IDEA
International Dialogue for the Evaluation of Allergens

Rapporteur's Progress Report on the IDEA Expert Workshop on

Pre- and prohaptens

October 20th, 21st, 2015

Martin Klooster's Hotel
Onze-Lieve-Vrouwstraat 18,
3000 Leuven,
Belgium

1. Introduction and workshop objectives.

Pre- and pro-haptens were the subject of workshops in May 2013 and June 2015. The primary aims of this workshop were:

i. To identify the progress in addressing the recommendations of the first and second workshops.

ii. Obtaining agreement on the key knowledge gaps and how these should be addressed including the mechanistic understanding of pre- and pro-hapten formation and the bridge of laboratory data to clinical data.

iii. To identify priorities for further work including how to improve the documentation of current knowledge and its implementation for risk management purpose.

2. Chemical analysis of hydroperoxides

Background.

Human exposure to oxidized materials (incl. hydroperoxides) can arise from many sources including the environment, and use of commercial (consumer) products. It is important to try to identify all the relevant sources. In addition to the formation of oxidation products abiotically, oxidation products may also be formed biotically from the parent compound(s).

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Identification.

An update (since the 2nd pre- and pro-hapten workshop in June 2015) of the work of the Hydroperoxides Task Force was provided. Problems with the purity of various reference standards were noted. The reference method for purity measurement is NMR + internal standard. Standard solutions of oxidized materials (incl. hydroperoxides) have been shown to contain a number of unknown products. A major one appears to be a hetero dimer, the presence of which was confirmed by LC-MS. The conditions to prevent or optimise its formation are not clear yet. Properties of the unknowns (including the heterodimer) need to be understood as those could serve as a reservoir of hydroperoxide, thereby reducing the reliability of the analytical determination. It is therefore very relevant to understand the chemistry and do the mass balance to be able to finally judge on the performance of a method.

While the uncharacterized component fraction presents a challenge, however, the remaining 'free' fraction might be measured reliably for hydroperoxide.

In this context it was suggested to also look into the commercially available patch test preparations, including the potential presence of the heterodimer.

3. Assessment of the allergenic inducing potential of hydroperoxides.

It is important to address the question as to whether hydroperoxides are the only/key allergens. A key consideration is whether the exposure amount is above the levels to trigger an induction. Aggregate exposure must be taken into account to reach a conclusion. One issue in comparing findings is that different units of expression of findings are used by different disciplines.

Limonene and Linalool are widely used in consumer products (added as such but also via natural sources like orange oil or Lavender oil) and are commonly used as the model to study the effects of hydroperoxides. The oxidised forms of limonene and linalool show much greater irritancy and allergenic potential than the neat materials. There are very few claimed cases of reaction to its non oxidised form. Auto-oxidation of unprotected linalool or limonene occurs, even in case of storage at low temperatures.

Scenarios regarding the effects in patch tests of hydroperoxide standards.

Any effects observed may be due to:

- A direct effect of hydroperoxides
- Other components of the matrix
- Other oxidation (reactive) products in the product or formed in the skin by reaction with skin molecules such as tryptophan
- Active sensitization by too high amounts of hydroperoxides present in the oxidation mixture

i) *Direct effect of Hydroperoxides* (estimated by reduction to alcohol) – those are inevitably present in low concentrations in pre formulated materials and it is not clear to what extent they also form post formulation? In the case of linalool various factors (like air exposure, increased temperature, light) might enhance oxidation.

The formation of oxidation products is influenced by the nature of the matrix. The extent to which their formation can be prevented or controlled requires further investigation. It was noted that limonene hydroperoxide is less stable than linalool hydroperoxide.

ii) *Other chemicals*. There is no evidence so far that other chemicals in formulation are a cause/exacerbating factor.

The development of the analytical methodology will be very important to make more reliable conclusions.

4. Patch testing and Cross reactivity.

Patch testing by suitably experienced clinicians is currently the only suitable indicator of elicitation in man. Thus the only reliable means of confirming whether a material is a pre- or pro-hapten is through testing in through a clinical assessment. Testing is inevitably largely based on the knowledge of what people are exposed to. In the past identification and characterization of causative agents has been limited by the lack of suitable package labelling. High quality patch testing often requires repeat studies, blind tests, dose response using the pure fragrance ingredient of concern. In this workshop the focus was on cross reactivity aspects. Cross reactivity was defined at the second workshop and this definition was agreed at this workshop. Namely: 'the receptor of a memory cell for antigen 1 cannot distinguish between antigen 1 and an antigen 2 created by another hapten and will thus react also to antigen 2'. The following situations therefore need to be considered:

- Haptens A and B have very similar structures,
- Hapten A is metabolized to a compound similar to compound B,
- Hapten B is metabolized to a compound similar to compound A,
- Haptens A and B are metabolized to the same hapten.

Although a number of patients are reactive to more than one contact allergen the majority do not show cross reactivity between linalool and limonene.

Guinea pig studies on cross reactivity indicate that cross reactivity between fragrances is uncommon. In patients reactions to hydroperoxides are compound specific. Limonene 1 and limonene 2 hydroperoxides show different reactions with limited cross reactivity. Oxidation of endogenous compounds such as tryptophan provides a potential common mechanism whereby some different oxidising agents might apparently cross react; however whether this is significant in practice is unclear.

Cross reactivity /co-sensitisation between haptens may be due to:

- 3D structural similarities enabling them to fit with the same receptor
- Metabolism to a common metabolite
- Polyclonal response profile

5. Pre-haptens: hydrolysis.

The potential for hydrolysis is an important consideration for many fragrance ingredients in a variety of consumer products. It was not discussed in any detail at the previous two workshops. Data were presented regarding 124 chemicals with potential for hydrolysis to potential haptens. Four categories were identified and a representative of each was chosen. Acid and alkaline pH and increased temperature facilitated the hydrolysis process. Smaller leaving group (acetate, formate) were found to be more readily hydrolysed than larger ones. The nature of the alcohol group nature also affected the process. Stability of esters in a given matrix and properties of released alcohol should be an important element to consider in the formulation of fragrance compounds for different consumer product types.

6. Identification and characterization of prohaptens.

It was emphasized that classification is usually based on negative criterion i.e. that a chemical is not a direct acting hapten.

Overall the so called drug metabolizing enzyme system is a detoxification one, rather than an activation process. However, the initial step in metabolism quite often generates reactive metabolites. A critical question in terms of whether current tests allow for prohapten metabolism to haptens is whether the mouse LLNA test and the guinea pig test produces the same haptens and in comparable amounts to those which occur in human skin. With the restriction on the use of such animal models there will be a heavy reliance in future on in vitro preparations to identify and characterise prohaptens.

Use of HRMAS Cells and spinning NMR.

The relationship of metabolic capability in intact human skin to that in various in vitro models is unclear although it appears that the hydrolytic drug metabolizing enzymes are particularly well preserved. It has been shown that in the HRMAS model for example hydrolysis occurs significantly within 5 min. There has been insufficient research of the variability in drug metabolism due to enzyme induction by substances applied to the skin.

Around 200 chemicals have been tested in in vitro models, around 50% of which were fragrances. Assays used included the keratinocyte assay, the peptide activity assay, the hCLAT assay. The cell line (COCAT) has been used as a metabolite generating system. There is some indication that upregulation of cytotoxicity may facilitate induction of allergic response.

7. In silico prediction for pre and pro haptens

Prediction requires:

- Identification of the relevant active metabolite based on chemical and metabolism data (can be predicted to some extent)
- Estimation of the rate of formation and enzymatic or abiotic degradation or binding to buffer proteins, etc. (more difficult to predict)
- Assumptions on the ability of the ultimate hapten to reach and combine with the target protein(s).

An update was provided on the TIMES –SS model which was discussed at the previous workshop. It is based on a linear model of phase 1 metabolism in which reactive metabolites are generated that may react with the target, react with buffer proteins or be further metabolized by phase 2 metabolism. The reliability of the model has been checked with selected chemicals for abiotic activation and the findings reviewed by experts. The model has great potential value although in its current form it fails to identify some haptens (pro or pre haptens).

8. The RIFM approach to the non-animal identification of haptens.

A staged approach is under development involving:

i) Use of structure activity analysis.

ii) Application of model for identifying reactivity with protein.

iii) Development of more sophisticated tests to enable the hazardous properties to be further characterized and dose response relationships to be determined.

9. Conclusions

What we know

i) Haptens, pro haptens and pre haptens should not be considered as mutually exclusive entities unless there is evidence to justify this. The fact that a chemical has been shown to be a pre hapten doesn't exclude the likelihood that it is a prohapten. Criteria are needed to be applied to each fragrance to ascertain that it is not a prehapten and is not metabolised in the skin to the final active form(s) for induction.

ii) Cross reactivity is uncommon both in humans and in the guinea pig model. For predictive purposes SAR analysis needs to include 3D considerations.

Remaining information gaps and questions

- The ability of chemicals to reach sites for biotic and/or abiotic transformation.
- The nature and relative quantities of each product generated and for the chemical/products generated. Only two types of activation have been considered in these workshops namely oxidation (particularly hydroperoxide generation) and hydrolysis. This focus is mainly due to clinical data indicating an issue. In published studies on the metabolism of other chemicals reduction, various types of conjugation are also sources of reactive metabolite generation.
- The processes for transfer to and react with target proteins, (e.g. 3D structure, potential for inactivation in the skin)
- The impact of enzyme inducers, very mild stress/lipid peroxidation and stimulation of reactive oxygen defence mechanisms

Further challenges

- How to modify QRA 2 to allow for prehapten/prohapten potential.
- The need to establish a robust non animal tests which incorporate drug metabolizing capacity comparable to that of human skin in vivo.

Recommendations to follow up have been made in respective breakout groups (attachments 1 and 2)

Jim Bridges, *Rapporteur*.

February 2016

APPENDIX 1 WORKSHOP PROGRAMME

IDEA Workshop

Pre- and Pro-haptens

October 20-21, 2015

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Program

The WS will build on what has been discussed and agreed at the June 2015 Expert WS on improved mechanistic understanding of pre- and pro hapten formation as well as on analytical developments.

The intention is to bridge what has been worked out in the June Expert Workshop and progressed over the summer, with clinical findings.

Monday, October 19th – Arrival and Registration

16:00 -18:30 Welcome and registration

18:30 - 21:30 Standing buffet

Tuesday, October 20th (09:00 to 17:00) – Day 1 (Predikheren meeting room)

09:00 - 09:15 Workshop opening – *Hans Bender and Matthias Vey*

<u>Hydroperoxide TF report</u>

09:15 - 09:45 Update on the work program, status, remaining activities and timelines. Proposals of next steps

Speaker: Alain Chaintreau (Firmenich)

09.45 – 10.15 Moderated discussion on proposals and agreement on conclusions

Pre-hapten – clinical data and interpretation

10.15 – 10.45	Update on clinical data including discussion on relevance of cross reactivity *Speaker: Ann-Therese Karlberg and Johanna Bråred Christensson (University of Gothenburg)*
10.45 – 11.00	Coffee Break
11.00 – 11.30	An alternative position on the role of cross reactivity and different theoretical scenarios to explain high frequency of patch test reactions. *Speaker: Andreas Natsch (Givaudan)*
11.30 – 12.00	Moderated discussion on proposals for next steps
12.00 – 12.30	What is known based on metabolism, induction and elicitation data (animal and clinical) regarding biotic pro-hapten on pro-hapten activation, 'cross' reactivity of e.g. esters and breakdown products - mechanistic understanding and quantitative follow up. *Speaker: Jean-Pierre Lepoittevin (University of Strasbourg)*
12.30 – 12.45	Moderated discussion, proposals, next steps
12.45 – 13.45	Lunch

Understanding Pro-haptens

13:45 - 14:30	State of knowledge on abiotic hapten formation (hydrolysis) using examples of fragrance ingredients and state of the art on the technical management of those transformations *Speaker: Chris Powell (Unilever)*
14:30 – 15:15	The methodological challenges – what is known about pro-hapten activation of fragrance materials in the in-vivo and in-vitro test methods we use for induction. *Speaker: David Basketter (Consultant) and Brunhilde Blömeke*
15:15 - 15:45	Moderated discussion
15:45 - 16:00	Coffee break

16:00 - 16:30	Usefulness of computer models in predicting pre- and pro-electrophilic activation of chemicals in skin sensitization assessment - modelling of metabolic pathways via QSAR/SAR – are quantitative considerations possible? *Speaker: Chanita Kuseva (University of Bourgas)*
16:30 – 16:45	Moderated discussion as basis for key conclusions on pro-haptens

Conclusion of Day I

16.45 – 17.00	Preliminary progress report *Speaker: Rapporteur of the workshop (Jim Bridges)*

End of Day I

19:00 – 22:00	Dinner

Wednesday, October 21th (09:00 to 15:00) – Day 2 (Predikheren meeting room)

09:00 - 09:15	Wrap-up of Day 1

Pre- and pro hapten consideration in current risk assessment (of sensitizers)

09.15 – 09.45	How pre-and pro haptens are currently incorporated in our risk assessment methodology build around the QRA? *Speaker: Anne Marie Api (RIFM)*
9.45 – 10.15	Moderated discussion and confirmation of Working Groups for day 2
10.15 – 10.45	Coffee break

Working group discussions

10:45 – 13:00	The participants will be subdivided into working groups
13:00 – 13:45	Lunch break
13:45 – 14:30	Presentation of the conclusions / recommendations of the working groups

Workshop conclusion

14:30 – 15:00	Conclusions of the workshop and next steps with regard to 'How confident can we be that pre-and pro haptens are adequately incorporated in our RA methodology build around the QRA II? *Speaker: Rapporteur of the workshop (Jim Bridges)*

End of Day 2

15:00	End of Day 2 and workshop closing

APPENDIX 2 PARTICIPANT LIST

Academic Community:	
Donald Belsito	*Columbia University Medical Center, USA*
Brunhilde Blömeke	*Trier University, Germany*
Johanna Brared-Christenson	*University of Gothenburg, Sweden*
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Peter Friedmann	*University of Southampton, UK*
An Goosens	*KULeuven, Belgium*
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Chanita Kuseva	*University of Bourgas, Bulgaria*
Jean-Pierre Lepoittevin	*University of Strasbourg, France*
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Ulrika Nilson	*University of Stockholm, Sweden*
David Roberts	*Liverpool John Moores University, UK*
Axel Schnuch	*IVDK / University of Göttingen, Germany*
Industry:	
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David Basketter	*DABMEB Consultancy*
Peter Cadby	*Firmenich, Chanel*
Alain Chaintreau	*Firmenich*
Graham Ellis	*Givaudan*
Dominique Favier	*IFF*
Carsten Goebel	*Procter and Gamble*
Etje Huzelbos	*IFF*
Boris Müller	*Symrise*
Vincent Murat	*Takasago*
Andreas Natsch	*Givaudan*
Neil Owen	*Givaudan*
Chris Powell	*Unilever*
Scott Schneider	*Firmenich*
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