

IMMUNO RESEARCH



The SENS-IS ASSAY

The ideal replacement test

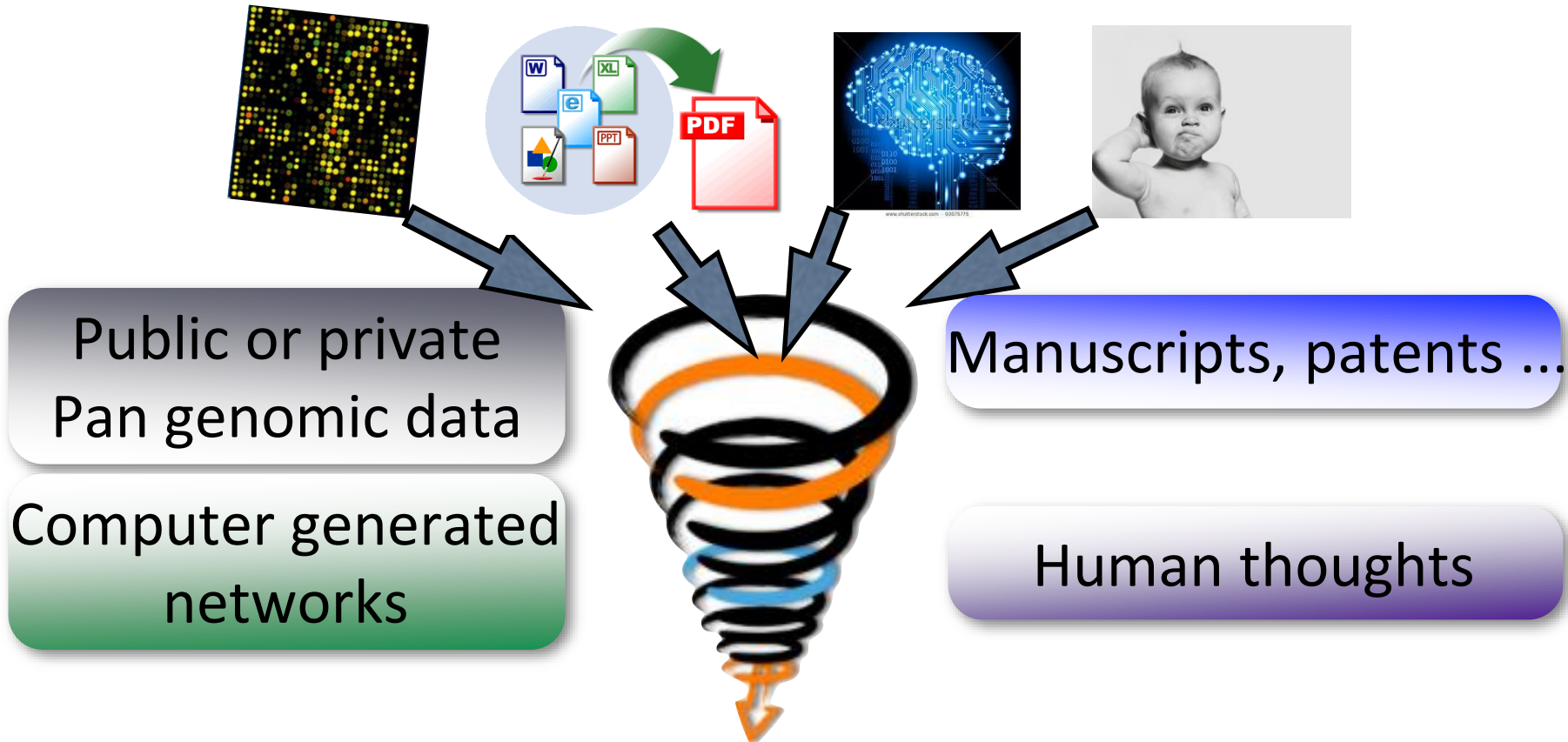


- **Discriminates irritant from sensitizers**
- **Takes into account chemical biodisponibility**
- **Can test any chemical, complex natural product and even mixtures applied onto skin**
- **Uses skin as the test system**
- **Defining a «genomic signature» of sensitization vs irritation**

DEVELOPMENT OF SENS-IS



STEP 1 : DATA MINING USING PROPRIETARY TOOLS



Selection of 900 target genes

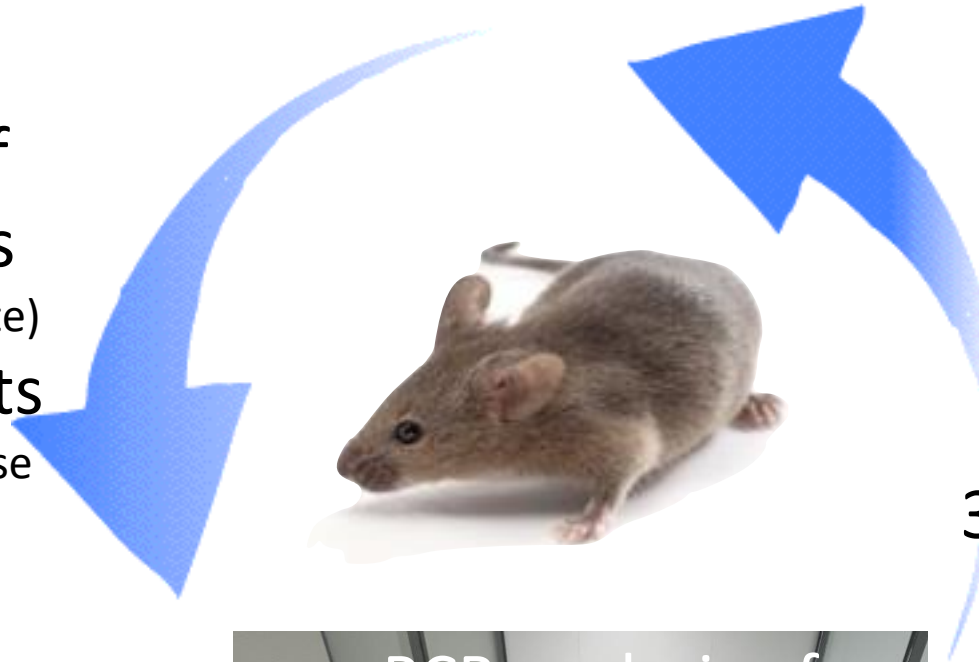


DEVELOPMENT OF SENS-IS

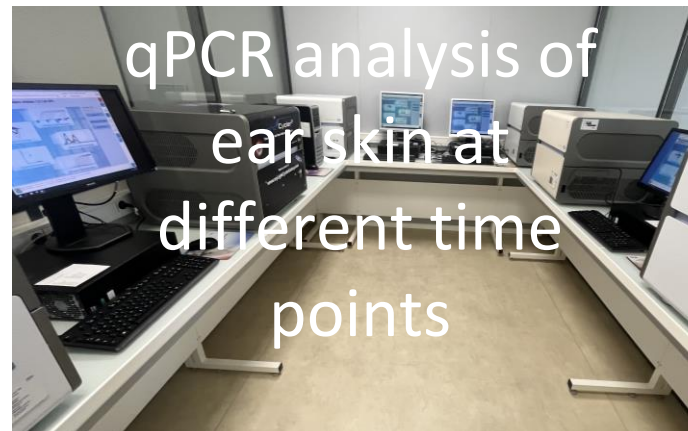


STEP 2 : ANALYSIS/REFINEMENT USING THE LLNA

Selection of
8 sensitizers
(weak and moderate)
and 4 irritants
(including LLNA false
positive)



Selection of
300 target genes



DEVELOPMENT OF SENS-IS

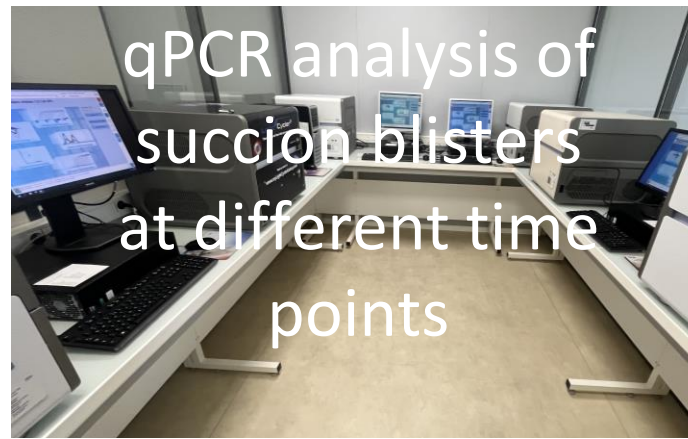


STEP 3 : REFINEMENT USING HUMAN BIOPSIS

Selection of
20 patients
sensitized to
Nickel or
fragrance mix



Selection of
200 target genes



qPCR analysis of
suction blisters
at different time
points

DEVELOPMENT OF SENS-IS



STEP 4 : USING EPISKIN AS TEST SYSTEM

Selection of
40 chemicals

(10 cat 1A, 10 cat 1B, 10
irritants and 10 NS/NI)



- Refinement of the protocole
- Développement of the prediction model

qPCR analysis of
200 targets genes



SENS-IS ASSAY METHOD

1-chemical application on Episkin



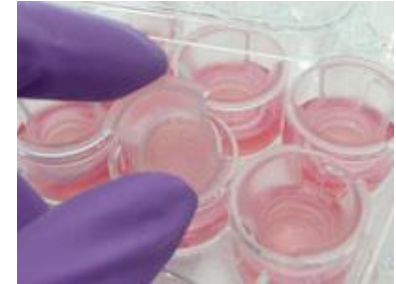
Human 3D reconstructed epidermis (Episkin®) are exposed for 15 min to 30µl of 100, 50, 10, 1, 0,1 % test chemicals in PBS, olive oil or DMSO.

2- Washing



After 15min exposure, the Episkin® are rinsed.

3- Post-incubation and sampling



After 6 hours of post-incubation, the samples are harvested and frozen in liquid nitrogen before tissue lysing and RNA extraction.

4-Tissue lysing and cDNA preparation



The tissues are mechanically disrupted using a tissue lyser (Qiagen). RNA extraction and cDNA preparation is done with classical methods.

5-RT-PCR quantification



Quantification by RT-PCR of 61 biomarkers classified into 3 groups : irritation, ARE and SENS-IS genes

6-Results analysis

Assay validation after analysis of :
negative controls (Olive oil, PBS and DMSO)
irritant control (5% SLS)
two sensitizer controls (50% HCA, 1% TNBS)

Irritation : positive response if at least 7/23 genes are over overexpressed

Sensitization : a molecule is classified as positive if at least:
- 7/17 genes in ARE genes group and/or
- 7/21 genes in SENS-IS genes group are significantly induced

Potency assessment :
-positive at 0,1% : extreme
-positive at 1% : strong
-positive at 10% : moderate
-positive at 50% : weak

Results analysis



- **Assay validation after analysis of :**
 - negative control (DMSO, PBS, Olive Oil)
 - irritant control (5% SLS)
 - two sensitizer controls (50% HCA, 1% TNBS)
- **Irritation :**
 - positive response if at least 15/23 genes are significantly induced
- **Sensitization : a molecule is classified as positive if at least:**
 - 7/17 genes in ARE genes group and/or
 - 7/21 genes in SENS-IS genes group are significantly induced
- **Potency assessment :**
 - positive at 0,1% : extreme
 - positive at 1% : strong
 - positive at 10% : moderate
 - positive at 50% : weak
 - positive at 100% : very weak

SENS-IS on RCPL chemicals



Name	CAS No	Potency Value (ug/cm2)	Predicted Potency Value (ug/cm2)	Category
5-chloro-2-methyl-4-isothiazolin-one (CMIT)	26172-55-4	2,3	<25	Extreme
2,4-dinitrochlorobenzene (DCNB)	97-00-7	3,4	<25	Extreme
1,4-Phenylenediamine (PPD)	106-50-3	3,9	25-250	Strong
Glutaraldehyde	111-30-8	20,0	<25	Extreme
trans-2-Hexenal	6728-26-3	39,3	25-250	Strong
1,4-Dihydroquinone	123-31-9	47,5	25-250	Strong
Benzyl bromide	100-39-0	50,0	25-250	Strong
1,1,3-Trimethyl-2-formylcyclohexa-2,4-diene (Safranal)	116-26-7	106	25-250	Strong
Methyl 2-nonynoate (Methyl octine carbonate)	111-80-8	109	25-250	Strong
Methyl 2-octynoate (Methyl heptine carbonate)	111-12-6	125	25-250	Strong
Isoeugenol	97-54-1	325	250-2500	Moderate
Phenylacetaldehyde	122-78-1	750	250-2500	Moderate
Allyl phenoxyacetate	7493-74-5	775	250-2500	Moderate
Cinnamic aldehyde	104-55-2	885	25-250	Strong
3-Propylideneephthalide	17369-59-4	925	2500-12500	Weak
4-Hydroxy-2,5-dimethyl-3(2H)-furanone (Furaneol)	3658-77-3	1181	12500-25000	very weak
Citral	5392-40-5	1450	250-2500	Moderate
p-Mentha-1,8-dien-7-al (Perillaldehyde)	2111-75-3	2175	250-2500	Moderate
Benzaldehyde	100-52-7	4094	2500-12500	Weak
Lylal (HICC)	31906-04-4	4275	2500-12500	Weak
Hydroxycitronellal	107-75-5	5275	2500-12500	Weak
Cinnamic alcohol	104-54-1	5775	2500-12500	Weak
Eugenol	97-53-0	7357	250-2500	Moderate
Geraniol	106-24-1	9197	250-2500	Moderate
Coumarin	91-64-5	11792	2500-12500	Weak
Carvone	6485-40-1	17573	250-2500	Moderate
Benzyl salicylate	118-58-1	17715	2500-12500	Weak
Hexyl cinnamic aldehyde	101-86-0	23620	2500-12500	Weak
Benzyl Alcohol	100-51-6	>25000	2500-12500	Weak
Benzyl benzoate	120-51-4	>25000	2500-12500	Weak
Isomethylionone (α -)	127-51-5	>25000	2500-12500	Weak
Methyl salicylate	119-36-8	No PV derived- very weak/nonsensitiser	2500-12500	Weak
Vanillin	121-33-5	No PV derived - very weak/nonsensitiser	NS	NS

RIFM data

Cosmetics Europe data

Outliers on RCPL chemicals



Name	CAS No	Potency Value (ug/cm2)	Predicted Potency Value (ug/cm2)	Category
1,4-Phenylenediamine (PPD)	106-50-3	3,9	25-250	Strong
3-Propylidenecephthalide	17369-59-4	925	2500-12500	Weak
4-Hydroxy-2,5-dimethyl-3(2H)-furanone (Furaneol)	3658-77-3	1181	12500-25000	very weak
Cinnamic aldehyde	104-55-2	885	25-250	Strong
Eugenol	97-53-0	7357	250-2500	Moderate
Geraniol	106-24-1	9197	250-2500	Moderate
Carvone	6485-40-1	17573	250-2500	Moderate

● UNDER-PREDICTION

- PPD from extreme to strong - old study should be retested to confirm
- 3-Propylidenecephthalide from moderate to weak - NS in keratinosens
- Furaneol from moderate to very weak - NS in keratinosens

● OVER-PREDICTION

- Cinnamal from moderate to strong - EC3 below 2 and human Cat 2
- Eugenol from weak to moderate - EC3 close to 10 and product positive at 10% but negative at 7,5%
- Geraniol from weak to moderate - product positive at 10% but negative at 7,5%
- Carvone from weak to moderate - EC3:3200, NOEL (RIFM) 2600

How to obtain a PoD with the SENS-IS assay ?



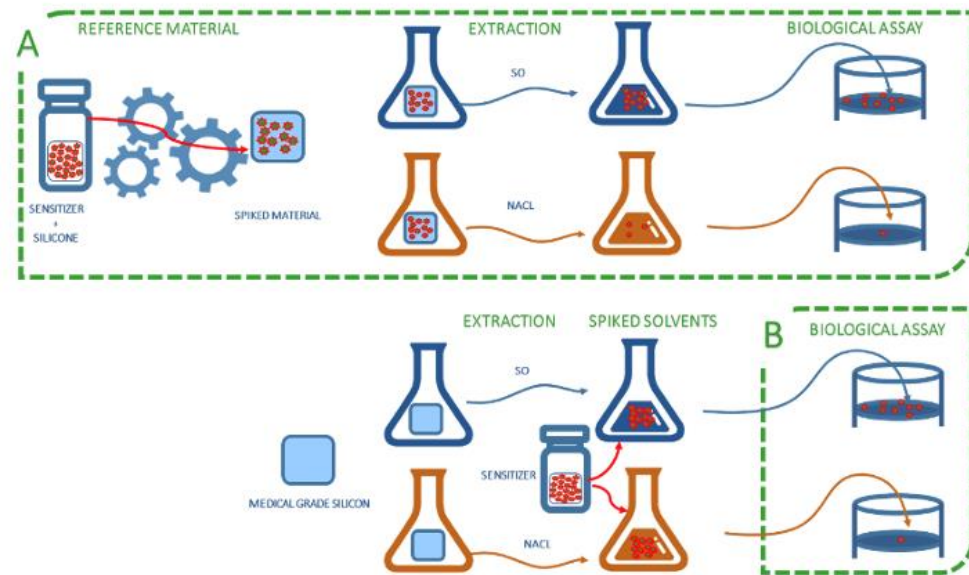
- Does the SENS-IS assay correlate with the EC3 value ? The MD study
- Use an « in between » concentration
- Do read across using SENS-IS data

Correlation between SENS-IS dose and EC3



- **Study for Medical Devices**
- **Technical specification : ISO/TS 11796:2023**
- **Biological evaluation of medical devices — Requirements for interlaboratory studies to demonstrate the applicability of validated in vitro methods to assess the skin sensitization of medical devices**

Biocompatibility testing of medical devices



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Is the SENS-IS assay sensitive enough to detect the sensitizer tested at the EC3 value ?

Correlation between SENS-IS dose and EC3



name SET 1	cas	Spiking concentration (EC3)	SENS-IS result
Glutaraldehyde	111-30-8	0,08	POSITIVE
1,4-Phenylenediamine	106-50-3	0,11	POSITIVE
Phthalic anhydride	85-44-9	0,16	POSITIVE
Cobalt chloride	7646-79-9	0,4	POSITIVE
Phenylacetaldehyde	122-78-1	3	POSITIVE
Hydratropic aldehyde	93-53-8	6,3	POSITIVE
Alpha-hexylcinnamaldehyde	101-86-0	10,8	POSITIVE
Linolenic acid	463-40-1	9,9	POSITIVE
Ethyl acrylate	140-88-5	10	POSITIVE
TPO (Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide)	75980-60-8	27	POSITIVE @50%
2,4,7,9-Tetramethyl-5-decyn-4,7-diol	126-86-3	34,3	POSITIVE
Isopropyl myristate	110-27-0	44	POSITIVE @100%
Tridecane	629-50-5	48,4	POSITIVE
Methyl methacrylate	80-62-6	75	POSITIVE
Diethyl phtalate	84-66-2	100	NEGATIVE
1,3-diphenylguanidine	102-06-7	100	NEGATIVE
Zinc oxide	1314-13-2	100	NEGATIVE

List of chemicals and doses



name SET 2	cas	Spiking concentration (EC3)	SENS-IS result
2,4-Dinitrochlorobenzen DNCB	97-00-7	0,06	POSITIVE
Formaldehyde (act. 37 %)	50-00-0	0,3	POSITIVE
Isobornyl acrylate (IBOA)	5888-33-5	1	POSITIVE
2-Mercaptobenzothiazole (MBT)	149-30-4	1,35	POSITIVE
2-hydroxyethyl acrylate	818-61-1	1,4	POSITIVE
Nickel(II) sulfate hexahydrate (NiSO4)	10101-97-0	4,8	POSITIVE
Abietic acid	514-10-3	15	POSITIVE
α -Methylstyrene	98-83-9	46	POSITIVE
Chlorobenzene	108-90-7	100	NEGATIVE
Octanoic acid	124-07-2	100	NEGATIVE
Glycerol	56-81-5	100	NEGATIVE
Lactic Acid	50-21-5	100	NEGATIVE

How to obtain a PoD with the SENS-IS assay ?



- Does the SENS-IS assay correlate with the EC3 value ? The MD study
- Use an « in between » concentration
- Do read across using SENS-IS data

In between concentrations analysis



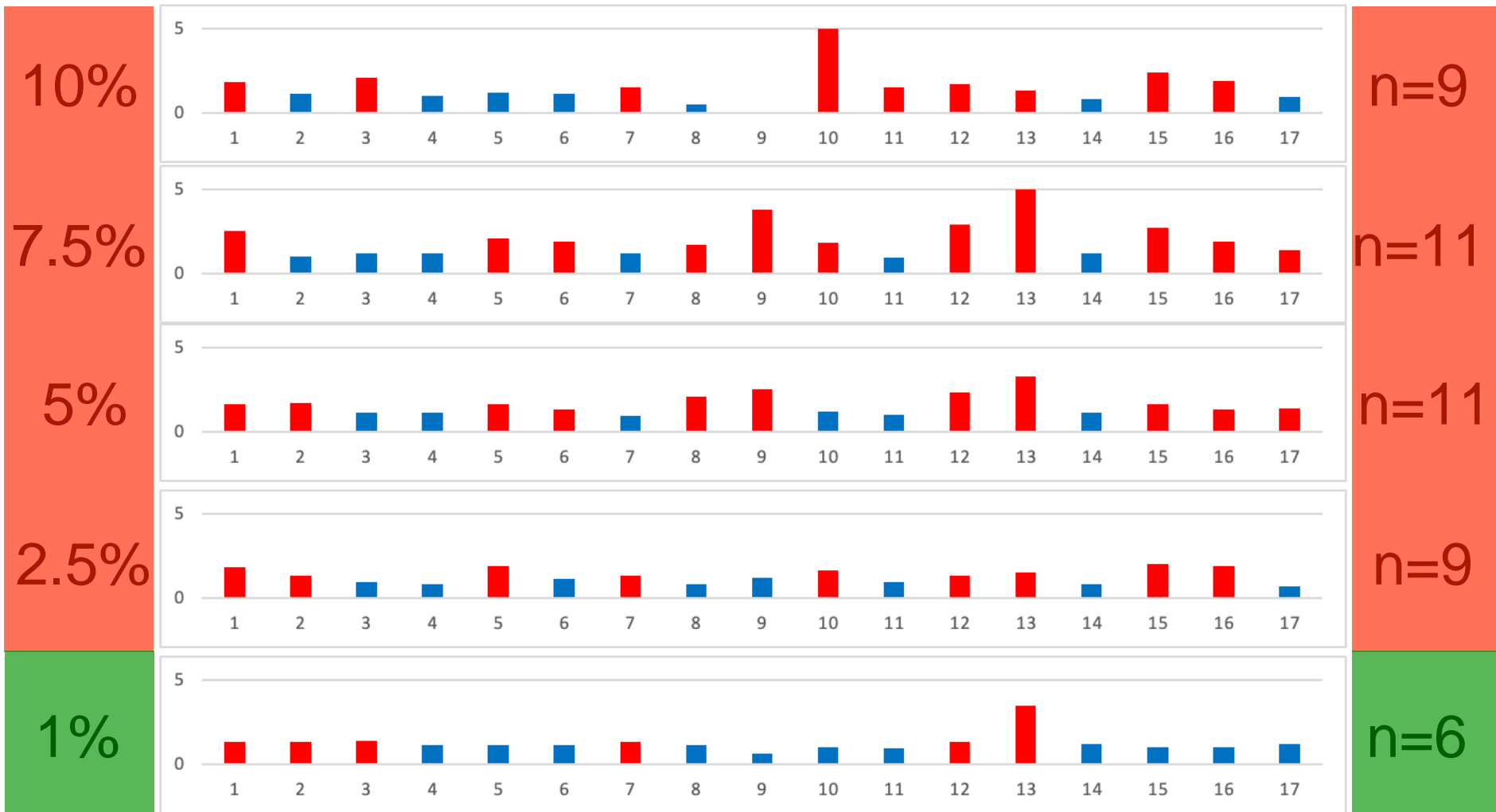
From API et al. 2014

name	cas	LLNA weighted mean EC3 (µg/cm ²)	NOEL HRIPT (Induction) (µg/cm ²)	NOEL HMT (induction) (µg/cm ²)	LOEL (induction) (µg/cm ²)	WoE NESIL (µg/cm ²)	RCPL WoE	SENS-IS (µg/cm ²)	SENS-IS category
Isoeugenol	97-54-1	498[18]	250	NA	775	250	NA	250-2500	moderate
Eugenol	97-53-0	2703[6]	5906e	5517e	NA	5900	7357	250-2500	moderate
Geraniol	106-24-1	4080[6]	11811e	4138e	NA	11800	9197	250-2500	moderate

In between concentration analysis



ISOEUGENOL - between 2,5% and 1%



In between concentration analysis



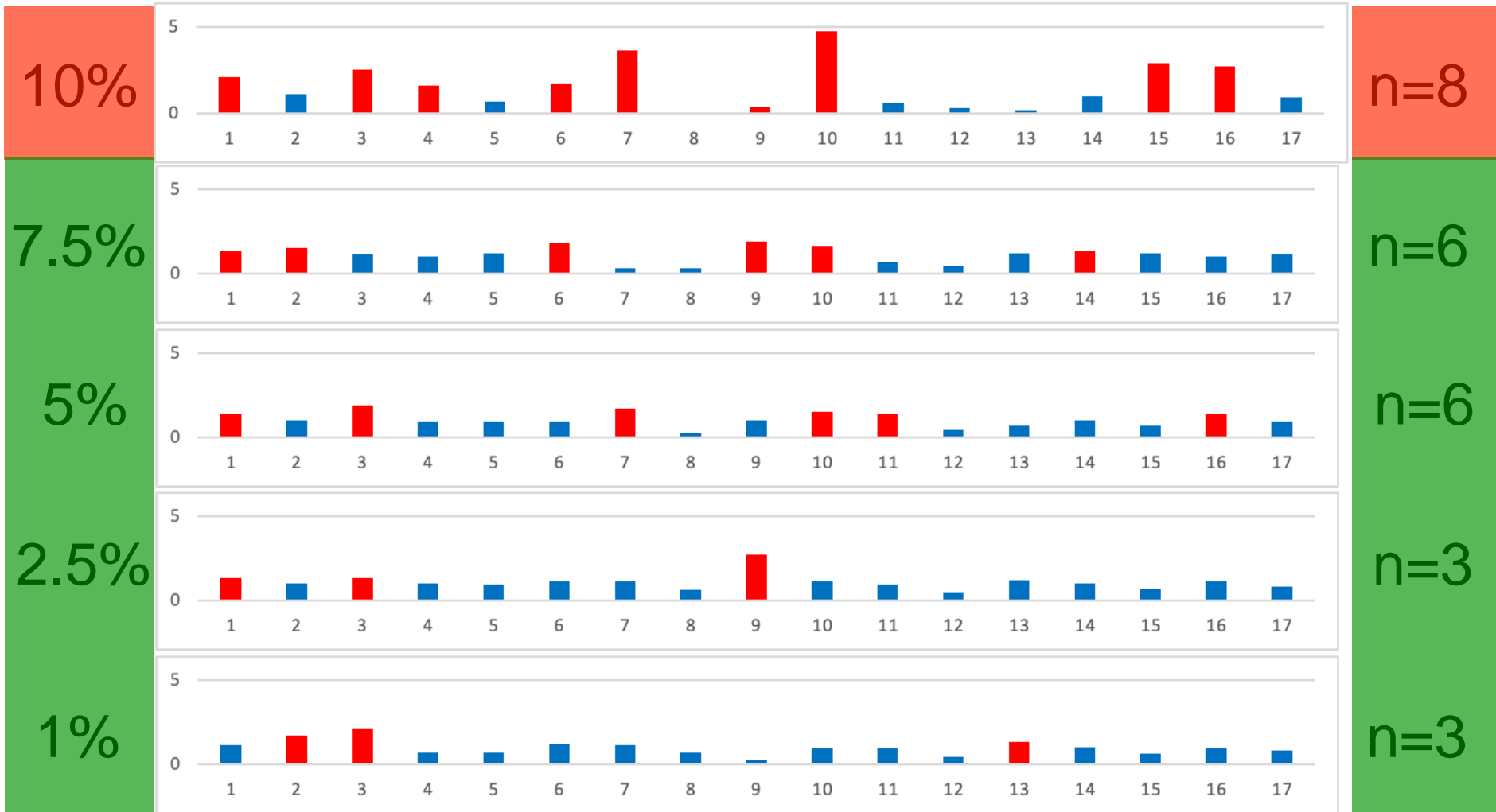
Eugenol - between 7,5% and 5%



In between concentration analysis



Geraniol - between 10% and 7,5%



In between concentrations analysis



From API et al. 2014 and RCPL

name	cas	LLNA weighted mean EC3 (µg/cm2)	NOEL HRIPT (Induction) (µg/cm2)	NOEL HMT (induction) (µg/cm2)	LOEL (induction) (µg/cm2)	WoE NESIL (µg/cm2)	RCPL WoE	SENS-IS (µg/cm2)	SENS-IS category
Isoeugenol	97-54-1	498[18]	250	NA	775	250	NA	250-625	moderate
Eugenol	97-53-0	2703[6]	5906e	5517e	NA	5900	7357	1250-1875	moderate
Geraniol	106-24-1	4080[6]	11811e	4138e	NA	11800	9197	1875-2500	moderate

How to obtain a PoD with the SENS-IS assay ?



- Does the SENS-IS assay correlate with the EC3 value ? The MD study
- Use an « in between » concentration
- Do read across using SENS-IS data

The NewgenTOX-iv Consortium



- Combine Physico-chemical data



- With SENS-IS data



- and perform read across using artificial intelligence



SENS-AI for potency



- **First grouping chemicals**
 - According to physico-chemical data as many as possible
 - According to SENS-IS gene signature
 - According to both
- **Then among the groups introduce the potency**
 - According to human data
 - According to animal data
 - According to SENS-IS + the gene signature
- **Then ask the machine to calculate a potency based on one positive determined concentration using the SENS-IS assay**

SENS-AI in practice for unknown chemicals



- Do read across to determine the likeliness of potency according to SENS-IS categories (very Weak, Weak, Moderate, Strong, Extreme)
- Perform a SENS-IS assay at one concentration according to the determination
- Calculate a PoD using SENS-AI

SENS-AI on RCPL chemicals

preliminary data



Name	CAS No	Potency Value (ug/cm2)	Predicted Potency Value (ug/cm2)	Category
2,4-dinitrochlorobenzene (DCNB)	97-00-7	3,4	6	Extreme
1,4-Phenylenediamine (PPD)	106-50-3	3,9	4	Extreme
Glutaraldehyde	111-30-8	20,0	22	Extreme
1,4-Dihydroquinone	123-31-9	47,5	38	Strong
Benzyl bromide	100-39-0	50,0	73	Strong
1,1,3-Trimethyl-2-formylcyclohexa-2,4-diene (Safranal)	116-26-7	106	89	Strong
Methyl 2-nonynoate (Methyl octine carbonate)	111-80-8	109	123	Strong
Methyl 2-octynoate (Methyl heptine carbonate)	111-12-6	125	117	Strong
Isoeugenol	97-54-1	325	289	Moderate
Allyl phenoxyacetate	7493-74-5	775	540	Moderate
Cinnamic aldehyde	104-55-2	885	206	Strong
3-Propylideneephthalide	17369-59-4	925	1345	Weak
Citral	5392-40-5	1450	1642	Moderate
Benzaldehyde	100-52-7	4094	5684	Weak
Hydroxycitronellal	107-75-5	5275	3587	Weak
Cinnamic alcohol	104-54-1	5775	7659	Weak
Eugenol	97-53-0	7357	2567	Moderate
Geraniol	106-24-1	9197	6540	Moderate
Coumarin	91-64-5	11792	10582	Weak
Carvone	6485-40-1	17573	9879	Moderate
Vanillin	121-33-5	No PV derived - very weak/nonsensitiser	NS	NS

■ RIFM data

■ Cosmetics Europe data

THANK YOU FOR YOUR ATTENTION

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