IDEA
International Dialogue for the Evaluation of Allergens

Rapporteur's Progress Report on the fourth IDEA Workshop on

Pre- and Pro- haptens

December 13th, 2016

The Hotel
Boulevard de Waterloo, 38
1000 Brussels

1. Introduction and workshop objectives.

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

- To identify the progress in addressing the recommendations of the previous workshops;
- To develop a risk assessment framework to incorporate pre- and pro-haptens into QRA2.

It focused on the identification and characterisation of fragrance ingredients which are abiotically and/or biotically converted to forms that lead to and/or enhance the induction of dermal sensitisation.

2. Identification of structural alerts for both pre- and pro-haptens:

The limitations of the tools

The strengths and limitations of in-silico tools (e.g. OECD (Q)SAR Toolbox, TIMES-SS and Meteor-Nexus) used to identify the potential skin sensitisation properties of materials were presented.
TIMES-SS and Meteor-Nexus provide an indication of metabolic pathways of the materials. In particular, TIMES-SS lists all the metabolic pathways that are possible for the material under study, but it does not provide further clarity on the relevance of the identified metabolic reactions for the skin sensitisation potential of the material. In addition, these tools are built with mainly hepatic metabolic data, and therefore their impact on the prediction in skin, is unclear. In addition, TIMES-SS gives strong emphasis on hydroperoxide formation. However, there is no evidence for hydroperoxide formation in the skin (HP formed mainly on forced aging of raw materials) - thus the HP formation in skin modelled in TIMES SS may not be relevant.
In vitro tests also may provide limited information on pre- and pro- haptens, due to the restricted metabolic capabilities of the systems.
TIMES-SS provides a good correlation for strong sensitizers, while it has more incorrect classification for weak sensitizers.

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The need for an integrative testing strategy

In silico prediction data are never used in isolation and the information obtained from their combination with in vitro systems can provide an indication on whether materials act as pre- or pro- haptens. The outcome of such an integrative testing strategy for the identification of pre- and pro- haptens can be used as a basis for risk assessment. However, these strategies do not provide the EC3 or the NESIL required to directly apply the QRA. This is still a challenge that needs to be addressed with non-animal methods.
To increase the metabolic capacity of in vitro methods, the addition of S9 enzymes into the systems is being explored. The use of reconstituted human skin instead was also mentioned.
As the metabolic rate of the skin is considerably lower than the liver, expert judgement is required to examine the reliability and the relevance of the outcome of the systems. In addition, a greater understanding on metabolic pathways is required in order to feed the in-silico systems, and enhance the information provided by the models on the relevance of the metabolic pathways for the material under study.

3. Understanding exposure levels of pre-haptens in products:

An update of the work of the Hydroperoxide Task Force was provided. The method selected, reduction followed by GC–MS, gives good inter-laboratory reproducibility and a good accuracy with a threshold of detection of 50ppm. So far, the analytical methodologies developed by the IDEA Hydroperoxides TF have proven to allow the quantification of limonene and linalool hydroperoxides in consumer products such as deodorants, fine fragrances, lotions and creams. The Hydroperoxides Task Force will now initiate a final ring test to confirm the preliminary results obtained in consumer products with complex matrixes such as face creams and body lotions, which will include a third party laboratory. An analytical general approach to measure hydroperoxide levels in a consumer product on the market was presented and adopted. Such quantifications will allow the provision of information on the extent of exposure to hydroperoxides contained in the product. The participants were invited to provide any suggestions that could be of help to select the products from the market that should be analysed in 2017.

The link between the formation of hydroperoxides in consumer products and the positive patch test reactions from the hydroperoxides and the oxidized mixtures for linalool and limonene observed in the clinics is still missing. The quantification of limonene and linalool hydroperoxides in the consumer products will help establish a correlation between the exposure of the consumer and the clinical data observed.

The majority of manufacturers know the detailed composition of their products and have information, such as impurities, which could be controlled. However, this is not the case with some companies buying materials of less controlled quality. In addition, there is an increasing use of aromatherapy and self-made products. These uncontrolled or unregulated exposures may play a non-negligible role and could partly explain the difference observed between the number of positive patch test reactions and the amount of hydroperoxides detected in the products. The IDEA Hydroperoxides TF will consider to analyse consumer products produced by IFRA members but it was suggested that also products outside of IFRA's control should be considered.

4. Understanding exposure levels for pre/pro-haptens

In vitro methods can predict both pre- and pro- haptens, but there are opportunities to improve the methods to provide further reassurance on the assessment of the potential skin sensitization properties of the material.

The LLNA allows the identification of a pre-/pro- hapten and the outcome of the test can be directly applied into QRA2. However, further work is required to replace the LLNA by alternative methods to animal testing. A negative DPRA in association with two positive cell tests could suggest a pro hapten.

For pre- haptens, the analytical method will provide information on the exposure. Pre-haptens that oxidize rapidly are easily predicted by validated in vitro methods. However, for the pre-haptens that undergo slow oxidation, we lack a system that would allow a reliable identification. However, this is also true for the LLNA. Both in the LLNA and in the in vitro methods, test materials oxidizing slowly must first undergo a forced oxidation to then be positively tested. However, a positive result from such a test must always be related to the likelihood that similar reactions occur under the intended use of the molecule. Clinical data may additionally be used to refine the risk assessment or risk management.

5. The framework for the inclusion of pre- and pro- haptens into QRA2

The following was agreed by the participants:

A) The current evidence with the 3 OECD validated in-vitro tests for skin sensitization hazard indicates that approximately 80% of pre- and pro- haptens are correctly identified (following the classification strategy outlined in ECHA guidance 2016) (Natsch et al., 2014; Patlewicz et al., 2016; Urbisch et al., 2016).

B) The general approach for the framework for the inclusion of pre- and pro- haptens into QRA2 is:
- Evaluate the chemistry to prepare an in vitro study plan
  - Structural alerts, in silico tools, read-across materials
  - Physicochemical data
  - Expert judgement
- In vitro testing adapted according to the outcome of the evaluation
  - DPRA, KeratinoSens and hCLAT
  - Consider S9 activated assays, peroxide/peroxidase assay and/or probable oxidized products
- Further considerations to refine the risk assessment
  - To what extent does the transition from pre-/pro- hapten to putative hapten occur?

C) Once the hazard is identified, there is no difference in the QRA approach for pre- and pro- haptens vs. haptens. On pre- haptens, exposure assumptions based on analytical quantification of oxidation product with sensitizing properties together with its NESIL (derived from LLNA or alternative methods) will allow for a QRA. On pro- haptens, direct use of LLNA data (or alternative methods) will allow for a QRA.

Annex 1: List of participants

Academia

Donald Belsito	University of Columbia
Brunhilde Blömeke	University of Trier
Johanna Bråred-Christensson	University of Gothenburg
Elena Gimenez-Arnau	University of Strasbourg
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David Lovell	University of London
Hans Merk	University of Aachen
Ulrika Nilsson	University of Stockholm
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SCCS Observer:

Vera Rogiers	University of VU Brussels

Industry:

David Basketter	DABMEB Consultancy
Michael Calandra	Firmenich
Graham Ellis	Givaudan
Carsten Goebel	Coty
Amaia Irizar	IFRA
Petra Kern	P&G
Boris Müller	Symrise
Vincent Murat	Takasago
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Other Observers:

Florian Schellauf	Cosmetics Europe

IDEA Management Team:

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IDEA Supervisory Group:

Alain Khaiat (*Rapporteur*)

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Alain Khaiat, December 19th, 2016
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