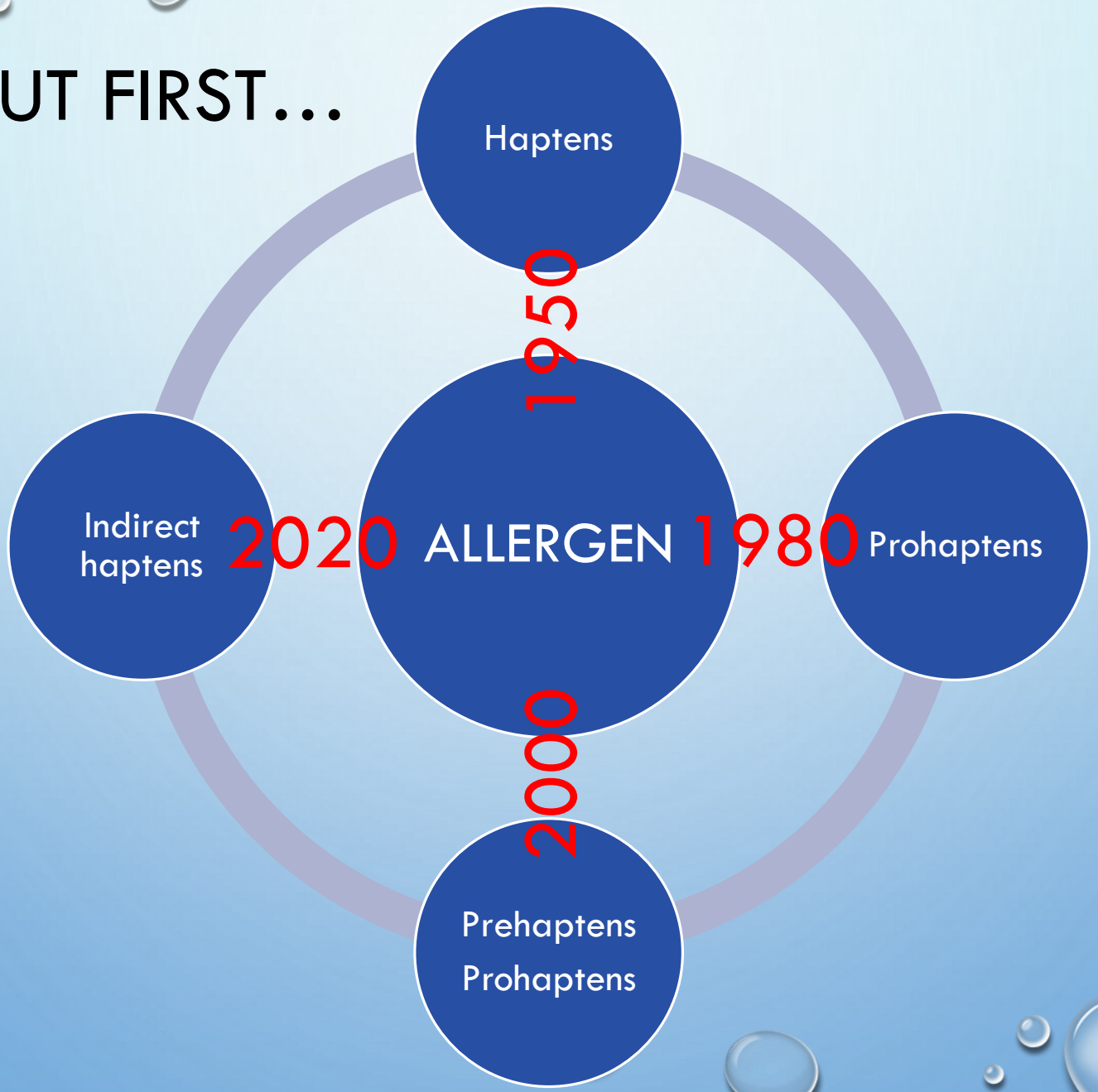
Viable metabolic systems and how to assess pro-haptens with NAMs. Summary of the pro-haptens workshop and outline of some key criteria to be fulfilled to build confidence in the use of new approach methodologies

PRE/PRO HAPTEN WORKSHOP OCT 16/17, 2019

David Basketter for Prof Jim Bridges,

Chair of the IDEA Supervisory Group

…BUT FIRST…


Haptens
1950
Indirect haptens
2020
ALLERGEN
1980
Prohaptens
2000
Prehaptens
Prohaptens

XENOBIOTIC METABOLISM SUMMARY

XENOBIOTIC REACTIVE METABOLITE PHASE 1 metabolite PHASE 2 metabolite

RH → → ~R→ → ROH↔ ROX

OXIDATION/REDCTION/ HYDROLYSIS ↓ CONJUGATION

binding to proteins (induction first phase)

SOME COMMON REACTIVE METABOLITES OF XENOBIOTICS

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- Epoxides
- Quinones
- Free radicals
- Unstable conjugates
- Reactive oxygen species

FRAGRANCE MATERIALS CONSIDERED TO ACT AS PRE- AND/OR PRO- HAPTENS

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Fragrance material	Air activated	Biologically activated
Cinnamic alcohol	yes	yes
Eugenol	no	yes
Geranial	yes	no
Geraniol	yes	yes
Isoeugenol	no	yes
Limonene	yes	no
Linalool	yes	no
Alpha-terpene	yes	yes

Karlberg et al., Contact dermatitis (2013) 69, 323

COMPARISON OF HUMAN SKIN WITH A CULTURED HUMAN SKIN CELL LINE (OESCH et al. 2018)

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Enzyme	NHEC	HaCaT	NCTC2544	KeratinoSens	LuSens	U937	THP-1
Cytochrome P450	md	r	md	md	md	md	md
Cyclooxygenase	v.low	md	md	-	-	-	-
Esterase	v.poor	-	-	v.poor	v.poor	v.good	v.good
Glutathione transferase	v.poor	v.poor	v.poor	-	-	-	-
N-acetyl transferase	v.good	v.good	-	md	md	md	md

PERFORMANCE WITH THE 26 FRAGRANCE LABELLED ALLERGENS

Fragrance	Pre/pro hapten?	KE1	KE2	KE3	Other
Amyl cinnamyl alcohol	Yes	-	-	+	+
Anise alcohol	Yes	+	-	+	+/-
Benzyl alcohol	Yes	-	-	+	+
Benzyl salicylate	Yes	-	+	-	+
Cinnamyl alcohol	Yes	+	+	+	+
Citronellol	Yes	+	-	+	+
Coumarin	Yes	-	+	-	+
Eugenol	Yes	+	-	+	+
Farnesol	Yes	-	+	+	+
Geraniol	Yes	-	+	+	+
Isoeugenol	Yes	+	+	+/-	+
Limonene	Yes	-	-	+	+
Linalool	Yes	-	-	+	+
Oakmoss (Evernia prunastri)	Yes	+	+	+	+
Tree moss (Evernia furfuracea)	Yes	+	+	+	+

About half of the indirect haptens are negative in the DPRA; overall 17/60 (28%) of the results are negative.

PERFORMANCE WITH THE 26 FRAGRANCE LABELLED ALLERGENS

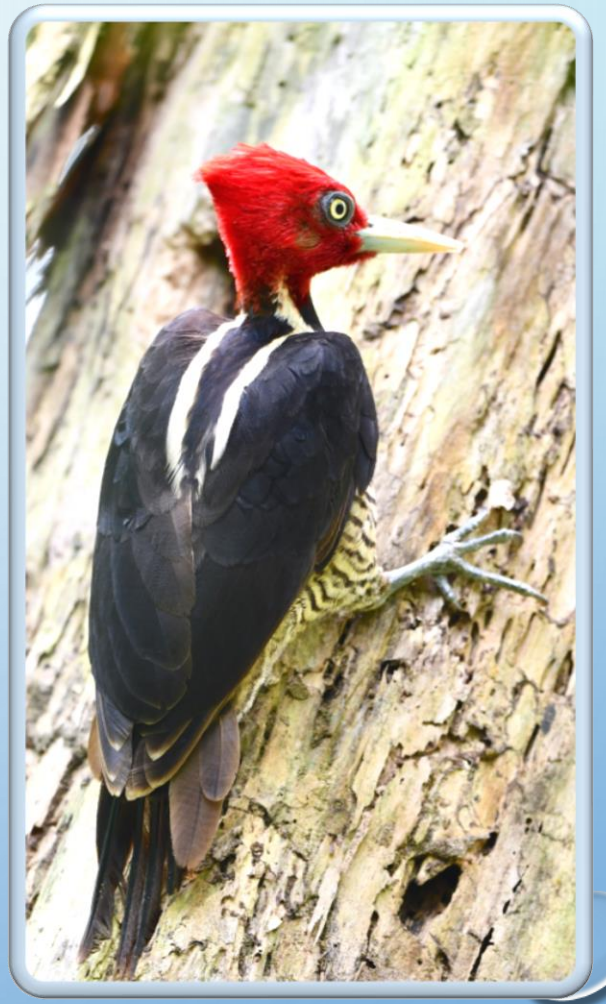
Fragrance	Pre/pro hapten?	KE1	KE2	KE3	Other
Alpha-isomethylionone	No	-	-	+	+
Amyl cinnamal	No	-	+	+	+
Benzyl benzoate	No	-	+	-	+
Benzyl cinnamate	No	-	+	-	+
Butyl phenyl methylpropional	No	+	-	+	+
Cinnamal	No	+	+	+	+
Citral	No	+	+	+	+
Hexyl cinnamal	No	-	+	-	+
Hydroxycitronellal	No	+	+	+	+
HICC/Lyral	No	+	+	+	+
Methyl 2-octynoate	No	+	+	+	+

About half of the direct acting haptens are negative in the DPRA; overall 10/44 (23%) of the results are negative.

TAKE OUTS…

SUBSTANCES THAT OXIDISE ***QUICKLY*** TO SKIN SENSITISERS ARE IDENTIFIED IN PREDICTIVE TESTS

SUBSTANCES THAT OXIDISE ***SLOWLY*** TO SENSITISING SPECIES CAN BE IMPORTANT CLINICALLY, BUT WE LACK AN IN VIVO OR IN VITRO SYSTEM FOR THEIR PREDICTIVE IDENTIFICATION



SLOW OXIDISERS REMAIN A SAFETY EVALUATION PROBLEM

REMEMBER PROHAPTENS MAY FEATURE IN OTHER INDUSTRY SECTORS

CONSIDERATIONS IN ASSESSING THE CONTRIBUTION OF XENOBIOTIC METABOLISM TO THE INDUCTION OF DERMAL ALLERGY

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- Are most pro-haptens also pre-haptens or is this only likely for reactive products formed by oxidation?
- Active oxygen species (e.g. OH*, O_{2^-}) formation - can this also arise as a consequence of inflammation?
- Is a close proximity of the enzymes producing reactive metabolites to the target proteins important for induction?
- What is the in vivo balance of activating, detoxifying and repairing enzymes

CONCLUSIONS FOR THE ASSESSMENT OF DERMAL INDUCTION OF FRAGRANCES

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- Although it is well established in other areas of toxicology it still remains uncertain how important metabolic activation is in the initiation of induction of dermal allergy.
- ***If*** it is a critical feature for the risk assessment of some fragrance materials then it is evident that the cultures in current use do not have an adequate XM activity.
- Relevant XM activity may need to be added to current test systems. The alternative, that such activity could be induced in these test systems, has not been studied.

Fifth IDEA pre- and pro- haptens workshop

Key conclusions – Day 1 (pre-haptens)

- The workshop took a deep dive into the extensive datasets generated both on the clinical and the analytical side to better understand positive patch test reactions to oxidized Limonene and Linalool.
- There is growing consensus that Limonene and Linalool containing products under the control of the fragrance industry may not be the main cause of the induction of contact allergy today. Other Limonene/Linalool exposure scenarios are more likely to be the major causes and need to be identified.
- Additional clinical studies are necessary:
  - ROAT with dilution series.
  - Use tests with suspected products.
  - Studies on exposure scenarios and confounding factors of affected patients (e.g. detailed and validated questioning).
- Further analytical work is needed:
  - On suspected products retrieved from patients to quantify elicitation levels.
  - On products with high terpene content to identify potential induction sources.
- Depending upon the outcome of the analytical work, the use of antioxidants and/or scavengers may be further optimized.
- Based on the above, further work may be needed to identify the circumstances involved in the induction of contact allergy to oxidized terpenes.

Fifth IDEA pre- and pro- haptens workshop

Key conclusions – Day 2 (pro-haptens)

- To set the scene, the workshop reviewed current knowledge on phase I and phase II metabolism with a focus on skin.
- The terminology of pro- (and pre-) haptens was revisited, reminding that the differentiation is based on biotic and/or abiotic activation. However, a chemical may be activated in both ways. Among fragrance chemicals, there is a lack of concrete and well documented examples of pure pro- haptens.
- Discussion of pro- haptens (that may also be pre-haptens), relevant to the fragrance industry concluded that:
  - Animal models (e.g. LLNA) provide a good identification of hazard and a reasonable assessment of potency for use in risk assessment.
  - There is a good correlation between *in vitro* assays with LLNA and human data, even if the underlying mechanisms are not fully understood. While there is room for improvement, it is questionable whether there is an urgent need.
  - *In silico* tools are a useful complement in hazard identification and potency characterization, assuming the chemical domain is covered.
- Looking ahead we need:
  - Concrete case studies on pure pro-haptens that could serve as example for the modeling and the hazard/risk assessment as we currently do.
  - Continue to improve *in silico* models as new data get generated.
  - Further data on xenobiotic metabolism including phase III would be useful.
  - Further work to better understand the impact of oxidative stress on the activation of pro- haptens should be considered.

Let’s keep things grounded…

Practical conclusions for fragrances…

- There is little evidence that current NAMs fail to identify the hazard of *fragrance* sensitisers dependent on indirect haptenation.
- Perhaps all known indirect fragrance sensitisers can function as pre-haptens.
- It may be that current NAMs do not properly assess potency, but metabolic failures may be only a part of this problem.