Risk assessment of pre- and pro- haptens

David Basketter

(..building on the work of the pre/pro haptens teams and their meetings, most recently December 2016...)

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Pre- and pro- haptens

- Three take home points:
  - This is **NOT** a new entity – haptens which require air and/or metabolic activation have been recognised for decades
  - Existing in vivo and in vitro methods already identify the great majority of these allergens
  - QRA2 accommodates them

Consider…..

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Guinea Pig Tests

- No guinea pig tests were ever validated or even assessed for their ability to detect pre- or pro-haptens
- Risk assessment was conducted using comparative toxicology

Local Lymph Node Assay

- The LLNA was formally validated, but not tested exhaustively regarding pre- or pro- haptens
- Risk assessment evolved to a more transparent, quantitative approach

The performance of the LLNA

Background information

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- 319 substances tested (Gerberick et al, 2005 and Kern et al, 2010).
- Of these 60 (19%) were identified as pre- and/or pro- haptens…
- …and of these, all except two were positive (97% accuracy)
- Such a level of performance is greatly in excess of what predictive toxicology normally achieves!

Are pre/pro haptens an issue?

- Guinea pig methods
  - substances originally described as “pro-haptens” were identified via positive data from “non-reactive” chemicals
- Murine LLNA
  - almost all pre/pro haptens are positive
- *In vitro*
  - the majority of pre/pro haptens are positive

The ECVAM work (1 year ago)

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Workshop report

Can currently available non-animal methods detect pre and pro-haptens relevant for skin sensitization?

Grace Patlewicz [a, *], Silvia Casati [b], David A. Basketter [c], David Asturiol [b], David W. Roberts [d], Jean-Pierre Lepoittevin [e], Andrew P. Worth [b], Karin Aschberger [b]

[a] US Environmental Protection Agency, National Center for Computational Toxicology, Research Triangle Park, NC, USA
[b] European Commission, Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Chemical Safety and Alternative Methods Unit, Ispra, VA, Italy
[c] DABMEB Consultancy Ltd, Sharnbrook, Bedfordshire, UK
[d] Liverpool John Moores University, School of Pharmacy and Biomolecular Sciences, Liverpool, UK
[e] Institute of Chemistry, CNRS UMR 7177 and University of Strasbourg, Strasbourg, France

How do *in vitro* methods perform?

- Until the ECVAM review, there was no independent/systematic analysis
- However, a range of commonly reported pre- and pro-haptens have been tested
- Natsch et al, 2014 reported on 145 substances: of 22 suspected pre/prohaptens, 17 (77%) were positive using the an ITS/IATA consistent with ECHA/REACH
- Reminder: analysis of individual assays is encouraged **only** for understanding applicability domains

The ECVAM work in 2015

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The conclusion was that in vitro tests do work.

It agrees with Urbisch et al, 2016 in Chem. Res. Toxicol.

ABSTRACT

Predictive testing to characterize substances for their skin sensitization potential has historically been based on animal tests such as the Local Lymph Node Assay (LLNA). In recent years, regulations in the cosmetics and chemicals sectors have provided strong impetus to develop non-animal alternatives. Three test methods have undergone OECD validation: the direct peptide reactivity assay (DPRA), the KeratinoSens™ and the human Cell Line Activation Test (h-CLAT). Whilst these methods perform relatively well in predicting LLNA results, a concern raised is their ability to predict chemicals that need activation to be sensitizing (pre- or pro-haptens). This current study reviewed an EURL ECVAM dataset of 127 substances for which information was available in the LLNA and three non-animal test methods. Twenty eight of the sensitizers needed to be activated, with the majority being pre-haptens. These were correctly identified by 1 or more of the test methods. Six substances were categorized exclusively as pro-haptens, but were correctly identified by at least one of the cell-based assays. The analysis here showed that skin metabolism was not likely to be a major consideration for assessing sensitization potential and that sensitizers requiring activation could be identified correctly using one or more of the current non-animal methods.

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"...sensitisers requiring activation could be identified correctly..."

Consequences for QRA2

- The practical reality is that in vivo and non animal methods do a good job of identifying direct and indirect acting skin sensitisers.

  Thus, when a substance is positive, whatever its type, it can enter the risk assessment process; if negative it can be assumed not to classify as a sensitiser.

Are there any gaps?

- All *in vivo* and *in vitro* tests are unable to predict very slowly oxidising prehaptens

- For the latest review, see Karlberg (2017) Contact Dermatitis, 76, 63-66

Current actions in the pre- / pro-haptens team

- An addendum is being finalised for addition to the QRA2 dossier to make their position in risk assessment clear.
- As will be seen in this meeting, work on hydroperoxides and associated analytical efforts continues.
- Special focus is being applied to linalool and limonene.

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Thank you for your attention