In silico toxicology protocols – defining standards and best practices towards mutual acceptance of data

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Agenda

- Background
- *In silico* toxicology protocol framework
- Worked example
- Discussion

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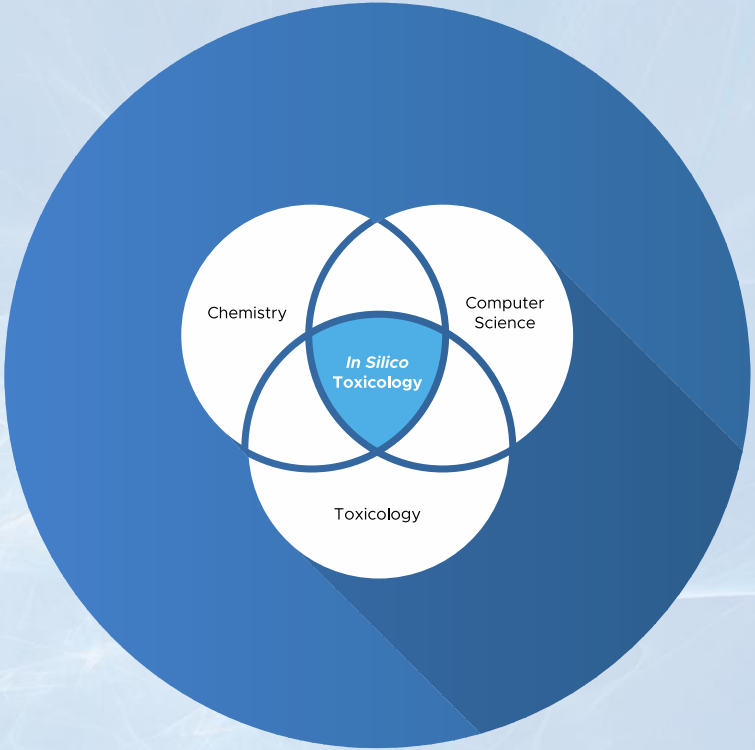
In silico toxicology

- *In silico* toxicology is used to make rapid predictions of the toxicity, generally based on the chemical structure alone



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Supporting regulatory guidelines

- The European Union's REACH regulation
- ICH M7 guideline ("Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk")
- This list of guidelines supporting regulatory applications is increasing

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Supporting chemical R&D

- **Discovery phase**: to prioritize candidates and design around potential toxicity
- **Pre-clinical assessment**: they are often used to develop testing strategies based on any predicted toxicity
- **Quality**: ensure the safety of impurities, degradants, metabolites, and excipients
- **Manufacturing**: to support worker safety, cleaning and transportation considerations

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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 ensure any assessment is performed in a transparent, accepted, consistent, documented and repeatable manner
- They incorporate:
  1. Best practices in computational toxicology, alongside
  2. The current science in assessing toxicity weight of the evidence (as encoded in AOPs, IATAs, and so on)
- This ensures good *in silico* processes and principles are adopted in the prediction of specific toxicological endpoints
- This supports the mutual acceptance of data

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IN SILICO TOXICOLOGY PROTOCOL FRAMEWORK

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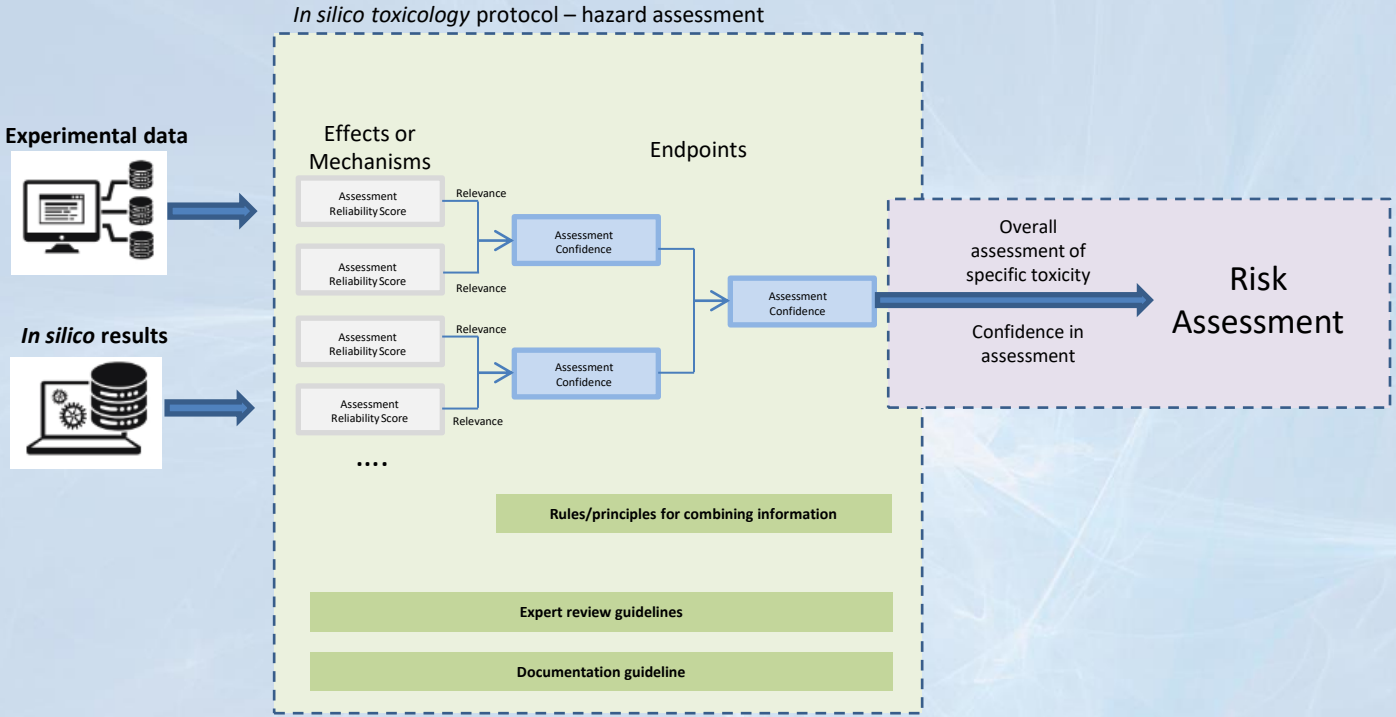
In silico toxicology protocol framework



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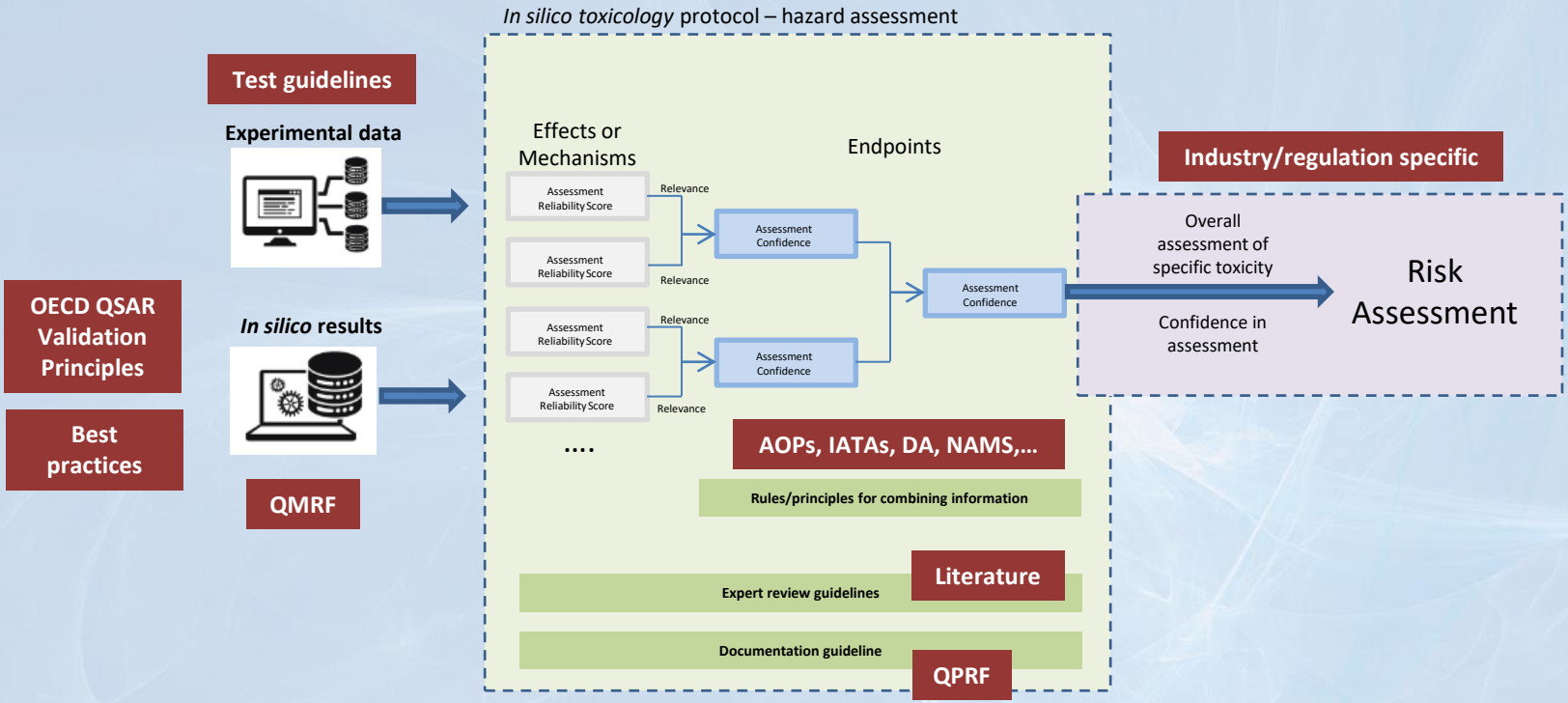
In silico toxicology protocol framework – leveraging existing work



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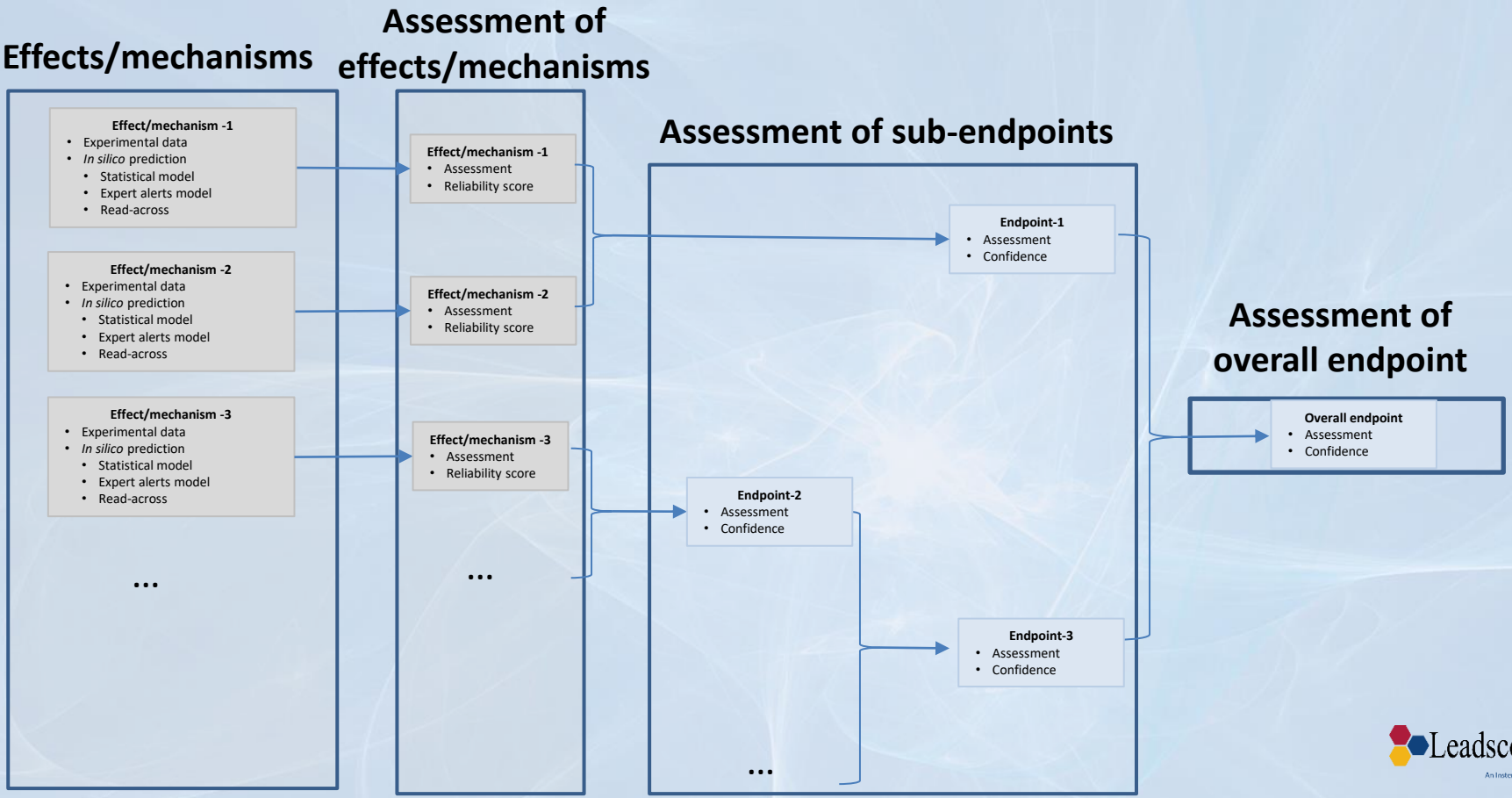
In silico toxicology protocol framework outline



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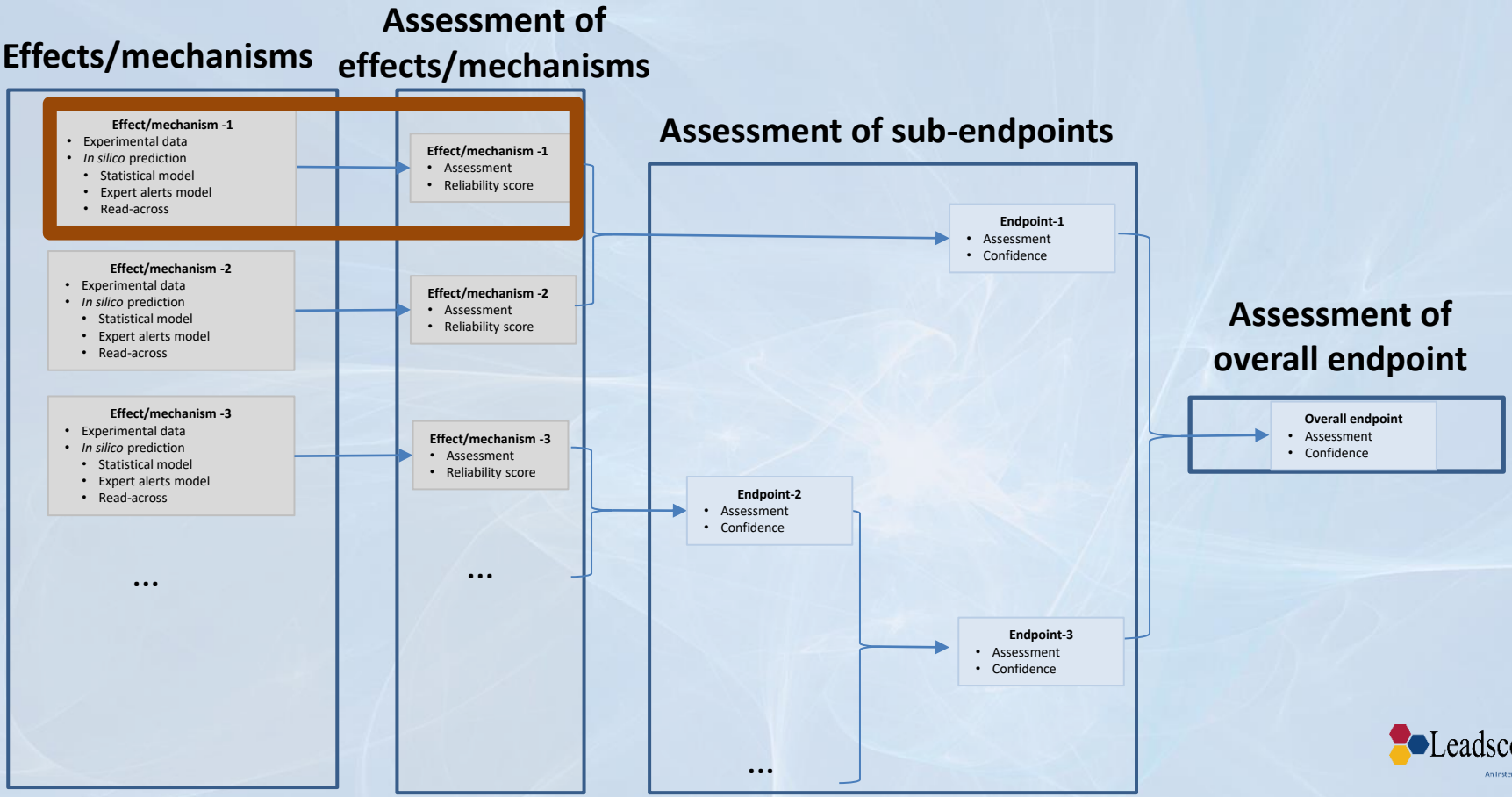
In silico toxicology protocol framework outline



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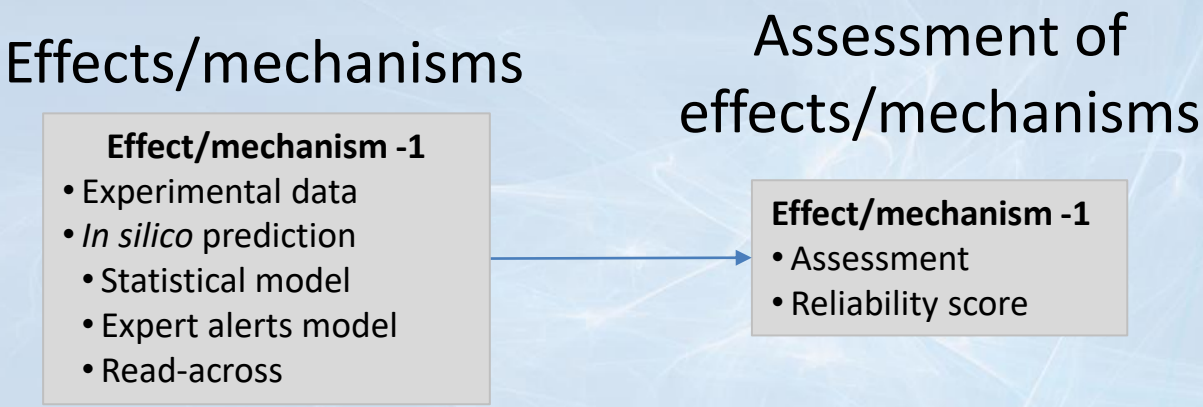
Assessment of effects/mechanisms



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Reliability score

Reliability of toxicity assessments based on computational models and experimental data (Myatt et al., 2018).

Reliability Score	Klimisch Score	Description	Summary
1	1	Data reliable without restriction	• Well documented and accepted study or data from the literature • Performed according to valid and/or accepted test guidelines (e.g., OECD) • Preferably performed according to good laboratory practices (GLP)
2	2	Data reliable with restriction	• Well documented and sufficient • Primarily not performed according to GLP • Partially complies with test guideline
3	–	Expert Review	• Read-across • Expert review of *in silico* result(s) and/or Klimisch 3 or 4 data
4	–	Multiple concurring prediction results	
5	–	Single acceptable *in silico* result	
5	3	Data not reliable	• Inferences between the measuring system and test substance • Test system not relevant to exposure • Method not acceptable for the endpoint • Not sufficiently documented for an expert review
5	4	Data not assignable	• Lack of experimental details • Referenced from short abstract or secondary literature

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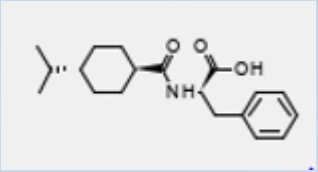
Assessment of effects/mechanisms example



Bacterial mutation

Experimental data

Negative (Reliability score = RS1)

Predictions

Statistical model = Negative (Reliability score = RS5)
Expert alerts = Negative (Reliability score = RS5)

Assessment

Bacterial mutation

Assessment = Negative
Reliability score = RS1

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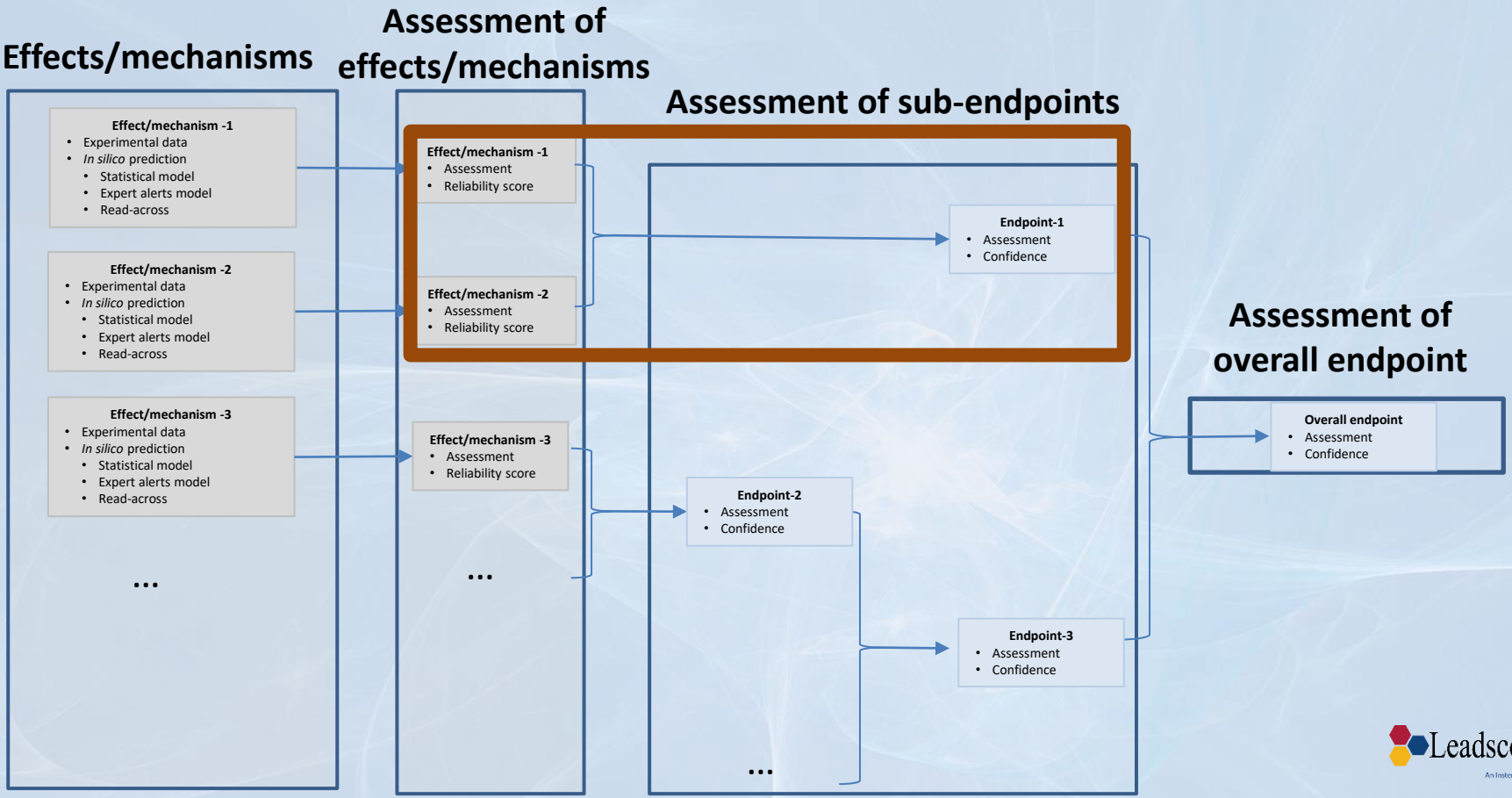
In silico toxicology protocol framework outline



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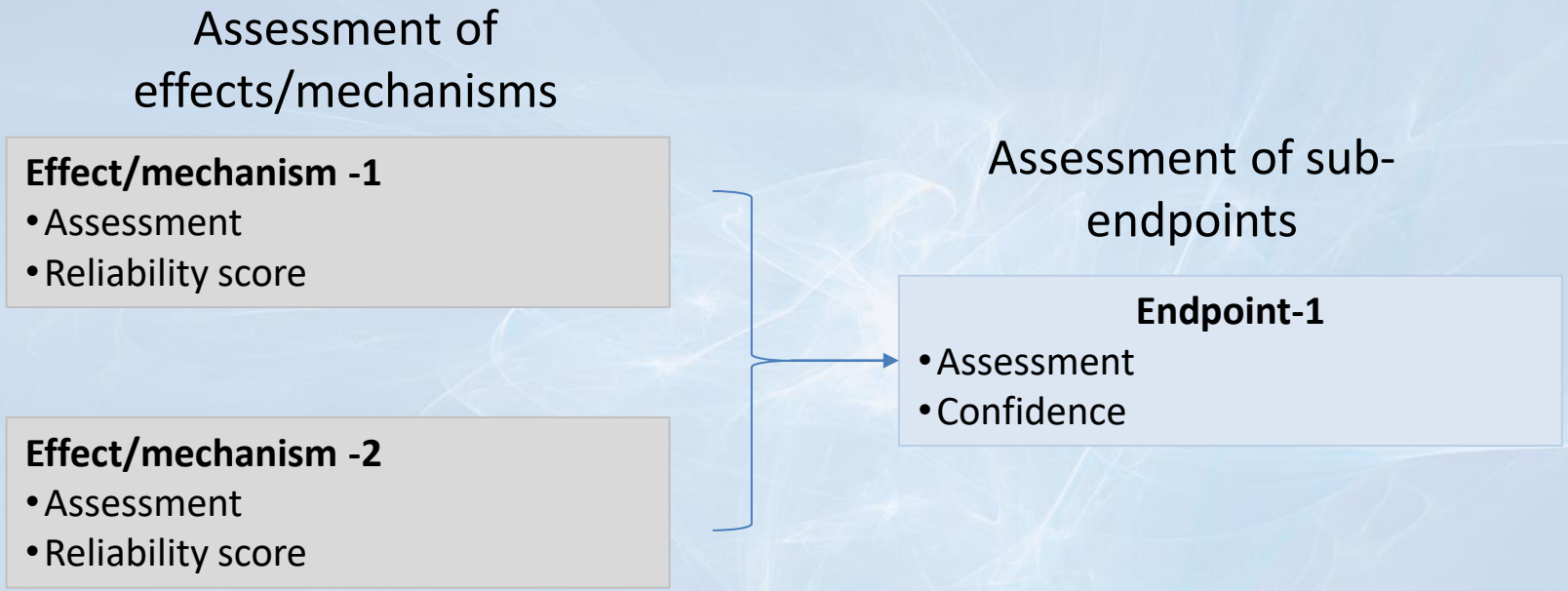
Assessment of sub-endpoints



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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

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.

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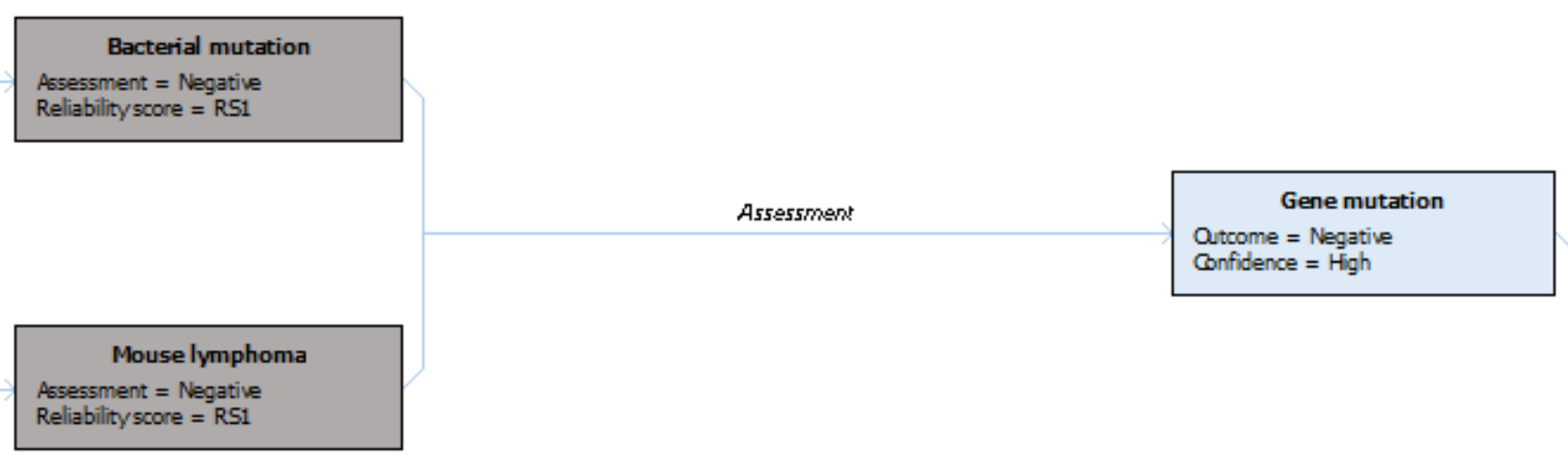
* Each protocol will include rules/principles to generate confidence

Assessment of sub-endpoint example



- The genetic toxicology *in silico* protocol included a series of rules for deriving the confidence score
- This included a rule to assign the endpoint to a high confidence if “both outcomes are negative with RS ≤ 2”

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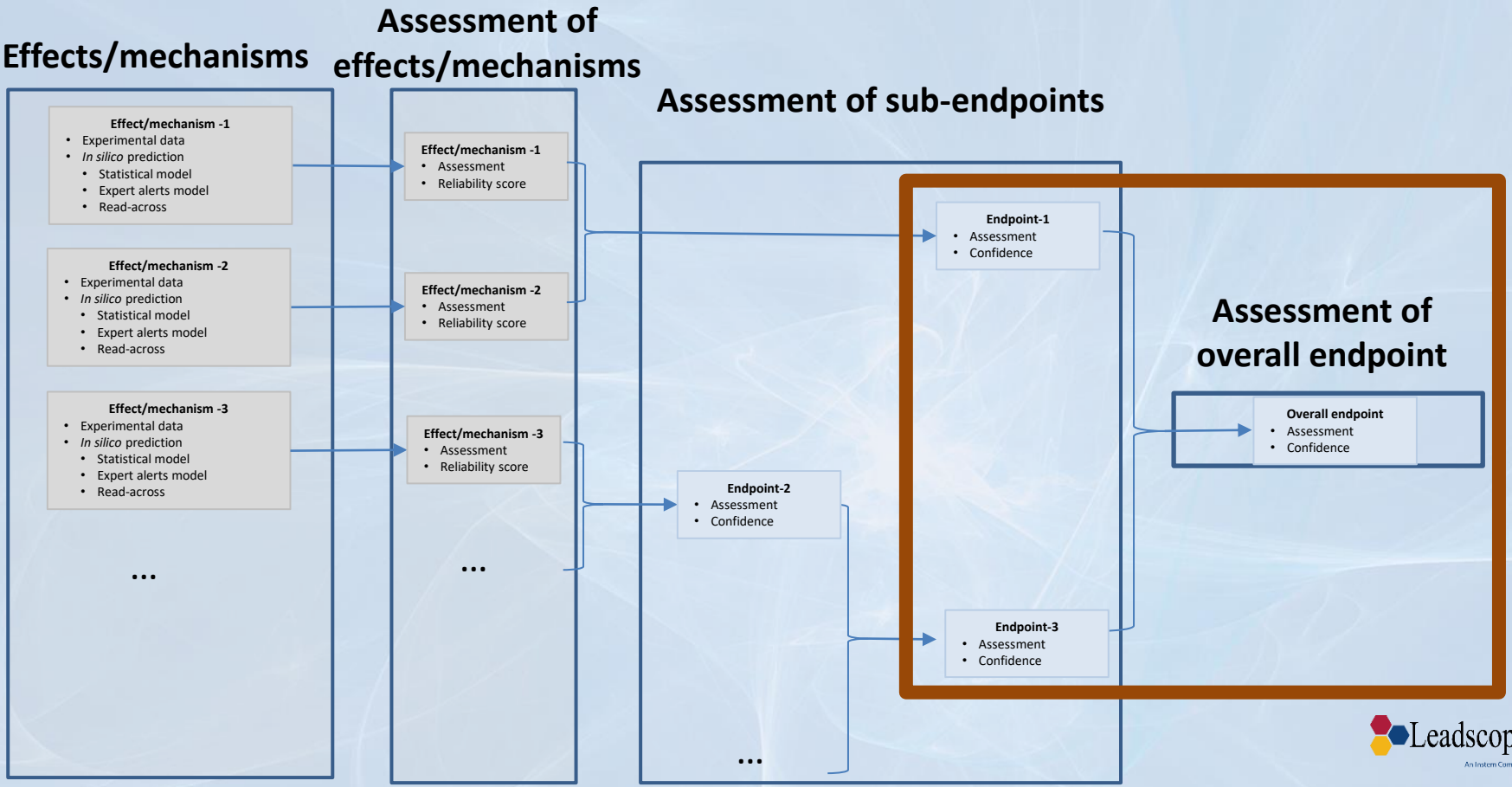
In silico toxicology protocol framework outline



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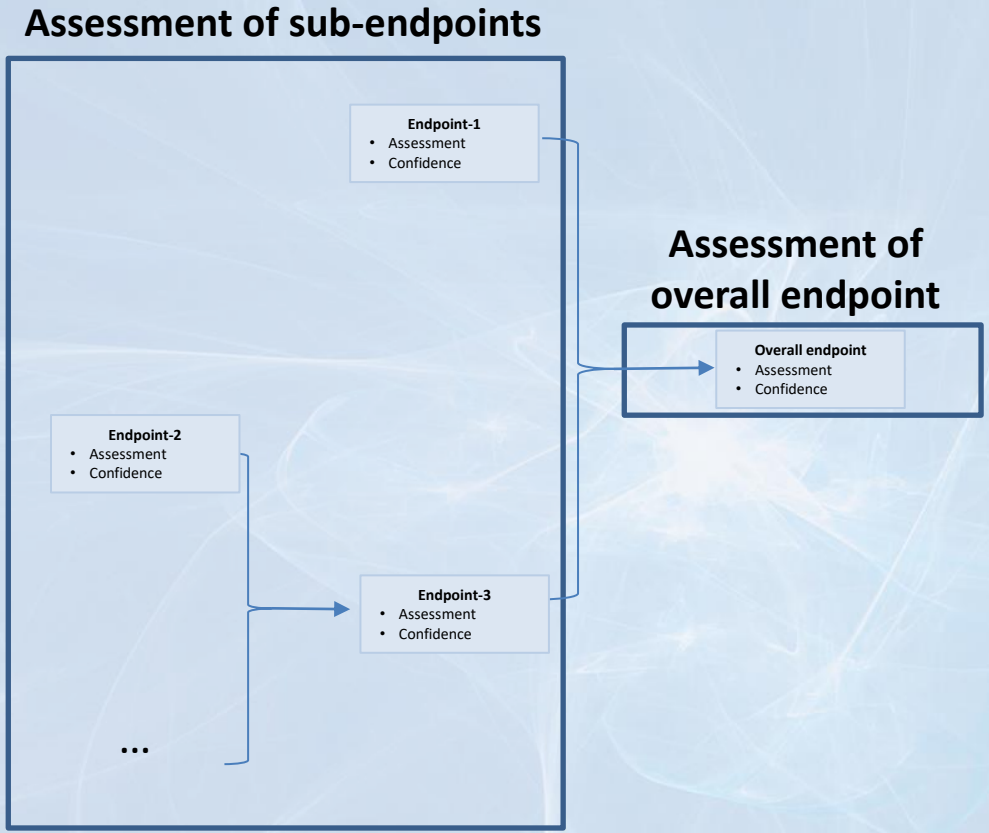
Assessment of overall endpoint



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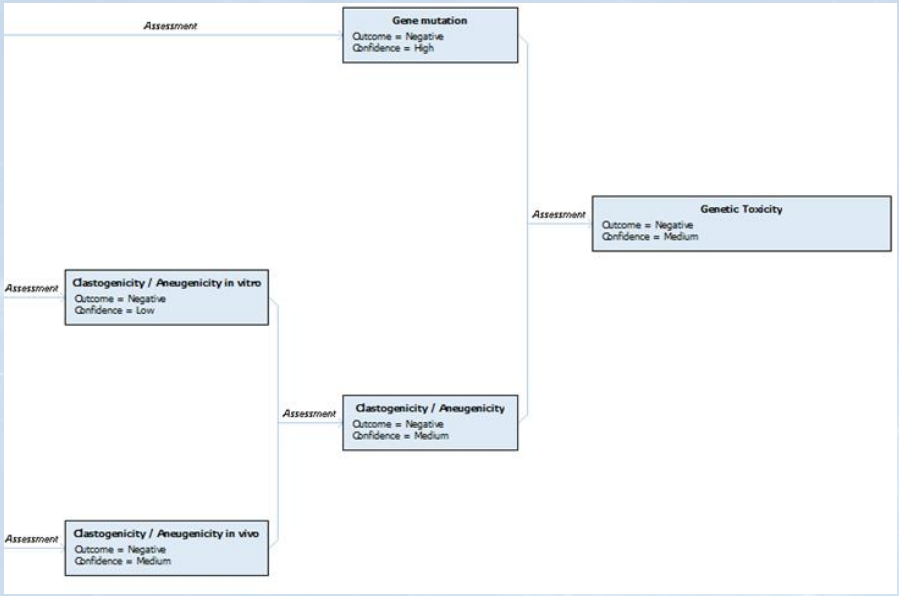
Assessment of overall endpoint example



- In this example, since both sub-endpoints were negative, the overall genetic toxicity endpoint is determined to be negative
- The confidence level is based on the rule for assessing “Genetic Toxicity” - “One with High and one with Medium confidence” to assign a medium confidence level

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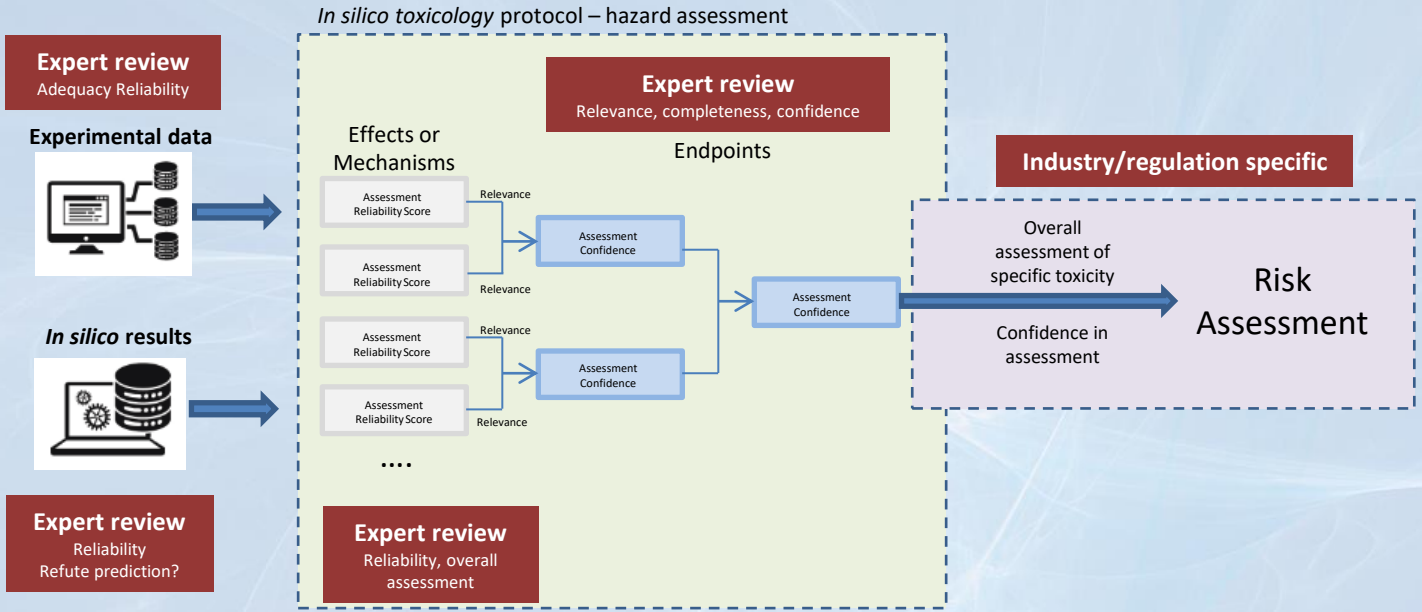
Expert review



Importance of documentation

Table 6
Elements of an *in silico* toxicology report (QMRF = QSAR Model Reporting Format).

Section	Content
Title page	- Title (including information on the decision context) - Who generated the report and from which organization - Who performed the *in silico* analysis and/or expert review, including their organization - Date when this analysis was performed - Who the analysis was conducted for
Executive summary	- Provide a summary of the study - Describe the toxicity or properties being predicted - Include a table or summary showing the following: ● The chemical(s) analyzed ● Summary of *in silico* results, reviewed experimental data and overall assessment for each toxicological effect or mechanism ● Summary of toxicological endpoint assessment and confidence ● Summary of supporting information
Purpose	- Specification of the problem formulation
Materials and methods	- QSAR model(s), expert alerts, and other models used with version number(s) and any parameters set as part of the prediction (e.g., QMRF format) - Databases searched with version number(s) - Tools used as part of any read-across with version number(s)
Results of Analysis	- Details of the results and expert review of the *in silico* models and any experimental data, including results of the applicability domain analysis - Report of any read-across analysis, including source analogs and read-across justifications
Conclusion	- Summarize the overall analysis including experimental data, *in silico* methods and expert review - Final prediction that is based on expert judgment
References	- Complete bibliographic information or links to this information, including test guidelines referred to in the experimental data, etc.
Appendices (optional)	- Full (or summary) study reports used or links to the report, detailed (or summary) *in silico* reports, reports on the models used (e.g., QMRF reports)

From Hasselgren et al. (2019) Genetic toxicology in silico protocol. Regulatory Toxicology and Pharmacology 107, 104403. doi:10.1016/j.yrtph.2019.104403

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WORKED EXAMPLE

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Skin sensitization

Regulatory Toxicology and Pharmacology 116 (2020) 104688

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Skin sensitization *in silico* protocol

Candice Johnson[a,*], Ernst Ahlberg[b], Lennart T. Anger[c], Lisa Beilke[d], Romualdo Benigni[e], Joel Bercu[f], Sol Bobst[g], David Bower[h], Alessandro Brigo[b], Sarah Campbell[i], Mark T.D. Cronin[j], Ian Crooks[k], Kevin P. Cross[a], Tatyana Doktorova[l], Thomas Exner[l], David Faulkner[m], Ian M. Fearon[n], Markus Fehr[o], Shayne C. Gad[p], Véronique Gervais[q], Amanda Giddings[r], Susanne Glowienke[s], Barry Hardy[t], Catrin Hasselgren[u], Jedd Hillegass[v], Robert Jolly[w], Eckart Krupp[x], Liat Lomnitski[w], Jason Magby[y], Jordi Mestres[z], Lawrence Milchak[a], Scott Miller[a], Wolfgang Muster[ab], Louise Neilson[ac], Rahul Parakhia[ad], Alexis Parenty[z], Patricia Parris[ae], Alexandre Paulino[af], Ana Theresa Paulino[af], David W. Roberts[j], Harald Schlecker[ag], Reinhard Stidl[ah], Diana Suarez-Rodriguez[ai], David T. Szabo[aj], Raymond R. Tice[ak], Daniel Urbisch[al], Anna Vuorinen[s], Brian Wall[a], Thibaud Weiler[a], Angela T. White[r], Jessica Whritenour[ak], Joerg Wichard[am], David Woolley[an], Craig Zwickl[aom], Glenn J. Myatt[a]

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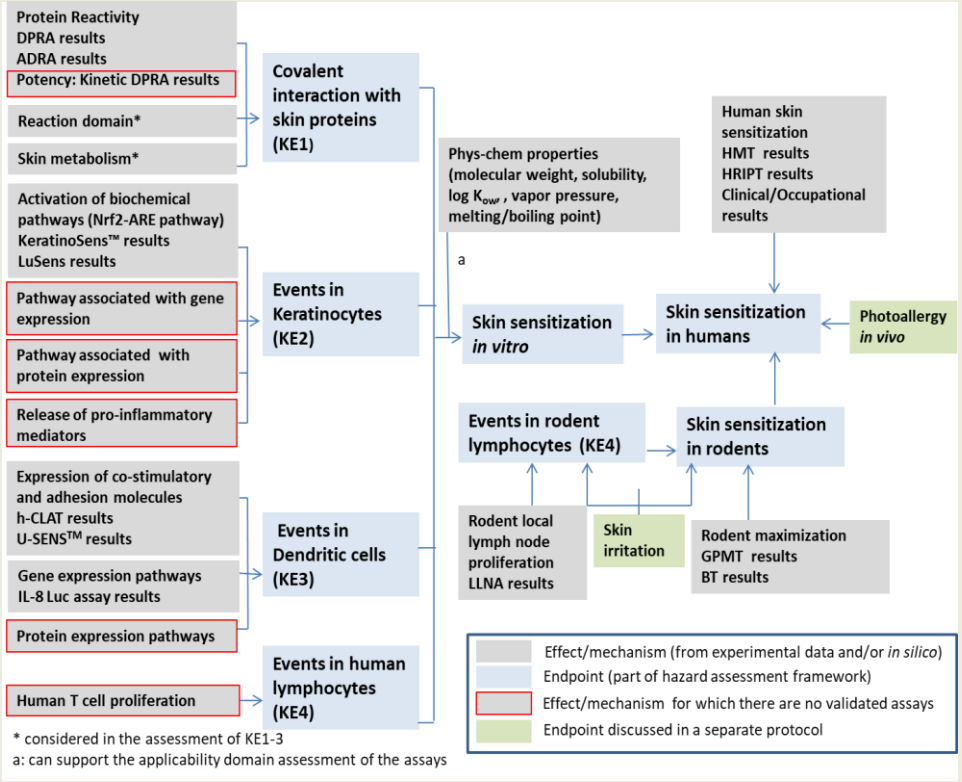
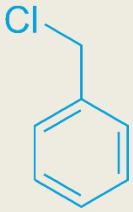
[g] Texas Advisors LLC, 2016 Main Suite 1901, Houston, TX, USA
[h] Roche Pharmaceutical Research & Early Development, Pharmaceutical Sciences, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Grenzacherstrasse 124, 4070, Basel, Switzerland
[i] Nelson Laboratories, LLC, 6280 South Redwood Road, Salt Lake City, UT, 84123, USA

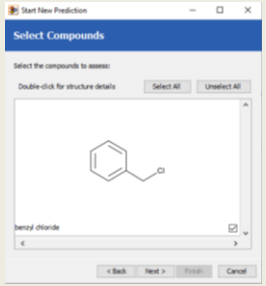




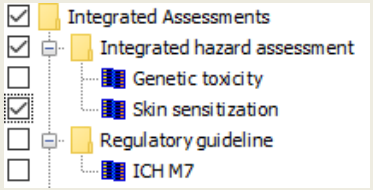
Benzyl Chloride

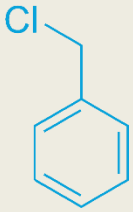
Is benzyl chloride a skin sensitizer?

Select the chemical



Select the skin sensitization





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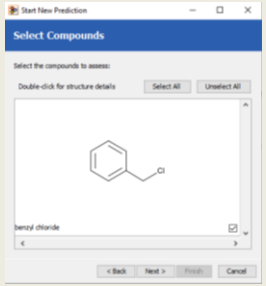
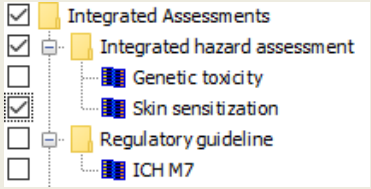


Select the skin sensitization

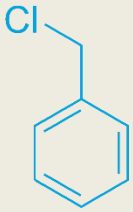


Integrated hazard assessment | **Individual models**

	Integrated hazard assessment: Skin Sensitization					
Structure	Skin Sensitization in Humans assessment	Skin Sensitization in Humans confidence	Skin Sensitization in Rodents assessment	Skin Sensitization in Rodents confidence	Skin Sensitization in vitro assessment	Skin Sensitization in vitro confidence
 benzyl chloride	Positive	Medium	Positive	Medium	Positive	Medium

Explain... Export Table...

Results based on methodology outlined in the *in silico* protocol



Benzyl Chloride

How was this assessment made?

Integrated hazard assessment | **Individual models**

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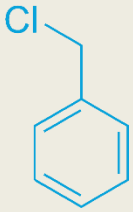
Explain... Export Table...



Benzyl Chloride

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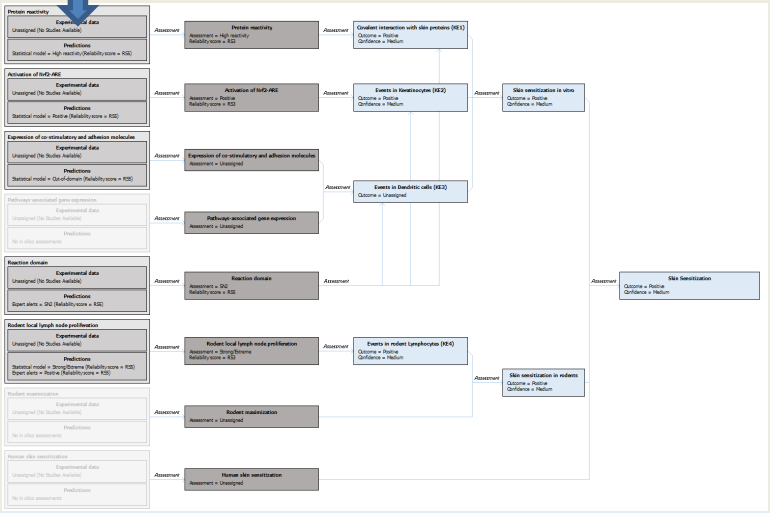
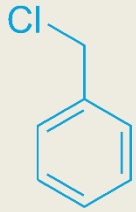
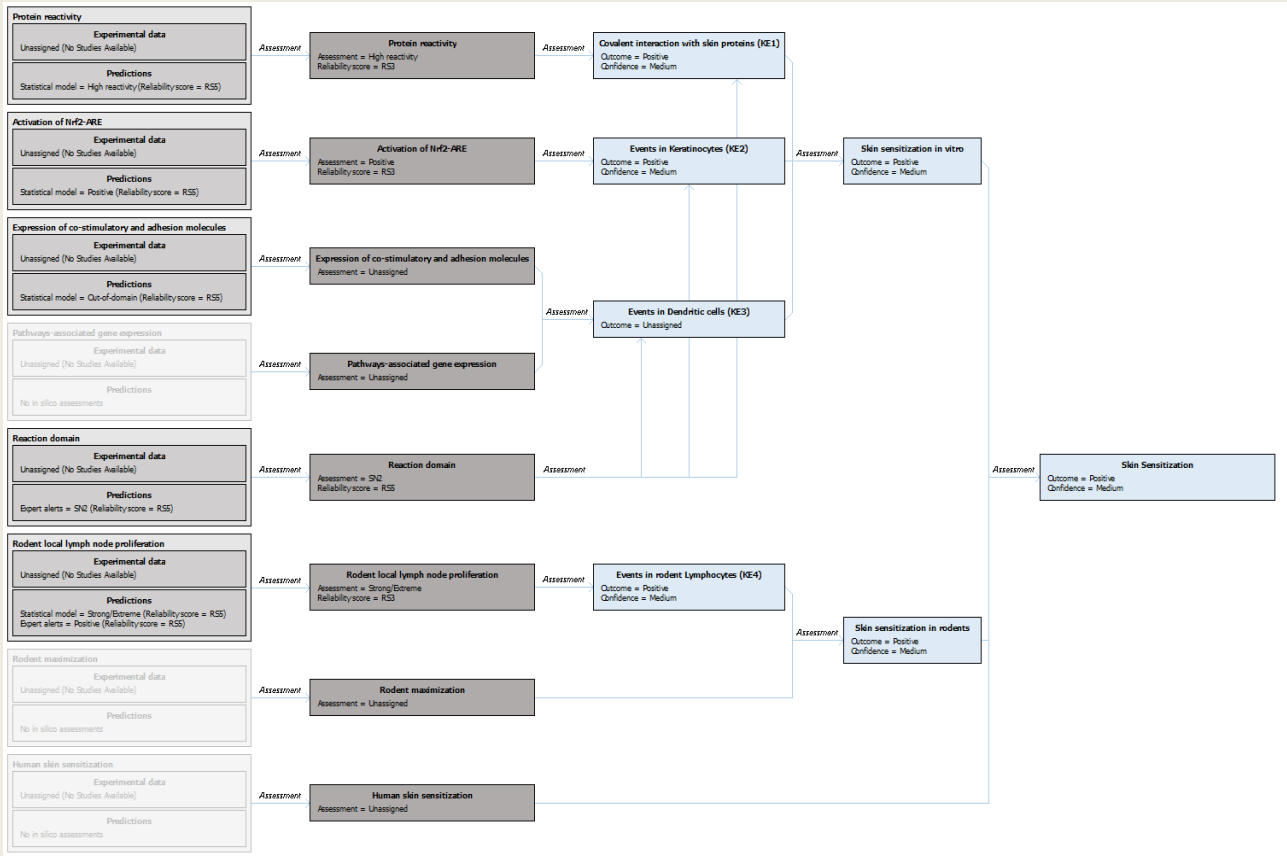


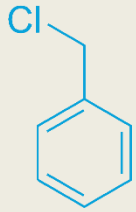
Hazard assessment framework (defined in the protocol) – describes how experimental data and/or *in silico* results are used in the prediction



Benzyl Chloride

Reviewing the results



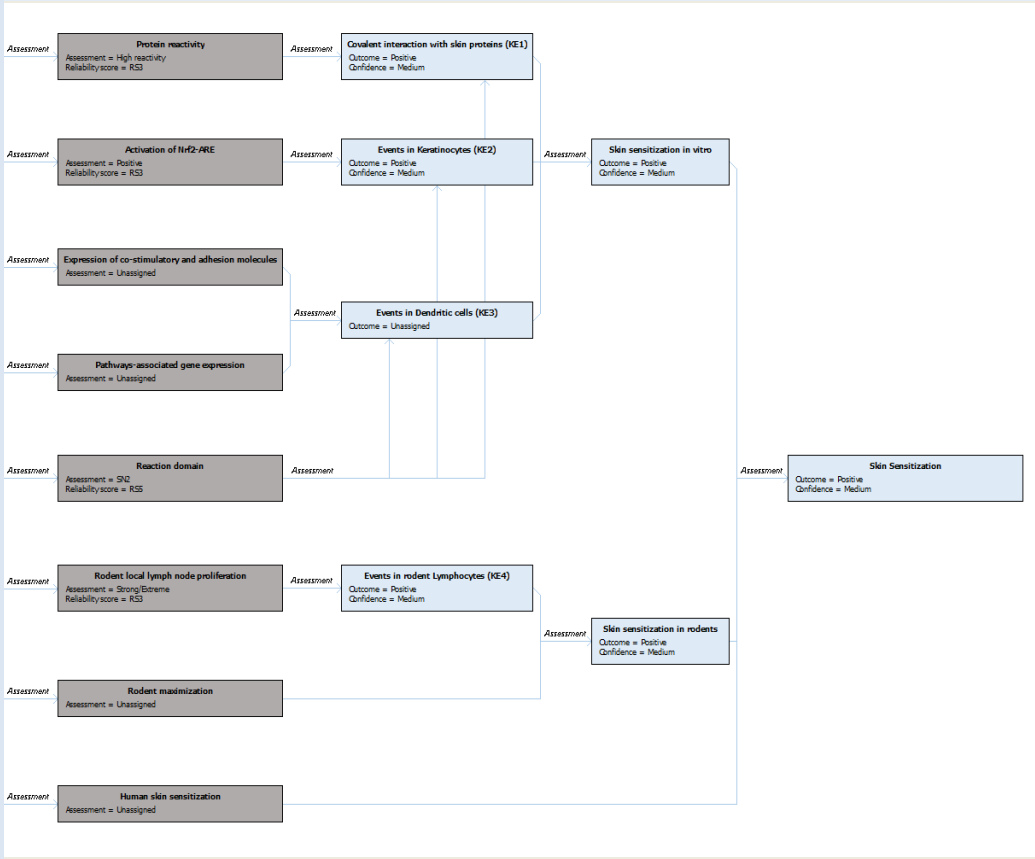


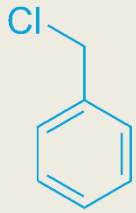
Benzyl Chloride

Effects or Mechanisms (e.g., protein reactivity, activation of Nrf2-ARE, …)

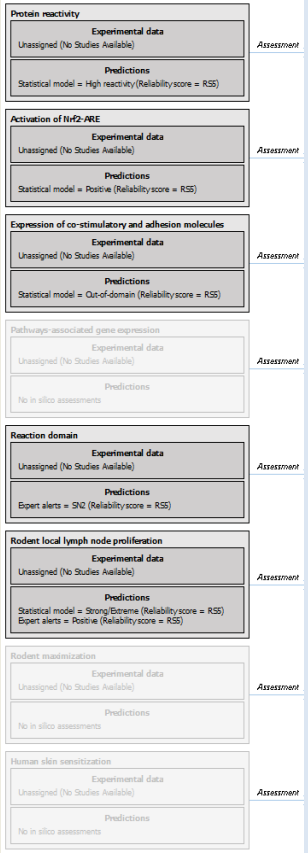
Experimental data

In silico results

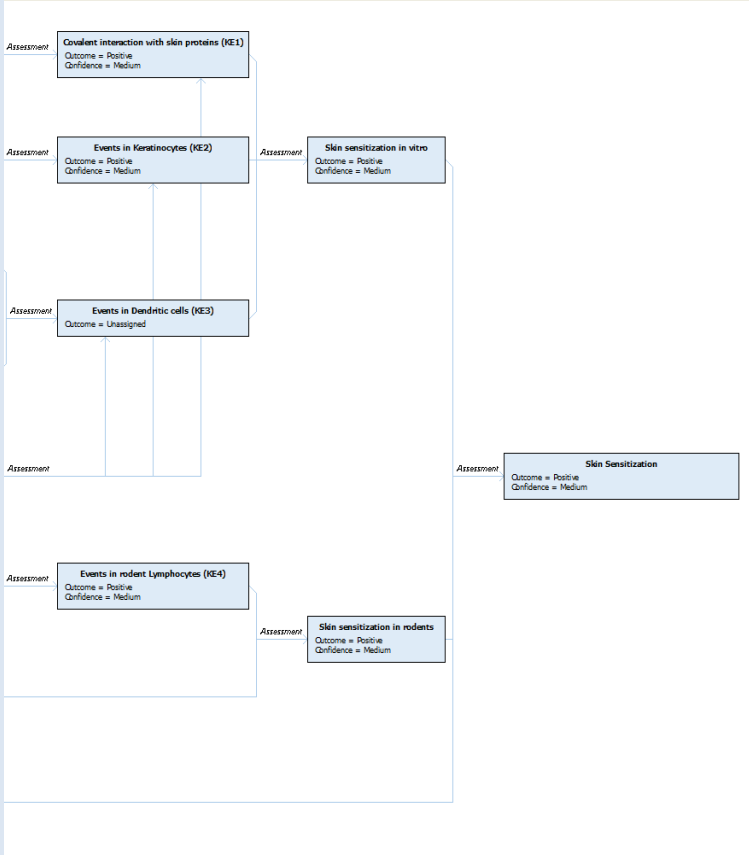


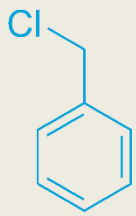


Benzyl Chloride

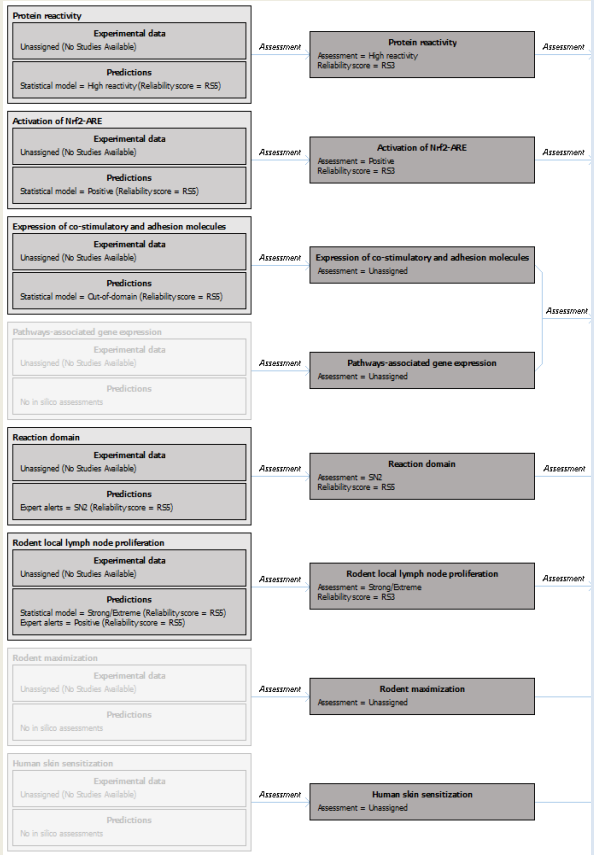


Generating an assessment and documenting the reliability of the information

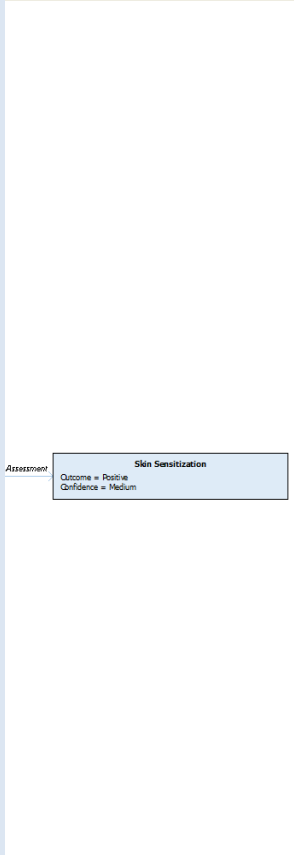


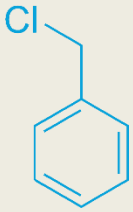


Benzyl Chloride

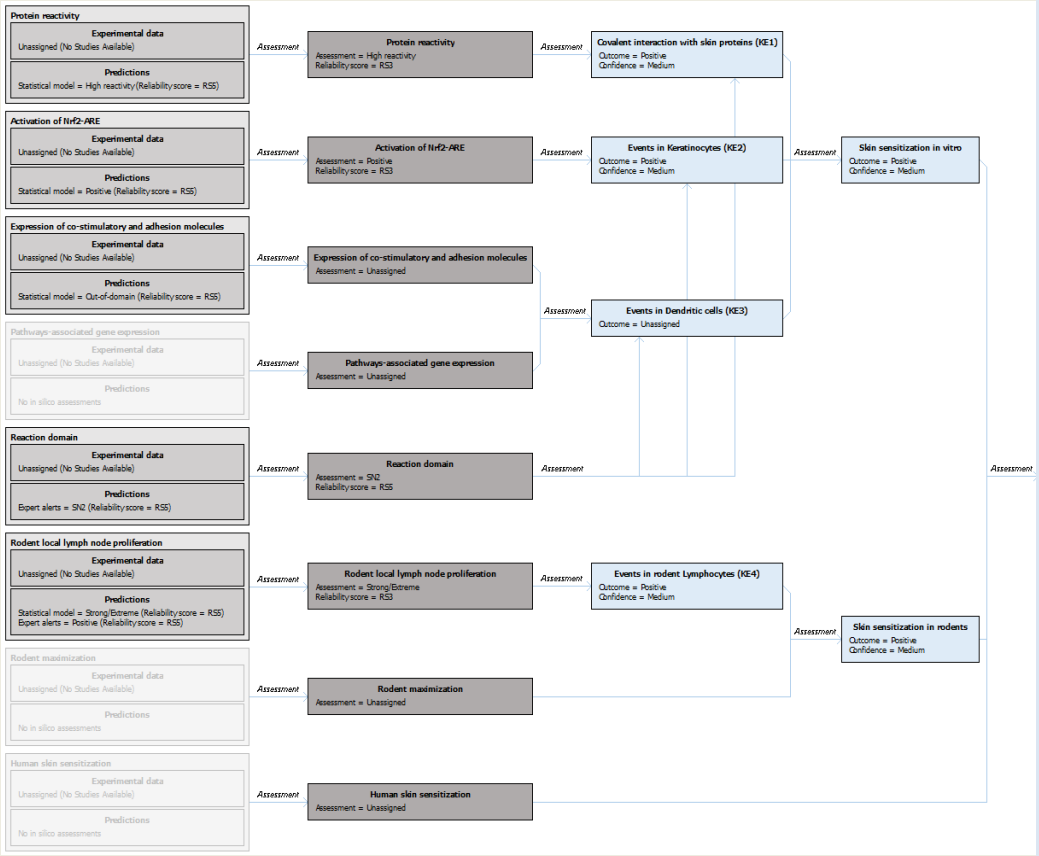


Generating an overall assessment for sub-endpoints (e.g., covalent interaction with skin proteins (KE1)) alongside a confidence score

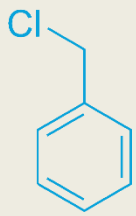




Benzyl Chloride

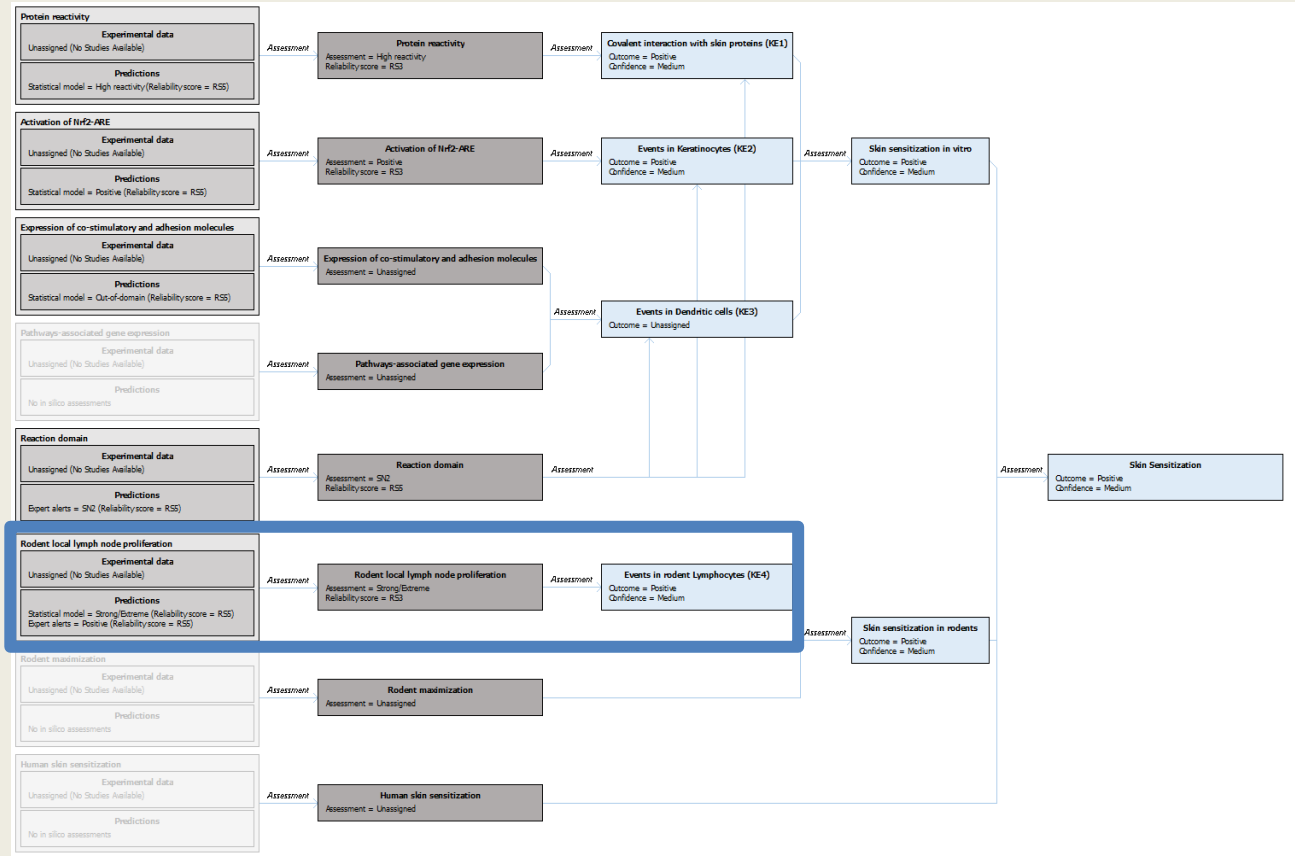


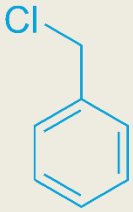
Generating an overall assessment for the major endpoint (e.g., skin sensitization in humans) alongside a confidence score



Benzyl Chloride

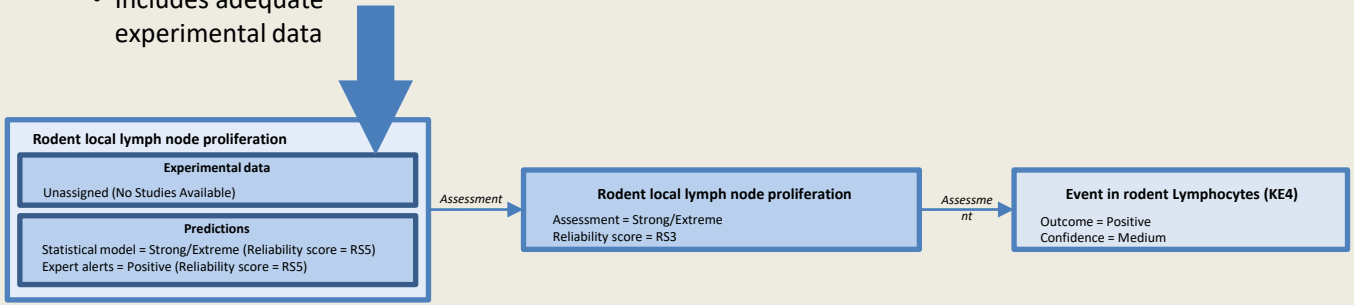
Focus on part of the framework

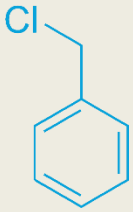




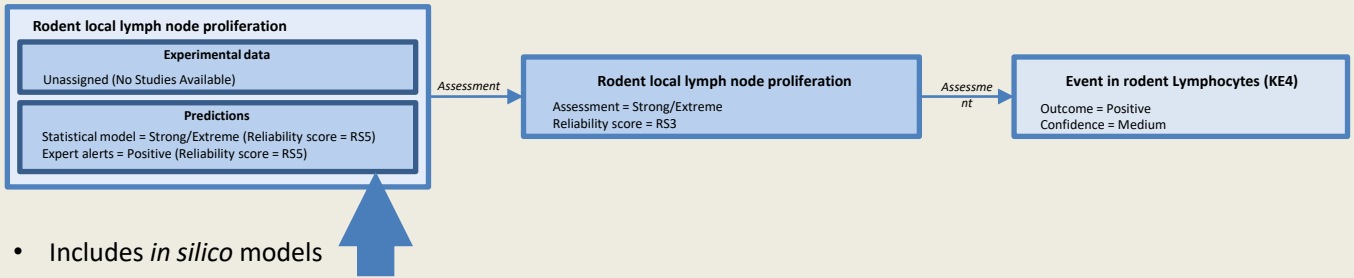
Benzyl Chloride

- Includes adequate experimental data

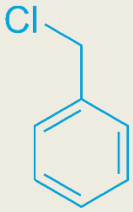




Benzyl Chloride



- Includes *in silico* models



Benzyl Chloride

- Inspect the results and underlying information
- Manually change results or reliability score
- Create an expert review

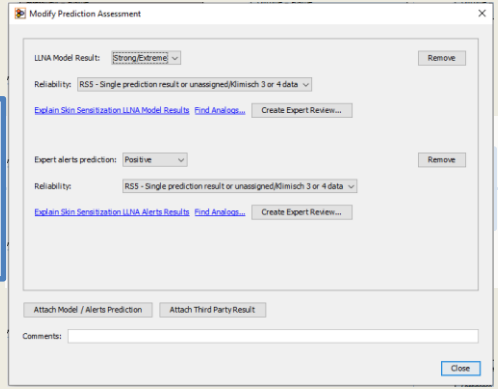
Rodent local lymph node proliferation

Experimental data

Unassigned (No Studies Available)

Predictions

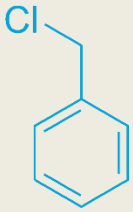
Statistical model = Strong/Extreme (Reliability score = RS5)
Expert alerts = Positive (Reliability score = RS5)



Assessment

Event in rodent Lymphocytes (KE4)

Outcome = Positive
Confidence = Medium



Benzyl Chloride

Rodent local lymph node proliferation

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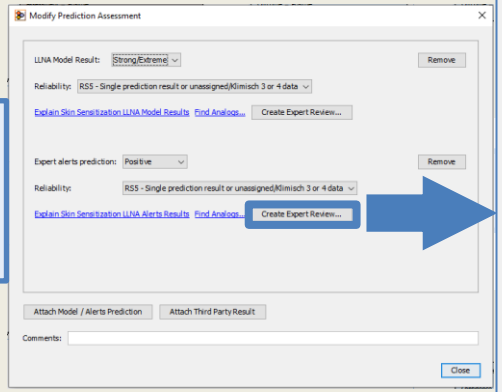
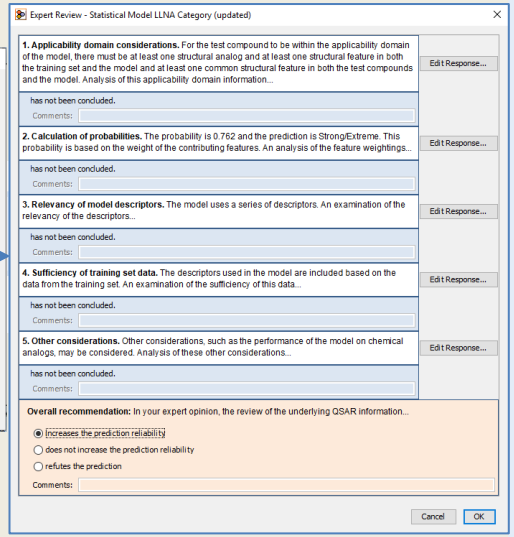
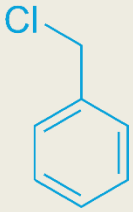


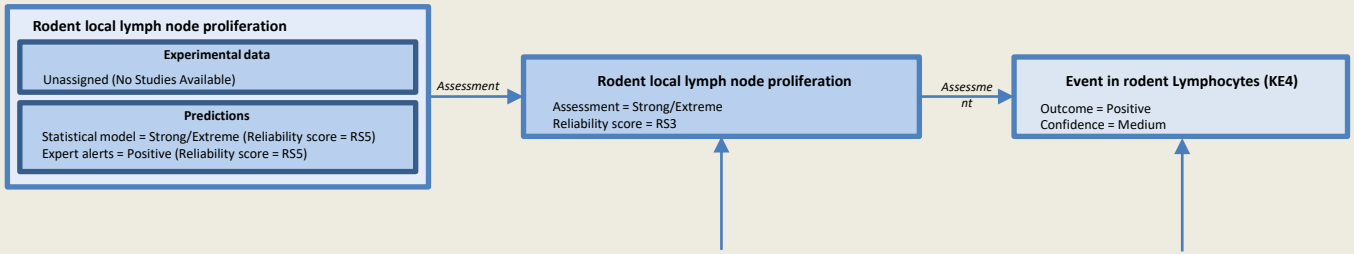


- Checklist* of items to consider as part of expert review
- Documenting the conclusions

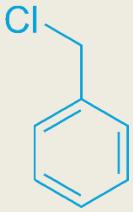
* Based on the protocol



Benzyl Chloride

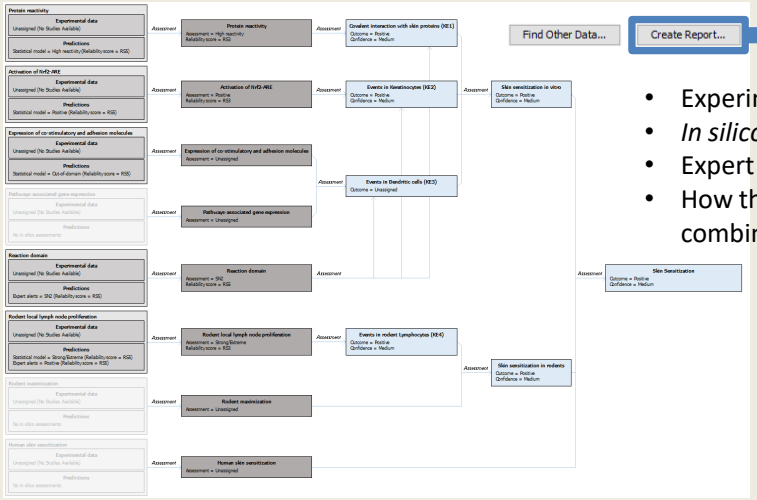


Review and manually update with sufficient evidence

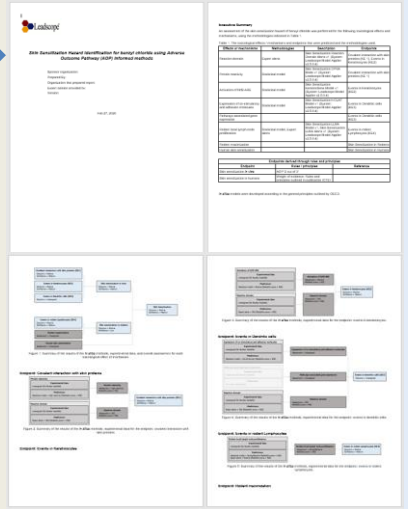


Benzyl Chloride

Documenting the results



Create report



- Experimental data
- *In silico* results
- Expert review
- How the information was combined

Protocols ensure results can be generated, recorded, communicated, and archived in a uniform, consistent, and reproducible manner

DISCUSSION

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Protocol vs. position papers

- *In silico* protocols for all toxicological endpoints is the ultimate objective of this project
- Current state-of-the-science dictates when the generation of such a protocol is possible:
  1. an accepted mechanistic basis to make decisions based on current knowledge
  2. adequate and robust databases and IST models
  3. clear regulatory or industrial drivers
- Position paper summarizing state-of-the-art is generated for other endpoints

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In silico toxicology protocol status

- The consortium has been organized into a series of working groups addressing individual endpoints:
- *In silico* toxicology framework completed
- Two protocols have been published
  - Genetic toxicology (Hasselgren et al., 2019)
  - Skin sensitization (Johnson et al., 2020)
- Additional protocols under-development for irritation/corrosion and endocrine activity

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Status of position papers

- Manuscripts have been drafted/completed covering
  - Acute toxicity
  - Liver toxicity
  - Kidney/lung/heart toxicity
  - Carcinogenicity
  - Neurotoxicity
- Overall conclusions for more complex endpoints
  - Generally missing the battery of *in silico* models necessary to predict mechanisms underpinning the toxicity
  - Existing models generally predict a potential hazard (i.e., toxic or non-toxic predictions)
    - Restricts their ability to support a risk assessment where information on dose is important

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Discussion

- Initiative developed to support toxicologists and regulators
  - Transparent and defendable protocol for performing such assessments
  - Enables mutual acceptance of data
- Project can also be used by scientists developing new tests and *in silico* model developers to focus attention on methods that:
  - Fill gaps in the hazard assessment frameworks
  - Provide the necessary information to support subsequent risk assessment

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Discussion

- 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)
    - “What if” questions
      - What if additional *in vitro* and/or *in vivo* data was included in the assessment - how would the results change?
- Currently under discussion how to expand framework to support risk assessments

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- All colleagues and collaborators on the project
- Funding:

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Thank you

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