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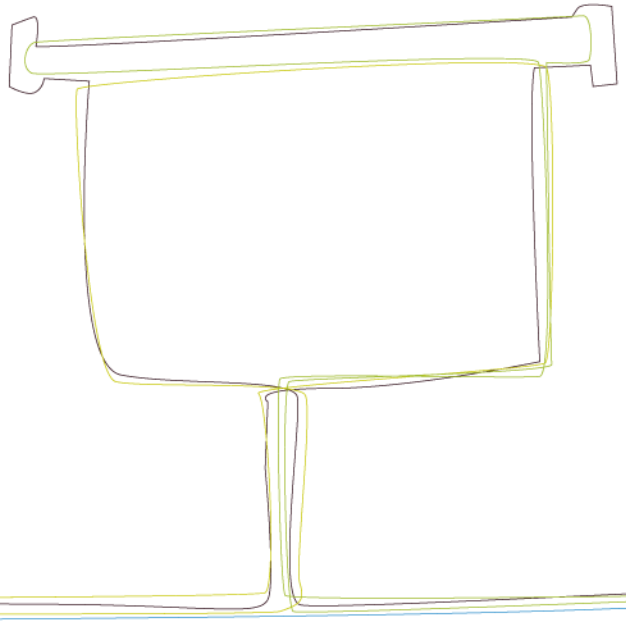
International Dialogue for the Evaluation of Allergens

Rapporteur's Progress Report on the IDEA Workshop on

Risk Assessment of Pre- & Pro-Haptens



May 28-29, 2013

Prof. Jim Bridges
Chairman of the IDEA Supervisory Group

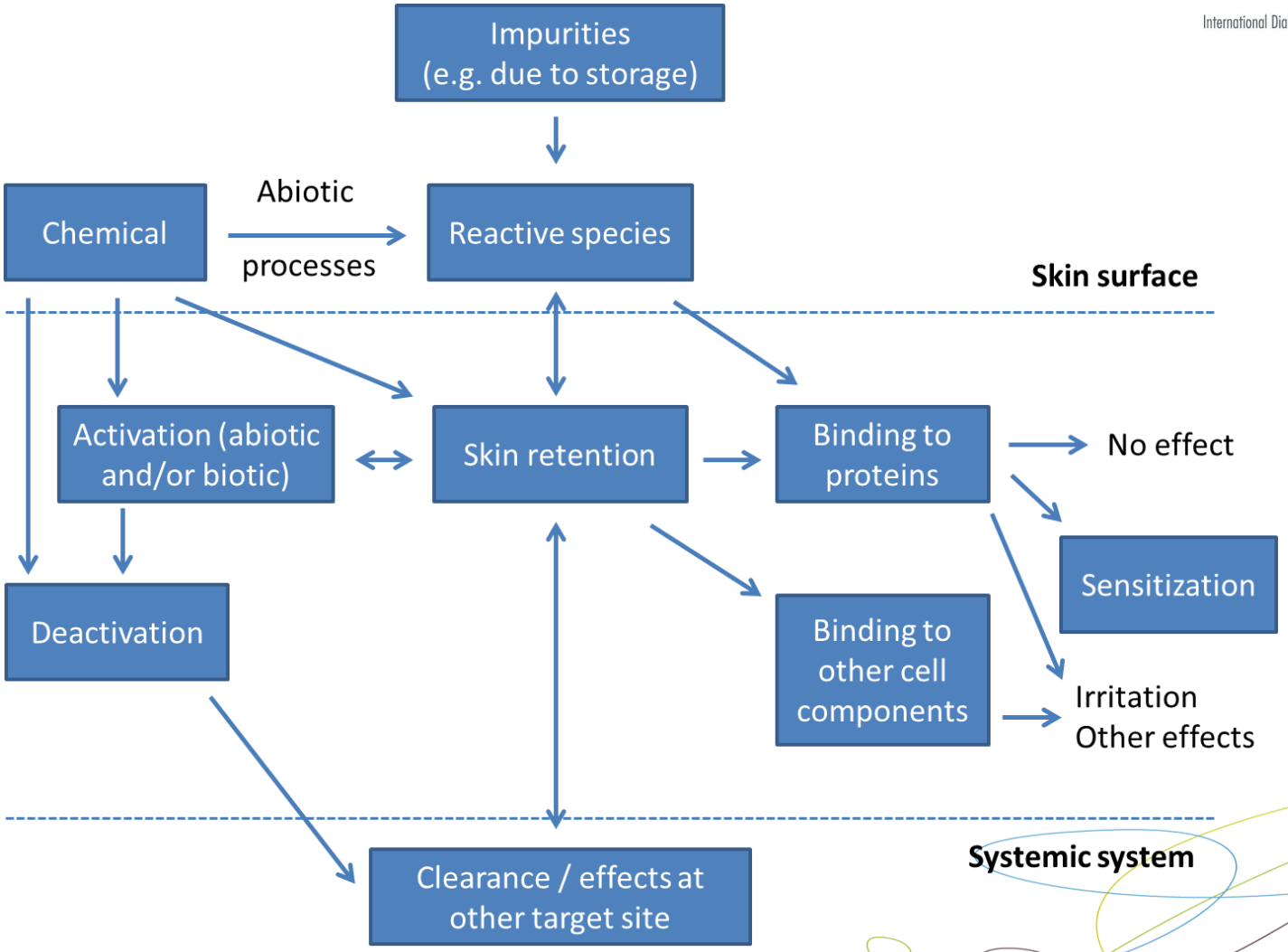
Current understanding of pre- & pro-haptens

- A **hapten** is a small low molecular weight molecule that can induce and elicit an immune response.

- **Pre- & pro- haptens** are hapten precursors and are often non-sensitizing themselves.

- **To be effective, a hapten needs to:**
  a. Gain access in sufficiently high concentrations to the target protein(s) responsible for triggering the induction of skin sensitization.
  b. React with these target protein(s) without also causing marked cellular damage.

Distribution, transformation and effects



The difference between pre- & pro-haptens

- A pre-hapten is a substance that needs to be chemically (**abiotically**) activated.
- A pro-hapten is a substrate for biotic activation (often involving the so-called drug-metabolising enzymes).
- Pre-haptens appear to be more common than pro-haptens.

The transformation of pre- & pro-haptens

- Most common pathways for hapten formation from precursors are **oxidation** and **hydrolysis.**

- Rate of abiotic / biotic activations and of **risk of sensitisation initiation** depends on:

  a. Chemical factors (reactivity, storage conditions, etc.)

  b. Inter-individual variability

Oxidation to form a hapten

- Functional groups that may oxidize and lead to the formation of haptens are: double bonds, allylic or benzylic C-H bonds, alcohols, aldehydes, …
- Factors affecting **abiotic** formation of haptens are:
  - Oxygen availability
  - Temperature
  - Competing alternative pathways that do not result in hapten formation.
- Other parameters for **biotic** transformation:
  - Availability of appropriate enzyme in the skin.
  - Ability of pro-hapten to reach the enzyme.

Hydrolysis to form a hapten

- Some functional groups are prone to hydrolysis: Schiff bases, esters (eg formates), acetals, …
- Parameters influencing hydrolysis are basically the same as for oxidative reactions except that:
  - Hydrolysis can also occur under anaerobic conditions.
  - Abiotic hydrolysis (and solvolysis) very much depends on the pH of the medium.

Current understanding of these transformations

- Pre-haptens:
  - Good qualitative evidence that sensitizers can be formed in some formulations under realistic conditions.
  - Studies on fragranced consumer products show concentrations of formed haptens are generally much lower than those generated under experimental conditions.
- Pro-haptens:
  - Literature on metabolic activation is very rich but primarily in organs other than skin and focused on endpoints other than sensitisation.
  - However, relevant enzymes also present in the skin.

Understand Inter-individual human variability

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- Further investigation is necessary to better characterize the potential inter-individual differences in the initiation of sensitization.
- For pro-haptens, it is likely that genetic or other differences in skin metabolising enzymes play a role in skin sensitization.
- For pre-haptens, it is not clear if inter-individual variation occurs or if it is important.

Needs: Prediction of pre- and pro-haptens properties

- Development of a **structure-activity model** would be valuable.
- Model should include:
  - Determination of bond energy at “vulnerable” site(s).
  - Determination of parameters influencing the kinetic rate of reactions (pH, oxygen, availability, temperature, etc.)
  - Ability of human skin to generate electrophiles, nucleophiles, radicals
  - Physical properties of precursors and resultant haptens.
- Targets the understanding of skin sensitisation potential of resultant haptens

Needs: Laboratory methods

- The development and use of:
  - Methods to measure / estimate hapten formation: magic angle spinning NMR, chromatography with use of trapping agents, oxidation stability studies, …
  - Models for biotic transformation: animal / human skin biopsy samples, skin culture models (further developments needed).
  - Tools to identify skin sensitization potency (LLNA, HRIPTs and in-vitro methods).
- Current methods and tools will need refinements and adaptations to provide adequate information on pre- and pro-haptens.

Needs: Clinical methods

- **Identify / characterize** pre- and pro-haptens via experimental methods.
- Assess **total real-life exposure of consumers** to haptens resulting from biotic / abiotic transformation of fragrance ingredients:
  - Attention should be paid to the development of viable methodology to determine hapten formation in the skin.

Conclusions on risk assessment/management

- No reliable way to identify, characterize and quantify haptens from fragrance ingredients. **Staged approach** to identify / characterize these chemicals was proposed and could be adopted (see progress report).
- Most tools (analytical, biological or clinical) for investigation in skin or in products exist but need to be adapted / refined for pre- and pro-haptens.
- Resulting knowledge to be incorporated into QRA ensuring high level of consumer protection.

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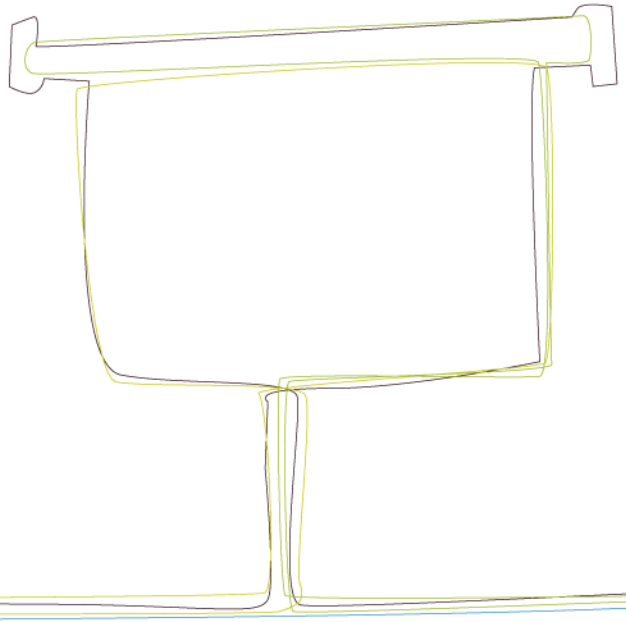
Progress report on actions taken

Risk Assessment of Pre- & Pro-Haptens



Process and timeframe

- The recommendations made during the workshop:
  - pertaining to risk assessment are addressed by RIFM
  - Recommendations pertaining to chemical analysis and risk management are addressed by IFRA.

- Preliminary results are available and consolidated results will be presented at the next workshop on pre- and pro-haptens (September 2014).

Risk assessment (ONGOING)

- Data on pre- and pro-haptens are currently being collected to develop a suitable Structure-activity model.
- A research project is underway developing human skin models for studying hapten formation and protein interactions. The role that skin metabolism has in this characterization is also being explored.
- Research is ongoing to incorporate pre- and pro-haptens in QRA methodology.
- A framework has been developed for the identification of important pre & pro haptens requiring further study.

Analytical development (ONGOING)

- IFRA Analytical Working Group (chaired by Dr. Alain Chaintreau, workshop participant) supervises the development of procedures to prepare, purify and assess the stability of haptens currently not commercially available (e.g. hydroperoxides).
- Objective is to get a suitable range of pure references, necessary to allow the development of an analytical method for the quantification of haptens.
- This analytical method is critical for the subsequent execution of *in vitro* / biological assays and clinical investigations.

Risk management (PENDING)

- Once fully developed, the quantification method of pre- & pro-haptens will be used to monitor the market:
  - First to gather useful information on actual consumer exposure
  - Then to ensure the level of haptens (e.g. hydroperoxides) in marketed products is acceptable.
- Raise awareness of good operating practices known to reduce the level of haptens in consumer products amongst all stakeholders along the supply chain.

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Thank you for your attention

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