Skin Sensitization
In Silico Protocol

Glenn J. Myatt
VP, Informatics
Instem
**In silico toxicology protocols**

- Equivalent to *in vivo* or *in vitro* test guidelines
- Developed through an international cross-industry consortium
- Individual working group set-up per major toxicological endpoint
- These protocols help ensure any assessment is performed in a transparent, accepted, consistent, documented and repeatable manner
- They incorporate:
  1. Best practices in computational toxicology
  2. The current science in assessing toxicity weight of the evidence (as encoded in AOPs, IATAs, and so on)
In silico toxicology protocol framework - leveraging existing work

Test guidelines
- Experimental data

In silico results
- OECD QSAR Validation Principles
- QMRF

Best practices

In silico toxicology protocol - hazard assessment

- Effects or Mechanisms
  - Assessment Reliability Score
  - Assessment Confidence

- Endpoints
  - Assessment Reliability Score
  - Assessment Confidence

Rules/principles for combining information
- Expert review guidelines
- Literature
- Documentation guideline
- QPRF

Industry/regulation specific
- Overall assessment of specific toxicity
- Confidence in assessment

Confidence in risk assessment

AOPs, IATAs, DA, NAMS,...
In silico toxicology protocol framework outline

Effects/mechanisms

- **Effect/mechanism -1**
  - Experimental data
  - *In silico* prediction
  - Statistical model
  - Expert alerts model
  - Read-across

- **Effect/mechanism -2**
  - Experimental data
  - *In silico* prediction
  - Statistical model
  - Expert alerts model
  - Read-across

- **Effect/mechanism -3**
  - Experimental data
  - *In silico* prediction
  - Statistical model
  - Expert alerts model
  - Read-across

... ...

Assessment of effects/mechanisms

- **Effect/mechanism -1**
  - Assessment
  - Reliability score

- **Effect/mechanism -2**
  - Assessment
  - Reliability score

- **Effect/mechanism -3**
  - Assessment
  - Reliability score

... ...

Assessment of sub-endpoints

- **Sub-endpoint -1**
  - Assessment
  - Confidence

- **Sub-endpoint -2**
  - Assessment
  - Confidence

- **Sub-endpoint -3**
  - Assessment
  - Confidence

Assessment of overall endpoint

- Overall endpoint
  - Assessment
  - Confidence
Skin sensitization

Skin sensitization in silico protocol

Protein Reactivity
DPRA results
ADRA results

Potency: Kinetic DPRA results

Reaction domain*

Skin metabolism*

Activation of biochemical pathways (Nrf2-ARE pathway)
KeratinoSens™ results
LSens results

Pathway associated with gene expression

Pathway associated with protein expression

Release of pro-inflammatory mediators

Expression of co-stimulatory and adhesion molecules
h-CLAT results
U-SENS™ results

Gene expression pathways
IL-8 Luc assay results

Protein expression pathways

Human T cell proliferation

Covalent interaction with skin proteins (KE1)

Events in Keratinocytes (KE2)

Phys-chem properties (molecular weight, solubility, log Kow, vapor pressure, melting/boiling point)

Pathway associated with gene expression

Pathway associated with protein expression

Events in Dendritic cells (KE3)

Events in human lymphocytes (KE4)

Human skin sensitization
HMT results
HRIP results
Clinical/Occupational results

Skin sensitization in vitro

Skin sensitization in humans

Photoallergy in vivo

Events in rodents lymphocytes (KE4)

Skin irritation

Rodent local lymph node proliferation
LLNA results

Rodent maximization
GPMT results
BT results

* considered in the assessment of KE1-3
a: can support the applicability domain assessment of the assays
Reviewing the results
Effects or Mechanisms (e.g., protein reactivity, activation of Nrf2-ARE, ...)

Reviewing the results

Experimental data

In silico results
Reviewing the results

Generating an assessment and documenting the reliability of the information

bis-GMA
Generating an overall assessment for sub-endpoints (e.g., covalent interaction with skin proteins (KE1)) alongside a confidence score.
Generating an overall assessment for the major endpoint (e.g., skin sensitization in humans) alongside a confidence score.
Reviewing the results
Reliability score

Reviewing the results
**Relevance and Completeness**

**Relevance:** *In silico* toxicology protocols consider the relevance of experimental study data or *in silico* results (i.e., usefulness for predicting the toxicological endpoint of interest, such as acute oral toxicity in humans)

**Completeness:** Invariably, information will not be available for all effects/mechanisms outlined in the protocol. The overall confidence in any assessment may be reduced when critical information is missing.
Confidence is established based on the weight-of-evidence*, incorporating reliability, relevance and completeness:

- A **high confidence** rating suggests that the assessment is likely to be true and that further research is unlikely to diminish its confidence.
- A **medium confidence** rating suggests that the assessment is likely to be true, but that further research might change its confidence.
- A **low confidence** rating suggests that further research is needed in order to improve its confidence. While regulatory submissions are not recommended, the low confidence rating could be useful for prioritization, and to determine data gaps.
- A **no confidence** rating suggests that further research is needed in order to derive an assessment.

* Each protocol will include rules/principles to generate confidence
Reviewing the results
Expert review

**Expression of co-stimulatory and adhesion molecules**

**Experimental data**
Negative (Reliability score = R55)

**Predictions**
Statistical model = Positive (Reliability score = R55)
The skin sensitization protocol outlines factors that could lead to false negative results in the h-CLAT experimental system and discusses the exclusion of chemicals with a Log P value greater than 3.5 from the applicability domain of the h-CLAT test.

Expert review

Access to structural features, and training set examples which map to those features and underlying information, probabilities, and feature coverage. This facilitates an expert review.

Presents the required information for examination of reliability and documents expert review findings.
Importance of documentation

- Supports internal or external decision-making
- Important to document both the model and the results of applying the model
- Include information with standard report, e.g., title page, executive summary, purpose, materials and methods, results of the analysis, conclusions, references, appendices
CONCLUSIONS

**Initiative developed to support toxicologists and regulators**
- Transparent and defendable protocol for performing such assessments
- Supports mutual acceptance of data

**Incomplete package of experimental data and/or in silico results**
- Possible to generate an overall assessment based on available information
- Associated confidence to determine whether the conclusion is sufficiently robust
  - Regulatory purposes (i.e., requiring a high level of confidence)
  - Prioritization or screening (i.e., scenarios that are tolerant of a lower level of confidence)
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In silico toxicity protocols


In silico skin sensitization


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Thank you

Glenn J. Myatt

glenn.myatt@instem.com
References
