Non-animal approaches to assessing the skin sensitization endpoint under REACH

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Introduction

The skin of consumers and workers is daily exposed to chemicals via use of household or cosmetic products, or in industrial settings. One of the adverse effects that can occur as a result of skin exposure to xenobiotics is skin sensitization, the clinical manifestation of which is termed allergic contact dermatitis. The evaluation of the skin sensitization potential of a substance is therefore of central importance for hazard and risk assessment of chemical exposures. Like most other toxicological endpoints, skin sensitization is in the regulatory context currently assessed by *in vivo* testing. Only animal tests like those described in OECD TG 406 (i.e., guinea pig maximization or Buehler test) or OECD TG 409/442 (i.e., murine local lymph node assays) are officially accepted by regulatory bodies for hazard classification purposes.

The increasing emphasis on ethical considerations and the need for reduction of animal testing has manifested itself at a regulatory level in the recent chemicals regulation 'REACH' ((EC) No 1907/2006), but even more in the amendments to the European Cosmetics Directive 76/768/EC and today's European Cosmetics Regulation ((EC) No 1223/2009). Even though skin sensitization is an endpoint for which information is already required at the lowest tonnage band of 1-10 tons per year, REACH mandates that every effort be made to avoid animal testing. Specifically, Annex XI of REACH allows for adaption of the standard testing requirements by use of non-animal approaches.

This presentation will describe consortium/SIEF approaches to the skin sensitization assessment of chemicals under the REACH regulation. It will describe the 'tool box' of non-animal and animal methodologies available for chemical registrations under REACH and look at its practical application based on case studies.

Hazard information and evaluation requirements under REACH

The assessment of skin sensitization follows a stepwise approach. The standard requirements are described in REACH Annex VII 8.3, column 1:

- **Step 1**: Assessment of all of the available human, animal and alternative data;
- **Step 2**: *In vivo* testing.

Step 2 does not need to be conducted if the chemical of interest (CoI) should be classified as skin sensitizer or corrosive, is a strong acid (pH < 2) or base (pH > 11.5), or is flammable in air at room temperature.

For hazard identification and dose response assessment, it is important to consider adequacy and completeness of the data:

- Adequacy assessment shall address reliability and relevance of the data;
- Completeness refers to the conclusions on the comparison between available adequate information and information requirements under REACH.

The conclusions rely on Weight of Evidence (WoE) approaches as categorized in REACH Annex XI section 1.2 based on methods used

- Guideline and non-guideline tests;
- 'Other types' of information justifying adaption of the standard testing regime;

Human and animal sensitization data used in REACH registrations

Human data on skin sensitization

- Epidemiological data, case reports and human experience (consumer, worker);
- Diagnostic clinical tests (e.g., patch tests; repeated open application tests);
- Confirmatory clinical or experimental studies (e.g., HRIPT, HMT).

Animal data on skin sensitization

- Mouse local lymph node assay (LLNA; OECD TG 429/442; 'REACH preferred method');
- Guinea pig maximisation or Buehler test (OECD TG 406); generation of new guinea pig testing for REACH registration purposes only if scientifically justified;
- Non-guideline tests (e.g., GP Draize or optimization test; mouse ear swelling).

Toolbox of 'alternative data' used for REACH registrations

Non-testing approaches to fill data-gaps for skin sensitization

- Reaction chemistry, metabolism, epidermal bioavailability – structure activity relationship models (e.g., DEREK, OECD Tool Box, Toxtree);
- Analogue-based assessments ('read-across');
- Chemical grouping/categories (interpolation, extrapolation)

***In vitro* approaches**

Today, no officially adopted *in vitro* test for addressing the skin sensitization endpoint exists; current approaches are being explored and used in combination:

- Chemical reactivity assays (e.g., protein binding/peptide reactivity assays (DPRA), human Cell Line Activation Test (h-CLAT);
- Cell-based assays (e.g., expression of surface markers, cytokine release);
- Epidermal bioavailability (i.e., dermal penetration is a pre-requisite for skin sensitization).

Overview of non-animal approaches under REACH

In a nutshell….all tools are being used to avoid generation of new animal data! Typical approach for addressing skin sensitization in the case substance specific animal data do NOT exist:

- **Step 1**: SAR analysis of chemical of interest (CoI);
- **Step 2**: Identification and categorization of suitable structural analogues, assessment of adequacy of analogue data and applicability of non-testing approaches (e.g., read-across, grouping)
  - '*Suitability evaluation*': based on chemical structure and reactivity (e.g., commonality of structural alerts, functional groups or double bonds), physico-chemical properties, bioavailability and metabolism
- **Step 3**: Weight of evidence analysis leading to use of a non-testing approach to meet REACH requirements or identification of further needs
  - '*Further testing*': in vitro and/or *in vivo* (LLNA or GPMT)

Case study I: Interpolation of data through grouping

Example: Surfactant class with varying alkyl chain length (i.e., Surf-C_8, Surf-C_{8-10}, Surf-C_{12}, Surf-C_{12-14}, Surf-C_{16-18})

Available data/information

- Negative LLNA for Surf-C_8, Surf-C_{12}, Surf-C_{16-18}
- Absence of structural alerts for skin sensitization and/or protein reactivity (e.g., DEREK™, OECD ToolBox);
- Surfactant group generally meets REACH grouping criteria: common constituents/chemicals and functional groups, incremental change of PC properties across category, common metabolism;
- Skin irritation potential decreases with increasing alkyl chain length (but no corrosivity of Surf-C_8);
- Long-term use of all surfactants in consumer products with significant skin contact and no market reports of skin sensitisation effects.

Conclusion: No further testing necessary; lacking data for Surf-C_{8-10} and Surf-C_{12-14}, are being read-across to existing data within the group (interpolation).

Case study II: Interpolation of data through grouping

Example: Group of complex acrylate-based UVCBs; substances are structurally similar but as a result of different starting materials and process conditions differ slightly with regard to oligomerization degree and chain length

Available data/information

- Positive LLNA studies for some, no animal data for others;
- Structural alerts for skin sensitization;
- Acrylate-based group generally meets REACH grouping criteria: common constituents/chemicals and functional groups, incremental change of PC properties across category, common metabolism;
- Substances are only mildly irritating to skin;
- No human data.

Conclusion: No further testing; all substances of the group were considered skin sensitizer and appropriate RM management measures proposed.

Case study III: Interpolation of data through grouping

Example: Polyfunctional silicone type substances slightly differing in MW, viscosity and a single functional group

Available data/information

- 4/5 substances weakly positive in the LLNA (no dose response); 5/5 substances negative in the GPMT (high dose);
- SAR analysis did not reveal a structural alert for skin sensitization for 4/5 substances; for one substance one path of reaction chemistry (activation) may explain some weak activity; neither study quality nor other chemical factors could explain the discordancy of the data;
- LLNA studies included ear thickness measurements to determine degree of irritation; except for one substance of those tested positive in the LLNA, only a very low level or irritation was determined;
- Absence of occupational allergic contact dermatitis; no in-market issues with structurally similar polyfunctional silicones used in cosmetic products.

Conclusion: WoE suggests that none of the examined silicone materials presents a skin sensitization hazard; the GPMT appears to provide more reliable results for identifying the skin sensitization hazard for this chemical class (Petry, T. *et al*., 2012).

Summary

- Consortia/SIEFs generally apply all tools and look at all suitable data that are available to address the skin sensitisation endpoint;
- Non-testing approaches such as (Q)SAR, grouping and/or read-across is extensively used to avoid unnecessary animal testing;
- Further *in vitro*, but sometimes also *in vivo,* testing may be necessary to confirm or identify the existence or absence of skin sensitisation properties;
- The type of chemistry often determines which tool is more suitable for skin sensitisation assessment. Hence, it is important to have all tools available;
- A weight of evidence approach is required for assessment of discordant skin sensitisation data sets.

References

EU, 2006. Commission Regulation (EU) No 1907/2006 of the European Parliament and Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals.
ECHA, 2013. Guidance on information requirements and chemical safety assessment. Chapter R.7a: Endpoint specific guidance. Version 2.1 August 2013.
Petry, T. *et al*., 2012. An Assessment of the Skin Sensitization Hazard of a group of Polyfunctional Silicones using a Weight of Evidence Approach. Regulatory Toxicology and Pharmacology 64, 305-314.