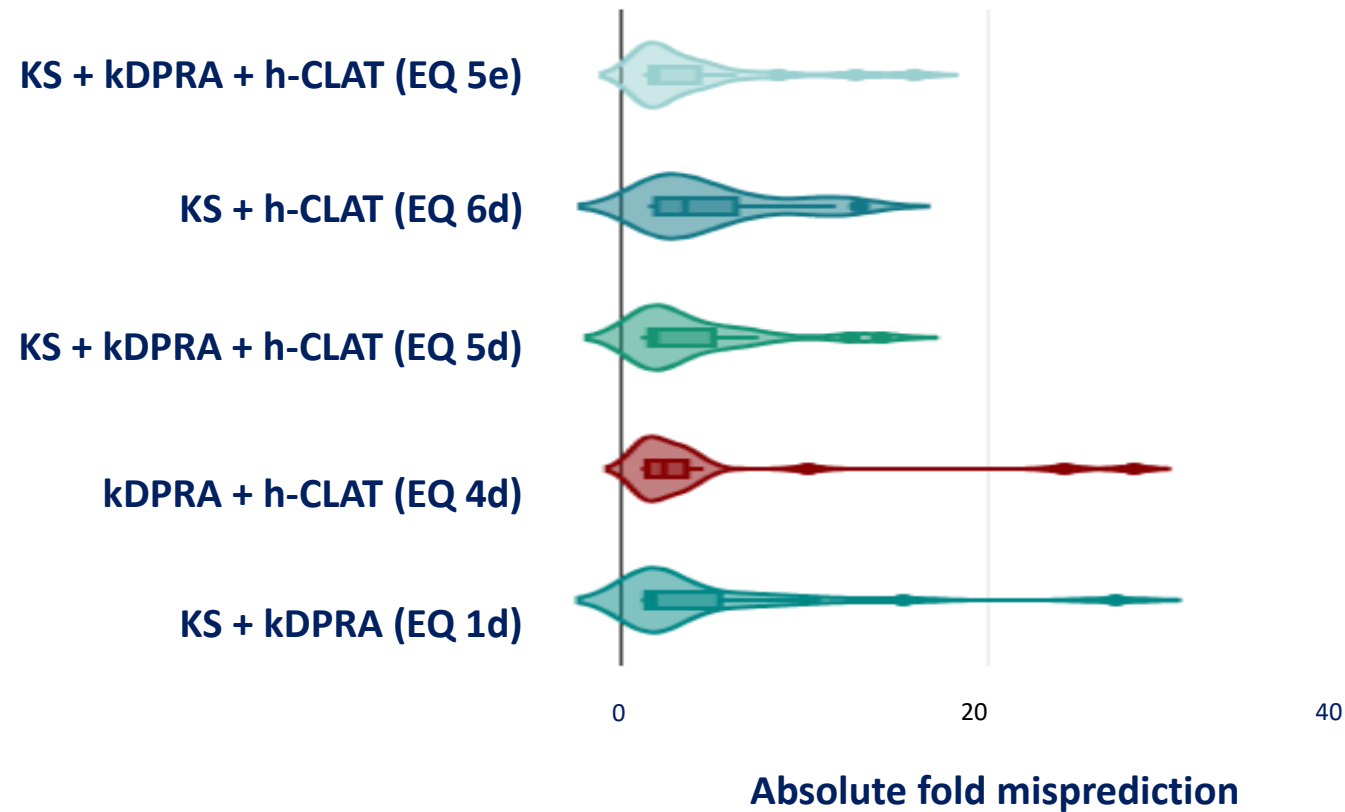
E.g.:
CAS # 104-55-2
Cinnamaldehyde

~60 materials

kDPRA
KeratinoSens
h-CLAT
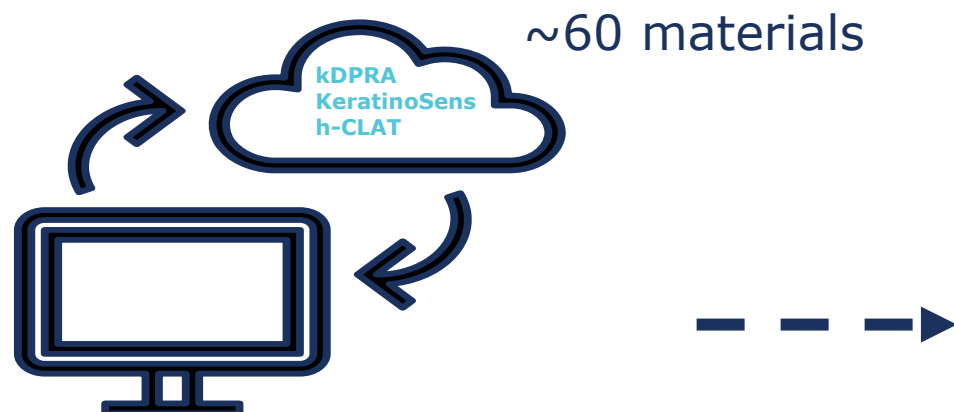
Regression Model	EC3	PV
KS + kDPRA	1.02	0.78
kDPRA + h-CLAT	0.81	0.67
KS + kDPRA + h-CLAT	0.80	0.56
KS (EC1.5) + h-CLAT	1.11	0.78
KS (EC3) + h-CLAT/ KS + kDPRA + h-CLAT	0.92	0.64

Research article by RIFM: Lee et al., 2024

Predicted PV match WoE categories with high accuracy: Equation 5d (KS + kDPRA + h-CLAT)

WoE↓/Equation 5d→	Extreme	Strong	Moderate	Weak	Very Weak	NS
Extreme	0	0	0	0	0	0
Strong	0	5	0	1	0	0
Moderate	0	2	3	7	1	0
Weak	0	0	9	19	2	0
Very Weak	0	0	4	10	7	0
NS	0	0	0	1	3	0

		Equation 5d prediction more conservative	Equation 5d prediction less conservative
Equation 5d prediction matched the WoE Potency Category	45.95%		
Equation 5d prediction was 1 category off from the WoE category.	44.59%	72.73%	27.27%
Equation 5d prediction was 2 or more categories off from WoE Category.	9.46%	71.43%	28.57%

The median fold misprediction for predicted PV vs human NOEL is between 1.7 and 3.4

KS + kDPRA + h-CLAT (EQ 5e)

KS + h-CLAT (EQ 6d)

KS + kDPRA + h-CLAT (EQ 5d)

kDPRA + h-CLAT (EQ 4d)

KS + kDPRA (EQ 1d)

0   20   40

Absolute fold misprediction

Conclusions

1. Distribution of predicted potency categories is similar to WoE categories; no extreme sensitizer category predicted

2. Both EC3 and PV equations provide good approximations of WoE potency categories

3. Equation 1 and 1d provide the highest prediction accuracy for WoE categories

4. Median fold misprediction for human potency values is between 1.7 and 3.4 for the 5 equations

5. Median fold misprediction for EC3 is between 1.8 and 2.5 for the 5 equations

Research article by RIFM: Lee et al., 2024

Point of departure assessment using the GARDSKin Dose response (DR) assay

Overview of the GARDskin DR assay

Step 1

GARDskin DV ≥ 0
= Skin sensitizer
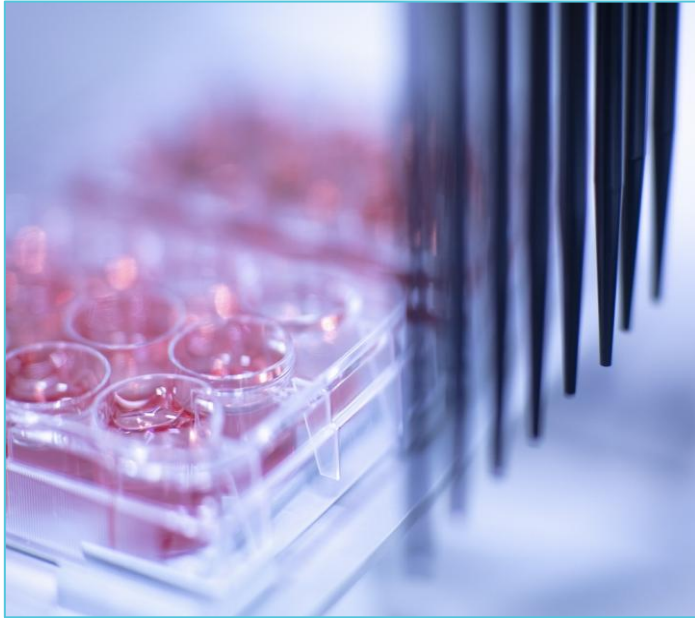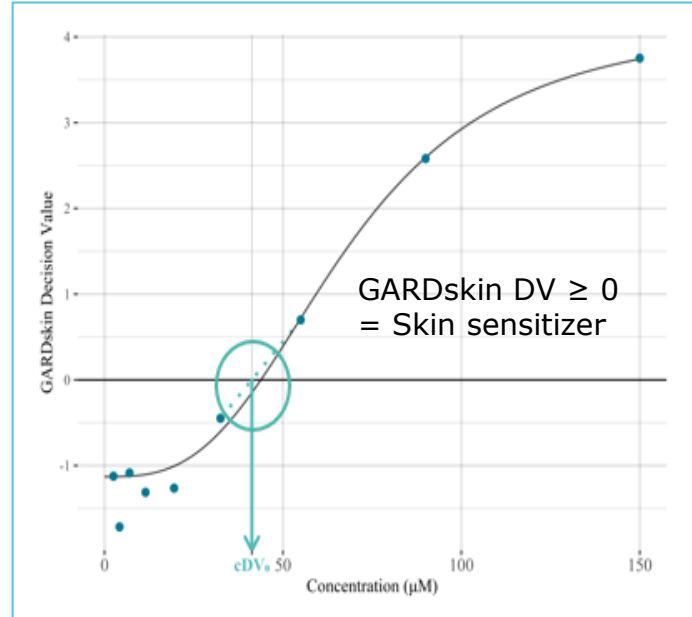
Step 2
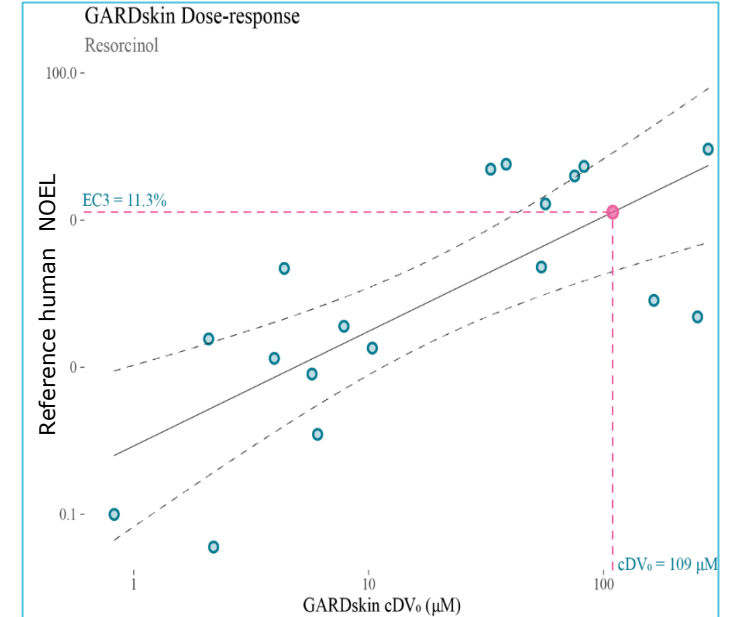
Step 3

GARDskin DR predicts hazard for 100 fragrance ingredients with an accuracy of 81%

		GARDskin DR Prediction	
		Negative	**Positive**
Reference Data	**Negative**	6	5
	Positive	14	75

Statistics	
Accuracy	**0.81**
Sensitivity	**0.84**
Specificity	**0.55**
Balanced accuracy	**0.69**
Positive Predictive Value (PPV)	**0.94**
Negative Predictive Values (NPV	**0.30**

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GARDskin DR predicts NESILs with an approximate accuracy of 81% for the reference potency categories
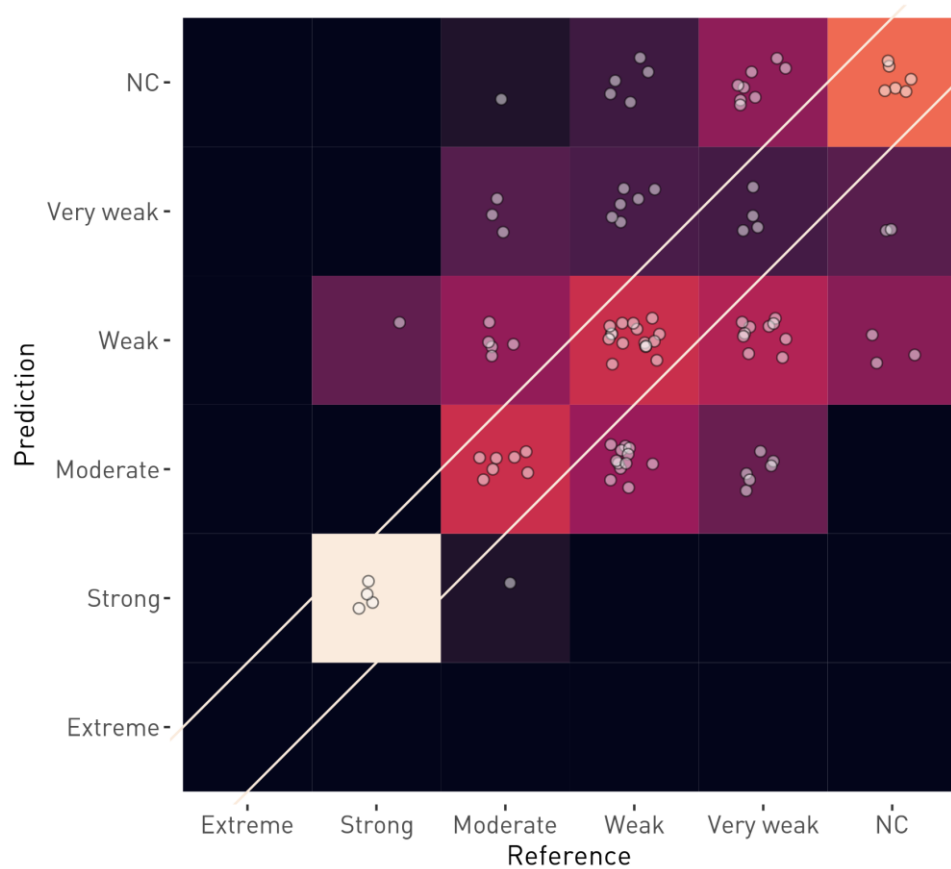
Total accuracy (%)	37.0	37/100
Approximate accuracy (%)	81.0	81/100
Fraction overclassified (%)	34.0	34/100
Fraction underclassified (%)	29.0	29/100
Accuracy [Extreme] (%)	NA	0/0
Approximate accuracy [Extreme] (%)	NA	0/0
Accuracy [Strong] (%)	80.0	4/5
Approximate accuracy [Strong] (%)	80.0	4/5
Accuracy [Moderate] (%)	41.2	7/17
Approximate accuracy [Moderate] (%)	76.5	13/17
Accuracy [Weak] (%)	41.0	16/39
Approximate accuracy [Weak] (%)	87.2	34/39
Accuracy [Very weak] (%)	14.3	4/28
Approximate accuracy [Very weak] (%)	78.6	22/28
Accuracy [NC] (%)	54.5	6/11
Approximate accuracy [NC] (%)	72.7	8/11

Total accuracy and approximate accuracy increase when weak and very weak categories are combined
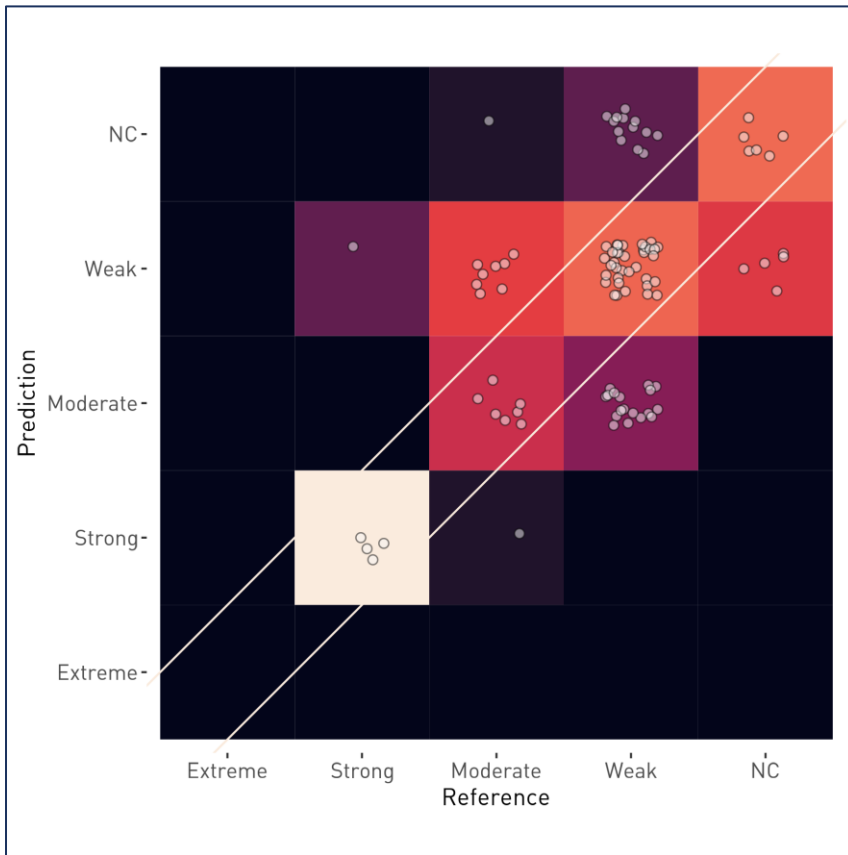
Total accuracy (%)	53.0	53/100
Approximate accuracy (%)	98.0	98/100
Fraction overclassified (%)	24.0	24/100
Fraction underclassified (%)	23.0	23/100
Accuracy [Extreme] (%)	NA	0/0
Approximate accuracy [Extreme] (%)	NA	0/0
Accuracy [Strong] (%)	80.0	4/5
Approximate accuracy [Strong] (%)	80.0	4/5
Accuracy [Moderate] (%)	41.2	7/17
Approximate accuracy [Moderate] (%)	94.1	16/17
Accuracy [Weak] (%)	53.7	36/67
Approximate accuracy [Weak] (%)	100	67/67
Accuracy [NC] (%)	54.5	6/11
Approximate accuracy [NC] (%)	100	11/11

The median fold misprediction for GARDskin DR predicted NESIL was 2.5 for reference materials with defined LOEL and NOEL
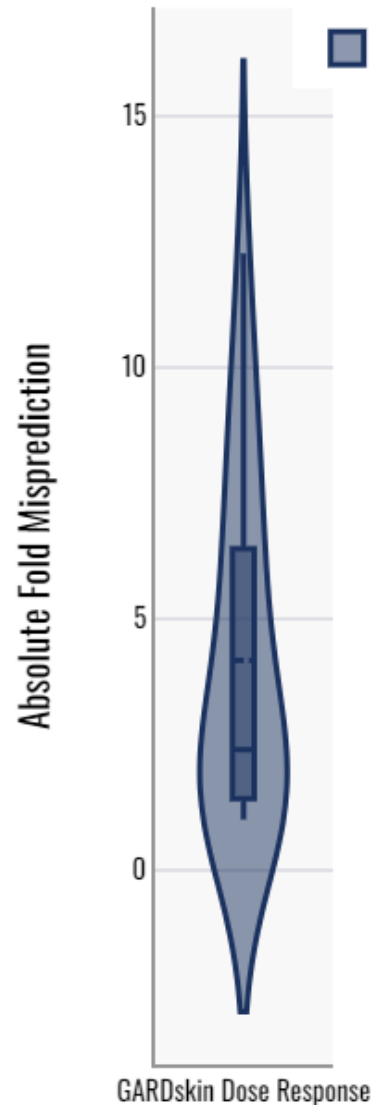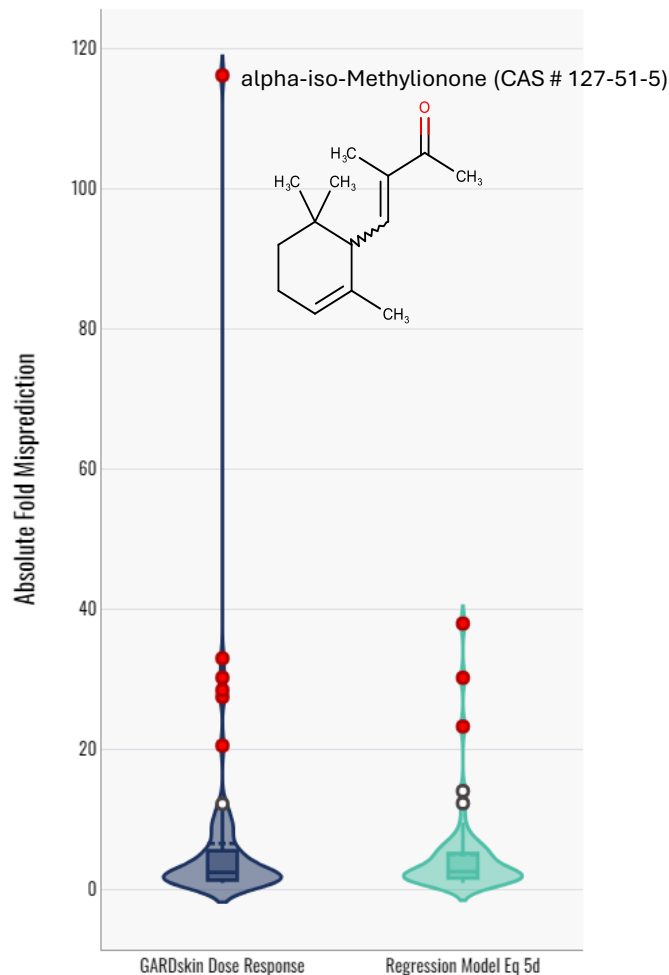

| Groups:	GARDskin Dose Response
Sample size (n):	23
Minimum:	1.0087
Lower boundary:	-6.0371
Q1:	1.422
Median:	2.3941
Q3:	6.3947
Upper boundary:	13.8539
Maximum:	12.2751
IQR	4.9728
Mean (x̄):	4.1739

Conclusions

1. GARDskin DR predicted human NOELs correlated well with reference NOELs, with a Pearson correlation of 0.73.

2. Predicted NOEL and LLNA EC3 values from replicate measurements were highly reproducible

3. The composite potency score was designed to account for main shared variance between predicted NOEL and EC3

4. An approximate accuracy of 81% was observed and mispredictions were mostly overclassified.

5. The approximate accuracy increased to 98% when the weak and very weak categories were combined

Research article by RIFM: Under peer review

Absolute fold mispredictions between predicted quantitative potencies are generally similar between the 2 models

alpha-iso-Methylionone (CAS # 127-51-5)

| Groups:	GARDskin Dose Response	Regression Model Eq 5d
Sample size (n):	78	57
Minimum:	1.0087	1.0044
Lower boundary:	-4.8163	-3.6209
Q1:	1.4266	1.6965
Median:	2.5391	2.6314
Q3:	5.5885	5.2414
Upper boundary:	11.8314	10.5588
Maximum:	116.1639	37.997
IQR	4.1619	3.5449
Mean ($\bar{x}$):	6.6344	5.0614

QRA2 remains critical as we incorporate NAMs in next generation risk assessment (NGRA) of fragrance ingredients
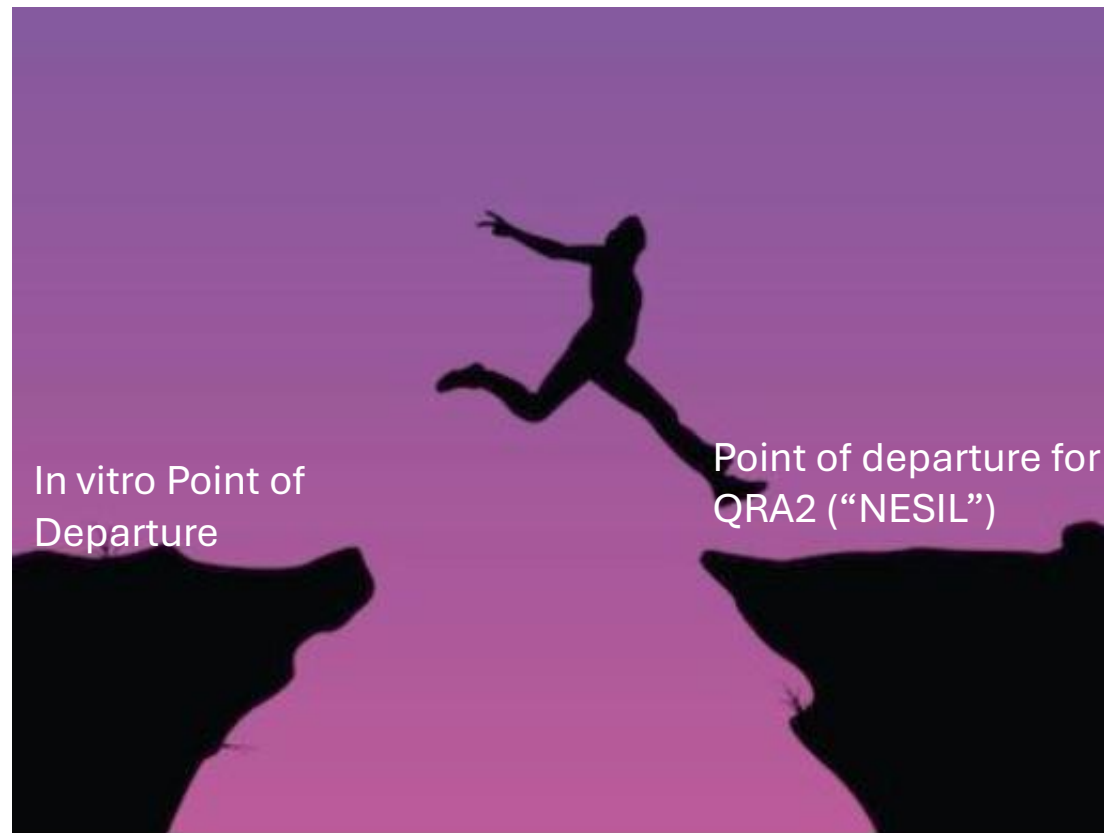
Defining uncertainty

Current framework for NGRA of fragrance sensitizers with incorporation of QRA2

Derive quantitative potency (PoD) → Apply uncertainty factor → Determine a NESIL → Conduct QRA2
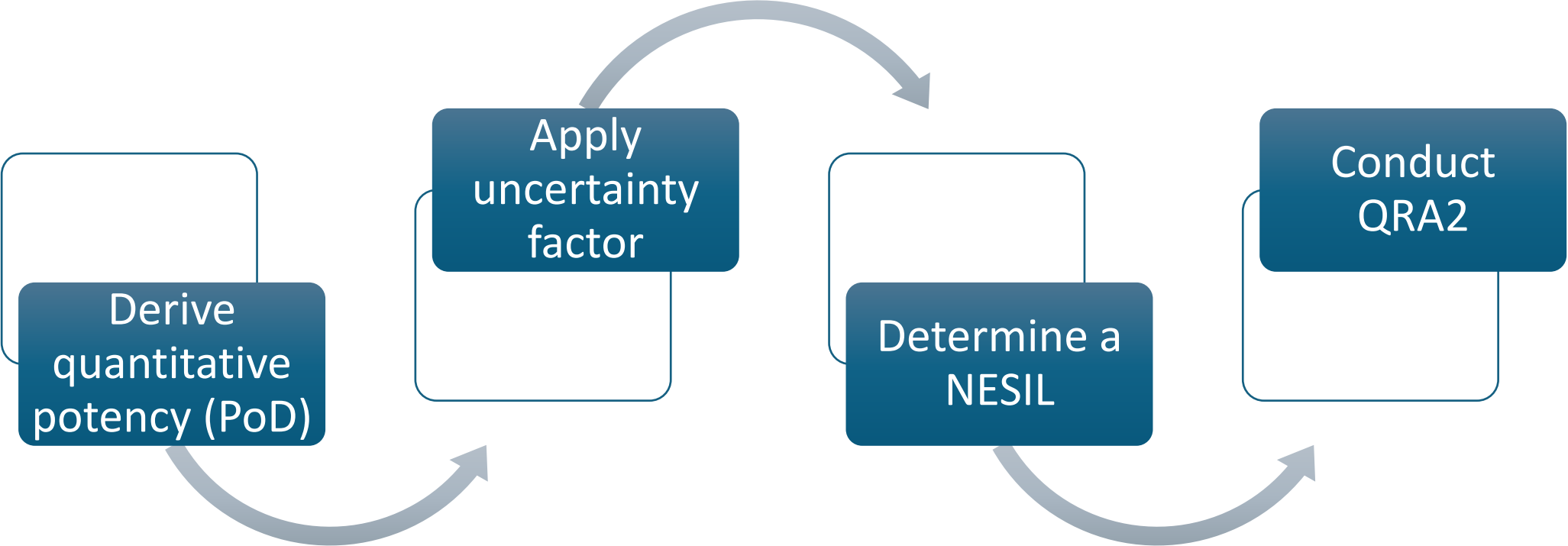
RIFM continues assess and integrate NAMs into our safety assessments through global collaboration

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Thank you!

Contact our team for more information, comments, and questions.