WEBINAR ON TESTING AND ASSESSMENT METHODOLOGIES

DEFINED APPROACHES FOR SKIN SENSITISATION

When: Wednesday 16 January 2019
1 pm (Paris time) / 7 am (EST)

The OECD is currently evaluating the utility of combination of methods to predict skin sensitisation potential of chemicals.
General workflow in Integrated Approaches to Testing and Assessment (IATA)

**Problem formulation**

**Gather existing information**

**Weight of Evidence Assessment:**
Adequate information for decision-making?

**Generate additional information**

**Weight of Evidence assessment:**
Adequate information for decision-making?

**Regulatory conclusion**

AOP

Multiple strategies e.g. in house data, mining of relevant data bases, literature search

Expert Judgement
Types of IATA – A Continuum

Flexible
Judgement-Based

Prescriptive
Rules-Based

Non-formalised approaches

- e.g. grouping and read-across

Structured approaches

- e.g. Integrated Testing Strategy
Defined Approaches

A **defined approach** to testing and assessment consists of a **fixed data interpretation procedure (DIP)** used to interpret data generated with a **defined set of information sources**, that can either be used alone or together with other information sources, to satisfy a specific regulatory need.

**OECD Series on Testing and Assessment**:
http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

- **Guidance Document Nº 255** on the Reporting of Defined Approaches to be Used within Integrated Approaches to Testing and Assessment.

- **Guidance Document Nº 256** on the Reporting of Defined Approaches and Individual Information Sources to be Used within Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitisation.
  - Includes **12 skin sensitisation case studies**
Using AOPs to develop novel testing and assessment approaches

Guidance Document Nº 260

http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm
Skin Sensitisation is an example of how OECD tools can be combined

- **AOP** for skin sensitisation ([https://aopkb.oecd.org/](https://aopkb.oecd.org/))
  - Provides a mechanistic basis including a molecular initiating event (MIE) and all key events (KE) leading to AO
  - Testable events

- **Test Guidelines** for evaluating skin sensitisation ([https://doi.org/10.1787/20745788](https://doi.org/10.1787/20745788))
  - In chemico/ in vitro TG
  - In vivo TG

- **QSARs** for skin sensitisation ([https://www.qsartoolbox.org/](https://www.qsartoolbox.org/))
  - The OECD Toolbox includes predictions based on profilers (e.g. protein binding) and also now includes the AOP
Defined Approaches can be developed based on AOPs: e.g. Skin sensitisation

- Chemical Structure/Properties
- MIE
- Cellular Level
- Tissue Level
- Organ Level

Electrophilic Chemicals → Covalent Protein Binding to Skin Proteins → Keratinocyte Activation → Dendritic Cell Activation → T-cell Activation and Proliferation → Skin Sensitisation

Expert Judgement

In vitro skin absorption (TG 428)

In vitro T cell priming/proliferation

Guinea Pig Maximisation Test

Local Lymph Node Assay

Buehler Test

In silico toxicokinetic models (TG 442C DPRA)

QSARs

TG 442D (ARE-Nrf2 luciferase test method, KeratinoSens™)

RhE IL-18

LuSens

Defined Approaches can be developed based on AOPs: e.g. Skin sensitisation

https://aopwiki.org/wiki/index.php/Aop:40
OECD Test Guidelines Programme Project 4.116 added to 2017 work plan
– JRC/US/Canada co-leads

Develop a Guideline on Defined Approaches (DA) for skin sensitisation
– Aims to substitute the need for animal testing for skin sensitisation based on a combination of methods which, individually, predict key event responses on the AOP
– DA will be evaluated based on their performance against the LLNA and human data sets/reference chemicals
– Resulting instrument will be amenable to the agreement on Mutual Acceptance of Data (MAD)

To meet regulatory requirements:
– DAs that discriminate skin sensitisers from non sensitisers (hazard ID)
– DAs that discriminate strong from moderate sensitisers (GHS potency)
– DAs that provide continuous quantitative measures used in risk assessment
GL for DASS will meet regulatory needs

- Cosmetics Regulation (Regulation (EC) No 1223/2009) prohibits animal testing and use of animal data

- Currently, OECD in vitro TGs are not considered stand-alone replacements for the animal tests
  - Industry is already submitting combinations of in vitro/in silico methods to meet regulatory requirements
    - there are not defined combinations that are officially endorsed replacements for animal data
  - Regulators are in a bind
    - Not currently GLs on what to accept or how to interpret the adequacy of the replacement data
Skin sensitisation: Cosmetics Europe database

Non-animal methods to predict skin sensitization (I): the Cosmetics Europe database

Non-animal methods to predict skin sensitization (II): an assessment of defined approaches
Skin sensitisation data streams in CE project

- 17 In vitro test methods
- 7 OECD validated methods
- In silico predictions
  - OECD QSAR TB
- GL in vivo studies
  - 3 OECD TG
  - Most data in LLNA

All data types available for 128 chemicals = CE Database
Development of a GL on Defined Approaches for Skin Sensitisation

- Project 4.116 added to 2017 work plan
  - JRC/US/Canada co-leads
- **Dec 2017**: Special session of the WNT to discuss project
Steps following Dec 2017 WNT meeting

- Convene an Expert Group for DAs for SS
  - Jan 2018: Request for nominations from WNT
    - 52 expert nominees representing 14 MC, BIAC, and ICAPO

- Survey to identify updates and new DAs to be considered
  - Jan 2018: Request sent to WNT and DA developers
    - 12 DAs for consideration (10 GD256 Annex 1 + 2 new)
<table>
<thead>
<tr>
<th>Define Approach</th>
<th>Bioavailability</th>
<th>Physico-chemical properties</th>
<th>In silico</th>
<th>Protein binding/reactivity</th>
<th>Events in Keratinocytes</th>
<th>Events in DC</th>
<th>Events in T cells</th>
<th>Adverse effect</th>
<th>Others</th>
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<tbody>
<tr>
<td>Sensitiser potency prediction Key event 1+2 (Givaudan)</td>
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<td>TIMES SS</td>
<td>Cor1C420-assay</td>
<td>TG 442D</td>
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<td>The artificial neural network model for predicting LLNA EC3 (Shiseido)</td>
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<td>X</td>
<td>SH Test</td>
<td>AREc32 assay</td>
<td>h-CLAT</td>
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<td>ITS/DS for hazard and potency identification of skin sensitisers (P&amp;G)</td>
<td>penetration</td>
<td>(PBPK model)</td>
<td>TIMES SS</td>
<td>TG 442C</td>
<td>TG 442D</td>
<td>h-CLAT U937 test</td>
<td>TG 429</td>
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<td>Tiered system for predicting sensitising potential and potency of a substance (STS) – (Kao Corporation)</td>
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<td>Score-based battery system for predicting sensitising potential and potency of a substance (ITS)– (Kao Corporation)</td>
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<td>IATA for skin sensitisation risk assessment (Unilever)</td>
<td>penetration</td>
<td>modified OECD TG428</td>
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<td>Weight of evidence in vitro ITS for skin hazard identification (BASF)</td>
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<td>STS for hazard identification of skin sensitisers (RIVM)</td>
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<td>IATA- (Dupont)</td>
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<td>Decision strategy (L'Oréal)</td>
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<td>Integrated decision strategy for skin sensitisation hazard (ICCVAM)</td>
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<td>Consensus model for hazard identification (EC-JRC)</td>
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## Defined approaches under consideration

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<thead>
<tr>
<th>#</th>
<th>Approach</th>
<th>Prediction</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>An Adverse Outcome Pathway-based &quot;2 out of 3&quot; integrated testing strategy approach to skin hazard identification (BASF)</td>
<td>Hazard identification</td>
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<tr>
<td>2</td>
<td>A non-testing pipeline approach for skin sensitisation (US EPA)</td>
<td>Hazard identification</td>
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<tr>
<td>3</td>
<td>Stacking meta-model for skin sensitisation hazard identification (L’Oréal)</td>
<td>Hazard identification</td>
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<td>4</td>
<td>Integrated decision strategy for skin sensitisation hazard (ICCVAM)</td>
<td>Hazard identification</td>
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<td>5</td>
<td>Consensus of classification trees for skin sensitisation hazard prediction (EC-JRC)</td>
<td>Hazard identification</td>
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<tr>
<td>6</td>
<td>Sensitizer potency prediction based on Key event 1 + 2: Combination of kinetic peptide reactivity data and KeratinoSens® data (Givaudan)</td>
<td>Potency category</td>
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<tr>
<td>7</td>
<td>The artificial neural network model for predicting LLNA EC3 (Shiseido)</td>
<td>Potency category</td>
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<tr>
<td>8</td>
<td>Sequential testing strategy (STS) for sensitising potency classification based on in chemico and in vitro data (Kao Corp)</td>
<td>Potency category</td>
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<tr>
<td>9</td>
<td>Integrated testing strategy (ITS) for sensitising potency classification based on in silico, in chemico, and in vitro data (Kao Corporation)</td>
<td>Potency category</td>
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<tr>
<td>10</td>
<td>DIP for skin allergy risk assessment (SARA) (Unilever)</td>
<td>Potency category</td>
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<tr>
<td>11</td>
<td>Decision tree integrated testing strategy with an in silico model and in chemico/in vitro data using exclusion criteria (Derek Nexus)</td>
<td>Potency category</td>
</tr>
<tr>
<td>12</td>
<td>Computational approaches for prediction skin sensitisation (US/UNC)</td>
<td>Potency category</td>
</tr>
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</table>
Steps following Dec 2017 WNT meeting

• Start with the easiest Defined Approaches to gain experience
  – “Group 1” DAs are simple, rule-based approaches that rely on OECD in vitro TG and in silico predictions
    • BASF 2 out of 3
    • Kao STS
    • Kao ITS
  – Substitute commercial software in silico predictions in DAs with predictions from OECD QSAR toolbox = Kao v2 ITS: Jun 2018

• Use an iterative approach as project progresses
  – learn from the outcome at each step

• Propose evaluation framework for DAs for SS
  – Feb 2018: Proposed criteria circulated to WNT and EG
    • Revised based on comments and finalised

• Put the evaluation framework and reporting template into practice
  – Apr 2018: Requested DASS EG reviewers to identify obstacles to MAD
Reviewers indicated no substantial omission and based on evaluation framework DAs could fulfil the criteria for inclusion in a guideline where resulting data would fall under mutual acceptance of data.
“Group 1” DAs

2 out of 3

Test Chemical
KE a
KE b
Concordant?
YES
NO
Classify based on concordance
Classify based on 2/3 concordance

Kao ITS v1

<table>
<thead>
<tr>
<th>Score</th>
<th>h-CLAT MIT</th>
<th>DPRA depletion</th>
<th>DEREK</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>≤10 µg/mL</td>
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<td>0</td>
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<td>No alert</td>
</tr>
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Potency: Total battery score
Strong: 7
Weak: 2-6
Not classified: 0-1

Kao ITS v2

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Kao STS

Test chemical
h-CLAT
Positive
MIT<10
Strong
Negative
MIT>10
Weak
DPRA
Negative
Not classified
Positive
3x3 Contingency Tables: DA GHS potency categories predictions of LLNA response*

<table>
<thead>
<tr>
<th></th>
<th>LLNA</th>
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<td>NC</td>
<td>1B</td>
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<td>Kao IT Sv1</td>
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<td>NS (NC)</td>
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<td>11</td>
<td>weak/mod. (1B)</td>
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<td>strong / extr. (1A)</td>
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<td>n=121</td>
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*LLNA reference data under consideration and subject to change.
Development of a GL on Defined Approaches for Skin Sensitisation

- **Project 4.116 added to 2017 work plan**
  - JRC/US/Canada co-leads
- **Dec 2017:** Special session of the WNT to discuss project
- **Oct 2018:** First draft of a GL and Supporting Document circulated for review/comments
- **Nov 2018:** Special session of the IATA case study project/QSAR TB Management Group
- **Dec 2018:** DASS EG meeting
Guideline ~30 pages
• Focuses on implementation of DAs
• Clearly distinguishes between
  – DAs for hazard ID
  – DAs for potency classification

Supporting document ~140 pages
• Similar to validation report
• Focuses on analyses of reproducibility, uncertainty, applicability domain, predictive performance
• Previously published information and new information on DAs available in a single document
Input from DASS Expert Group in Dec 2018

- **Technical limitations** need to be well described for each DA
  - e.g. not suitable for chemicals that are insoluble, highly cytotoxic, pre-/pro-haptens, metals, etc.

- **Reproducibility** of the DAs (not just individual elements) needs to be quantified

- **Applicability domain** of the DAs should be well defined
  - Defined differently for QSARs and in vitro methods

- **Quality assurance** of in silico data
  - Transparency and reproducibility to meet the standards of MAD
“Group 1” DAs retained for GL

2 out of 3

Test Chemical
KE a
KE b
Concordant?
YES
Classify based on concordance
NO
KE c
Classify based on 2/3 concordance

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Agreed on evaluation framework
Engaged EG to evaluate suitability of evaluation framework – no indications information/criteria were missing
Substituted open source for proprietary in silico predictions
Analysed all false positive–false negatives across DAs (hazard ID and potency categories)
Analysed uncertainty in animal reference data
Analysed the effects of uncertainty in in vitro assays through DA
Developed an approach to describe the applicability domain of DAs
Engaged QSAR EG to provide feedback
  Discussed how in silico data could be covered under MAD (description of workflow and reporting of prediction)
Next Steps

• Implement recommendation from Dec 2018 meeting of DASS EG to revise GL and supporting document
• Circulate to DASS EG for input
• Circulate to WNT for 2nd commenting round
  – Request input on timeline for GL
Additional efforts by co-leads in 2018/19: Expanding Substance Space Coverage

- US NTP is generating additional in vitro data:
  - DPRA, KeratinoSens™, hCLAT

- Additional chemicals include:
  - pesticide/agrochemical formulations, dermal excipients, personal care product products, “challenging” chemicals
  - 235 substances procured, testing underway
CE Chemical Use Space Coverage

U.S. EPA ACToR UseDB Categories

Average of 4.3 use cases per substance
CE Chemical PhysChem Space Coverage

PCA plot using six physicochemical properties:

- MW
- LogP
- LogS
- MP
- BP
- LogVP

**CE: 122 chemicals**

**Tox21: 8272 chemicals**

(combination of various regulatory lists of international importance)
Regulatory Progress

  - Applies to pesticide active ingredients, inerts, and single chemicals regulated under amended TSCA
  - Two Group I DAs accepted: “AOP 2 out of 3” and “KE 3/1 STS” in lieu of animal test
  - Includes other assays covered by the respective KE-based OECD TGs
  - Policy will be updated to accept more DAs as the OECD work progresses

[https://www.epa.gov/pesticides/epa-releases-draft-policy-reduce-animal-testing-skin-sensitization](https://www.epa.gov/pesticides/epa-releases-draft-policy-reduce-animal-testing-skin-sensitization)
Thank you!

General information on OECD testing of chemicals

http://www.oecd.org/chemicalsafety/testing/

New draft documents as they become available

http://www.oecd.org/env/ehs/testing/oecdguidelinesforthe-testing-of-chemicals.htm

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