Implementation of the *in silico* toxicology protocols within Leadscope

Leadscope Enterprise (LSE) version 3.9.0

Leadscope Model Applier (LMA) version 3.1.0

Leadscope Technical Support
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Contents

1. Introduction

Since 2016, Leadscope scientists have been leading an international consortium of over 60 organizations to develop *in silico* toxicology (IST) protocols (similar to *in vitro* or *in vivo* test guidelines). IST protocols describe how to perform a prediction in a consistent, transparent, and well-documented manner. This includes:

1) how to plan the *in silico* analyses including identifying what toxicological effects or mechanisms to predict, what *in silico* methodologies to use, and other selection criteria for the *in silico* methods
2) how to conduct the appropriate individual software predictions and further database searches
3) how to perform and document the *in silico* analysis including expert review,
4) how to combine all the information (both experimental, in silico, and expert reviews) into an overall assessment along with an associated level of confidence
5) how to report and share the information and assessment results, including information about uncertainties.

These protocols ensure a quality *in silico* assessment is performed and documented in a consistent and defendable manner.

In 2018, the project published a framework for *in silico* toxicology protocols[1], with the first protocol for genetic toxicology[2] published in 2019 following by a protocol for skin sensitization[3] in 2020. In addition, over 10 protocols and related position papers are currently being developed.

The Leadscope software now includes a complete implementation of these published protocols by providing immediate access to computation methodologies and toxicity databases outlined in the protocols. These are incorporated within a visual decision framework that combines all information, both experiment data and *in silico* results, in a manner consistent with the weight-of-evidence principles outlined in the protocols. This interactive framework includes the ability to inspect and perform an expert review of the experiment data and/or predictions at any point alongside an assessment of how the information was combined into an overall assessment and associated confidence score. This implementation supports the application of good *in silico* practices in the assessment of toxicological endpoints.

Key concepts from the protocol framework paper are summarized in Figure 1 and include:

- **Effects/mechanisms**: Each IST protocol defines a series of known toxicological effects and mechanisms relevant to the assessment of the major toxicological endpoint (Section 2.2 of Myatt et al., 2018). For each toxicological effect/mechanism, relevant information (as defined in the IST protocol) is collected, including any available experimental data (Section 2.5 of Myatt et al., 2018) as well as *in silico* predictions (Section 2.3 of Myatt et al., 2018). The experimental data and/or *in silico* results are then analyzed and an overall assessment of the toxicological effect or mechanism is generated.
- **Reliability score:** For each effect/mechanism a reliability score is calculated (Section 2.6.2 of Myatt et al., 2018) that reflects the quality of the results. This score is based on the information

[1] Myatt et al., 2018. *In silico* toxicology protocols. Regulatory Toxicology and Pharmacology https://doi.org/10.1016/j.yrtph.2018.04.014
[2] Hasselgren et al., 2019. Genetic toxicology *in silico* protocol. Regulatory Toxicology and Pharmacology https://doi.org/10.1016/j.yrtph.2019.104403
[3] Johnson et al., 2020. Skin sensitization *in silico* protocol. Regulatory Toxicology and Pharmacology. Accepted for publication

collected for a specific effect/mechanism and takes into consideration whether an expert review of the experimental data(Section 2.5 of Myatt et al., 2018) and/or the in silico results (Section 2.4 of Myatt et al., 2018) has been performed.

- **Endpoints**: The specific toxicological effects or mechanisms are used to support the assessment of a series of toxicological endpoints (Section 2.7.1 of Myatt et al., 2018). These toxicological endpoint assessments are, in turn, used in the overall assessment of the major toxicological endpoint (e.g., genetic toxicity or skin sensitization). This process may include an expert review of the information (Section 2.7.5 of Myatt et al., 2018).
- **Confidence**: Confidence, in this context, is defined as a score that combines the reliability and relevance of the associated toxicological effects or mechanisms (Section 2.7.4 of Myatt et al., 2018). This is an additional score associated with toxicological endpoints. The score may, in some cases, use other toxicological endpoint confidence scores. This score will also take into consideration the completeness of the information available; for example, the confidence score may be lowered when information on an effect or mechanism is missing.

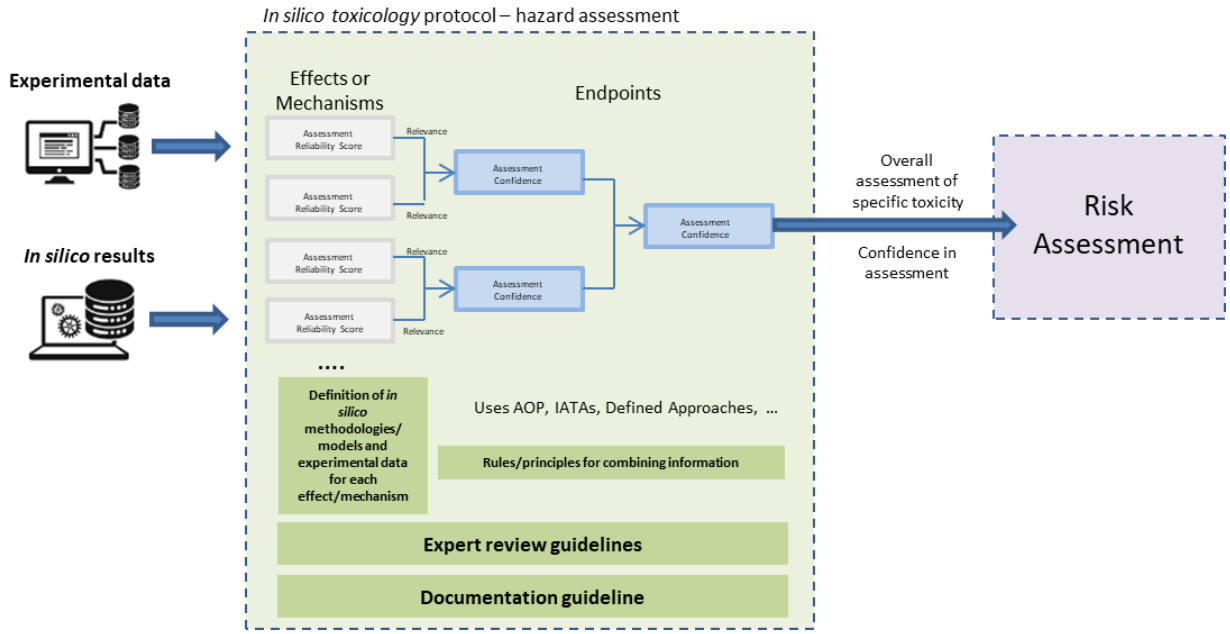


Figure 1: Overview of the *in silico* toxicology protocol framework

2. Initiating the assessment

The first step to performing an assessment on one or more chemicals based on the principles and procedures outline in the *in silico* toxicology protocol is to launch the tool by selecting the integrated hazard assessment option (shown in Figure 2 and 3).

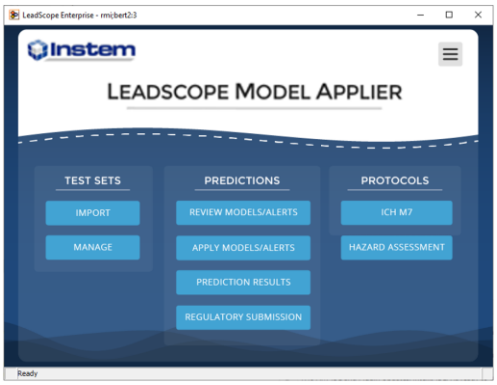


Figure 2: Launching the protocol-based assessment from the Leadscope model applier v3.1 selecting "Hazard assessments" (under "Protocols")

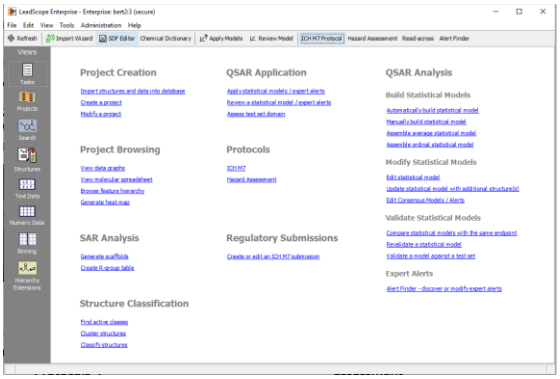


Figure 3: Launching the protocol-based assessments from the Leadscope user interface selecting "Create or edit an integrate hazard assessment"

The next step (shown in Figure 4) is to either select an assessment previously generated or "Start New Prediction". Selecting "Start New Prediction" will give you options to select the chemical(s) to analyze and to select the type of analysis, whereas opening a previous analysis will open the assessment where you previously left off.

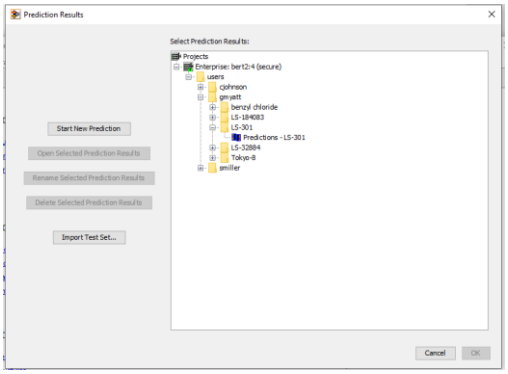


Figure 4: Initiating a hazard assessment

If you selected “Start New Prediction” then the next step is a standard dialog for selecting the chemical(s) to analyze, as shown in Figure 5. On the left-hand side, there are two options for selecting chemicals already loaded onto the database (it you are connected via a client-server configuration, there is also an option to select a specific server). The first is a new option whereby you can look-up a chemical (either by specifying a name or ID). The other option “Use a previously loaded test set:” provide a listing of projects (collections of chemicals that have been previously loaded) that can be selected. On the right-hand side there are 3 options for entering a single chemical: entering a SMILES string, pointing to a mol file or pasting a chemical structure in from the clipboard.

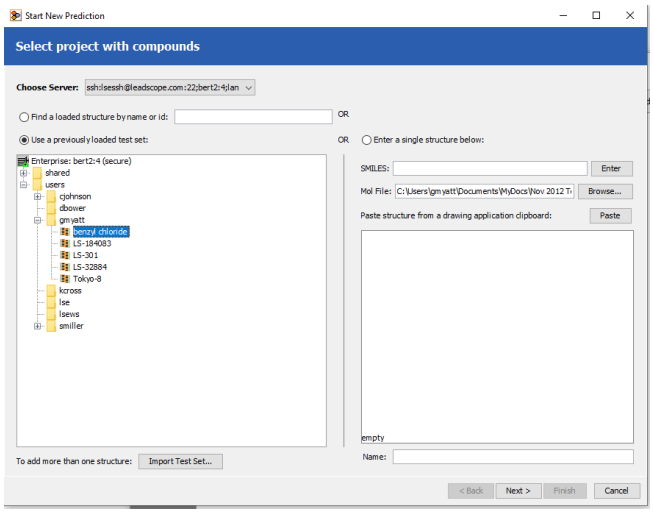


Figure 5: Selecting chemical(s) to analyze

As shown in Figure 6, once the chemical(s) of interest have been identified, the next dialog allows you the flexibility to select the specific chemicals to analyze. Alternatively, you can choose the “Select All” button to assess all the chemicals displayed.

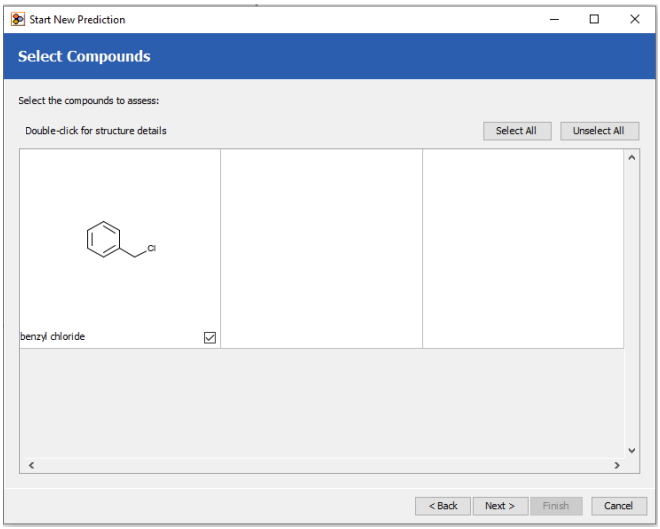


Figure 6: Selecting specific chemicals to analyze

As shown in Figure 7, the next step is to select which “Integrated hazard assessment” to perform. The three current options “Genetic toxicity”, “Skin sensitization” and “ICH M7 Protocol” are listed in the top left window. By selecting one of these options, the individual models included in these assessments are then automatically selected. In the case of the ICH M7 Protocol, an option to select the API (Active Pharmaceutical ingredient) as well as the impurities is provided. Clicking “Finish” will initiate an assessment based on the selected integrated hazard assessment.

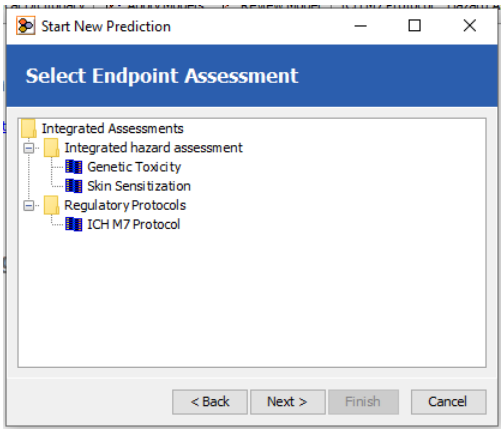


Figure 7: Selecting the integrated hazard assessment(s)

Once the analysis has completed, a summary of the initial results is presented in which a number of high-level endpoints are presented alongside the preliminary assessment of the confidence. As no expert review of the information has been performed the default assessment and confidence values are reported; however, these may be modified as part of any expert review. If the ICH M7 Protocol option was select, a customized view is generated (detailed in Section 8).

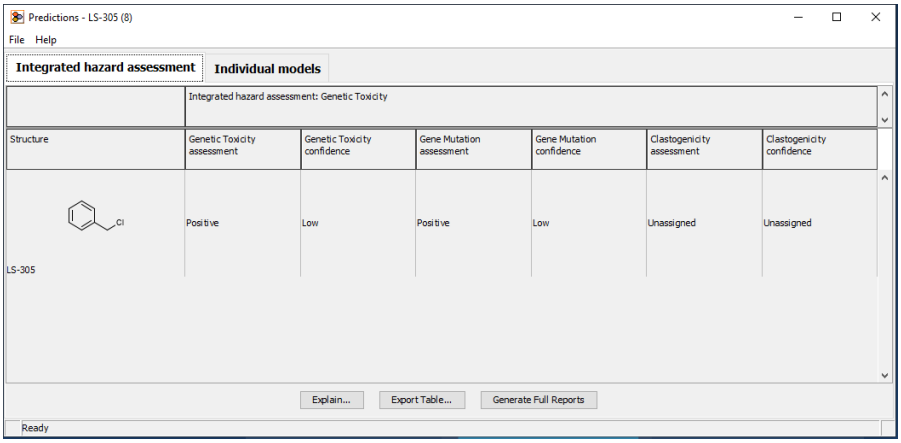


Figure 8: Summary of the results for high level endpoints for the selected hazard assessment

Individual model results can be inspected by clicking on the “Individual models” tab; however, to fully understand how these values were generated click on the “Explain” button to view the complete hazard assessment framework as described in the protocol.

3. Viewing the hazard assessment framework

Below is the genetic toxicity hazard assessment framework for the selected chemical (which is shown at the top left in Figure 10).

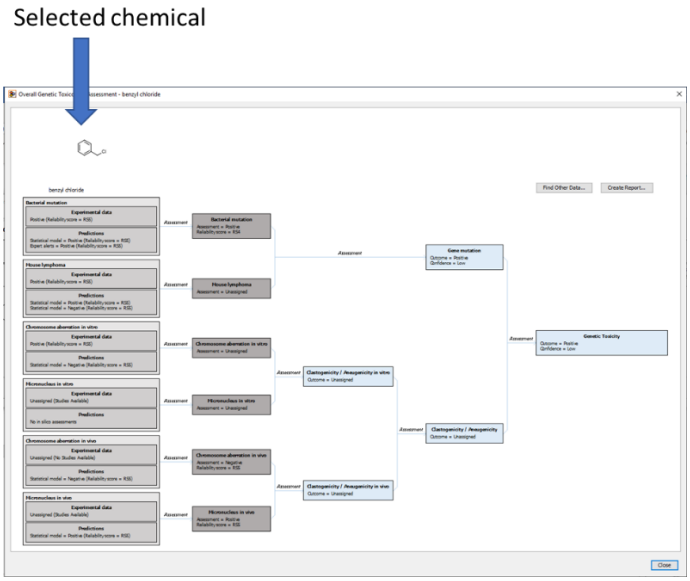


Figure 10: Hazard assessment framework with selected chemical highlighted

In Figure 11, the left-hand column of boxes are the effects/mechanisms[4] that are defined in the protocol (i.e., Bacterial mutation, Mouse lymphoma, ….). The software automatically performs a series of database searches and runs a series of models as outlined in the protocol. The "Experimental data" show the results from the database look-up and the "Predictions" shows the results from the *in silico* model(s). For example, the bacterial mutation experimental value is positive (with a default reliability score[5] of RS5) and there are two prediction results (the statistical model is positive with a reliability score of RS5 and the expert alerts is positive with a reliability score of RS5).

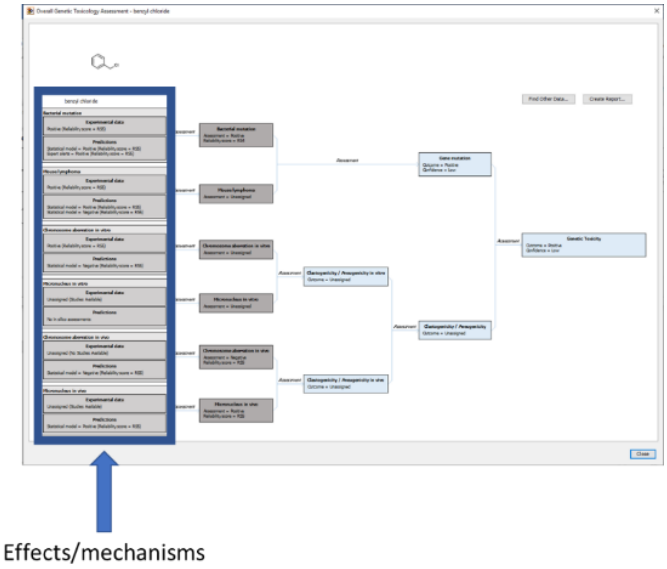


Figure 11: Effects/mechanisms highlighted in the hazard assessment framework

[4] As defined in Section 2.2 of Myatt et al., 2018
[5] As defined in Section 2.6.2 of Myatt et al., 2018

This information is then automatically assessed (based on the protocol rules) into a single overall assessment for the effect/mechanisms as well as an overall reliability score, as shown in Figure 12. For example, the Bacterial mutation assessment is positive, and the reliability score is RS4 (based on two concurring *in silico* models and an experimental data assessed as RS5).

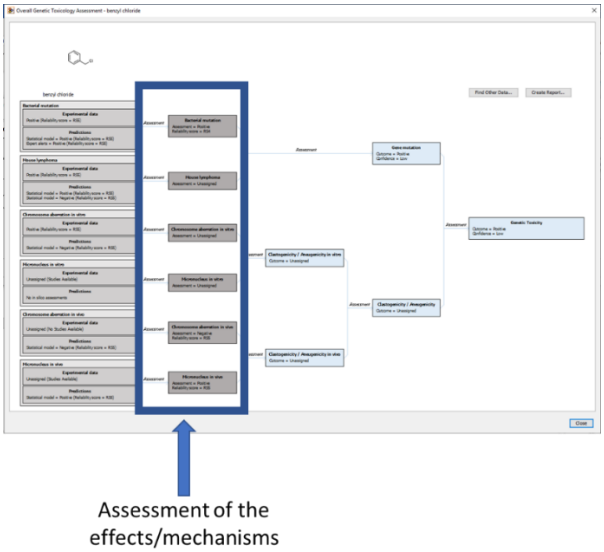


Figure 12: Assessment of effects/mechanisms highlighted in the hazard assessment framework

Figure 13 shows how multiple assessments of the individual effects/mechanisms are then combined into a (sub)endpoint[6] (shown in blue). For example, assessment for both "Bacterial mutation" and "Mouse lymphoma" are combined (based on rules described in the protocol) to generate an assessment and a confidence score for "Gene mutation". It is possible that an assessment is labelled "Unassigned" which reflects a lack of sufficient information, as documented in the protocol manuscripts.

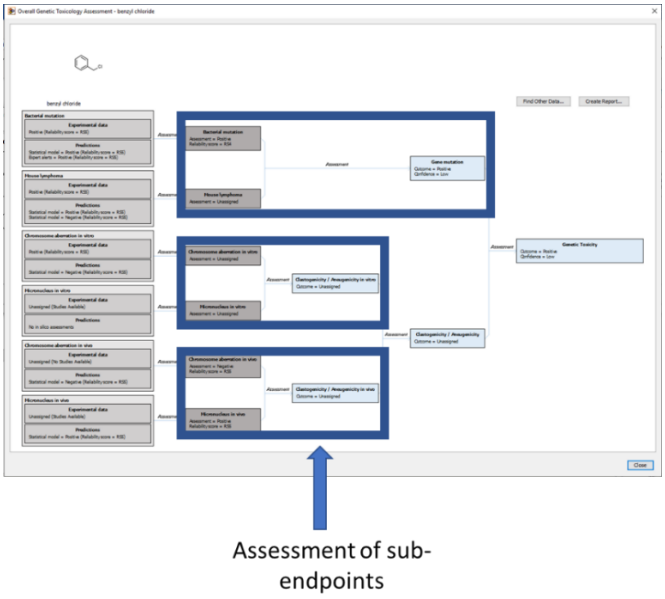


Figure 13: Assessing endpoints using information on the effects/mechanisms

[6] As defined in Section 2.7.1 of Myatt et al., 2018

Figure 14 shows how sub-endpoints are also combined to derive an assessment of an additional endpoint, for example, “Clastogenicity / Aneugenicity in vitro” and “Clastogenicity / Aneugenicity in vivo” are combined into a single “Clastogenicity / Aneugenicity” endpoint.

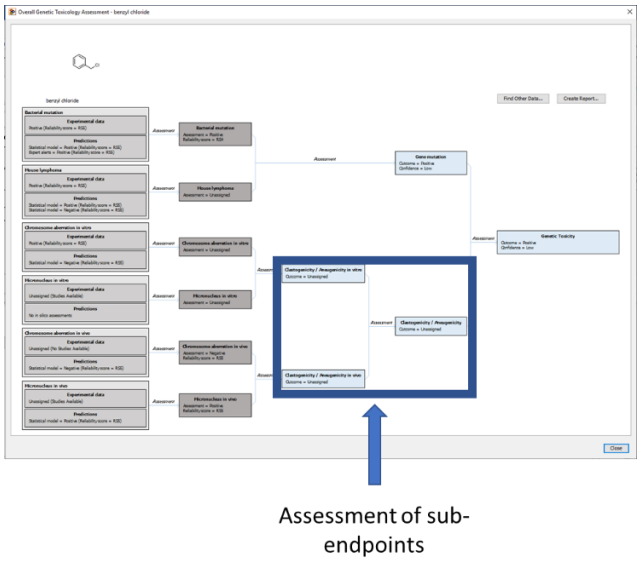


Figure 14: Assessing endpoints using information on other derived endpoints

In Figure 15, the endpoints are finally combined into a single overall assessment of “Genetic Toxicity” (alongside a confidence score) derived from the assessment of “Gene mutation” and “Clastogenicity / Aneugenicity”.

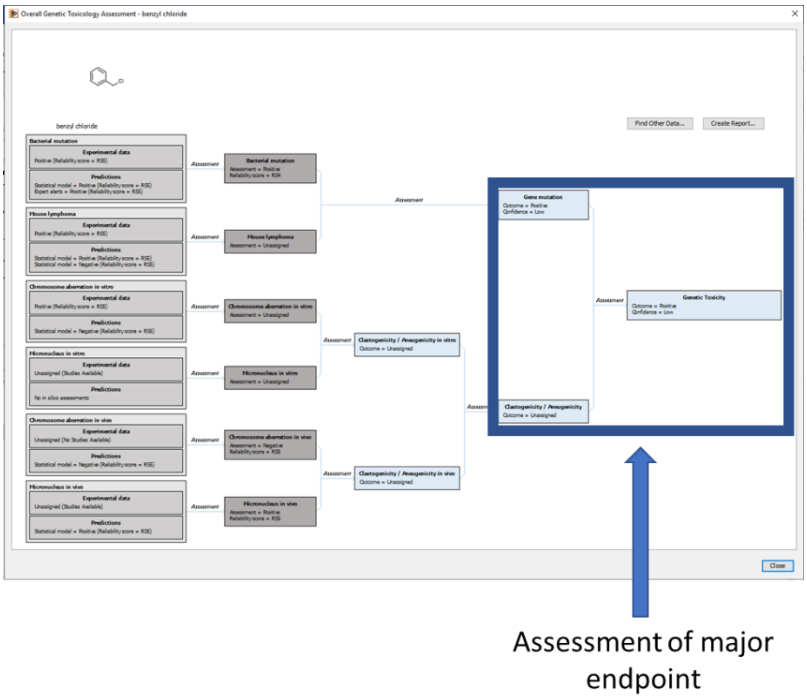


Figure 15: Assessment of the major endpoint

In Figure 16, the skin sensitization hazard assessment framework is presented following the same structure as the bacterial mutation assessment, with the first row detailing individual effects/mechanisms (described in the protocol), i.e., “Protein Reactivity”, “Activation of Nrf2-ARE”, … Each effect/mechanism is automatically linked to a database search and *in silico* models. An analysis aligned with the protocol is performed on each effect/mechanism to generate an overall assessment and reliability score. This information is then used to calculate a series of sub-endpoints (shown in blue) and these assessments and confidence scores are used to generate a final assessment for skin sensitization.

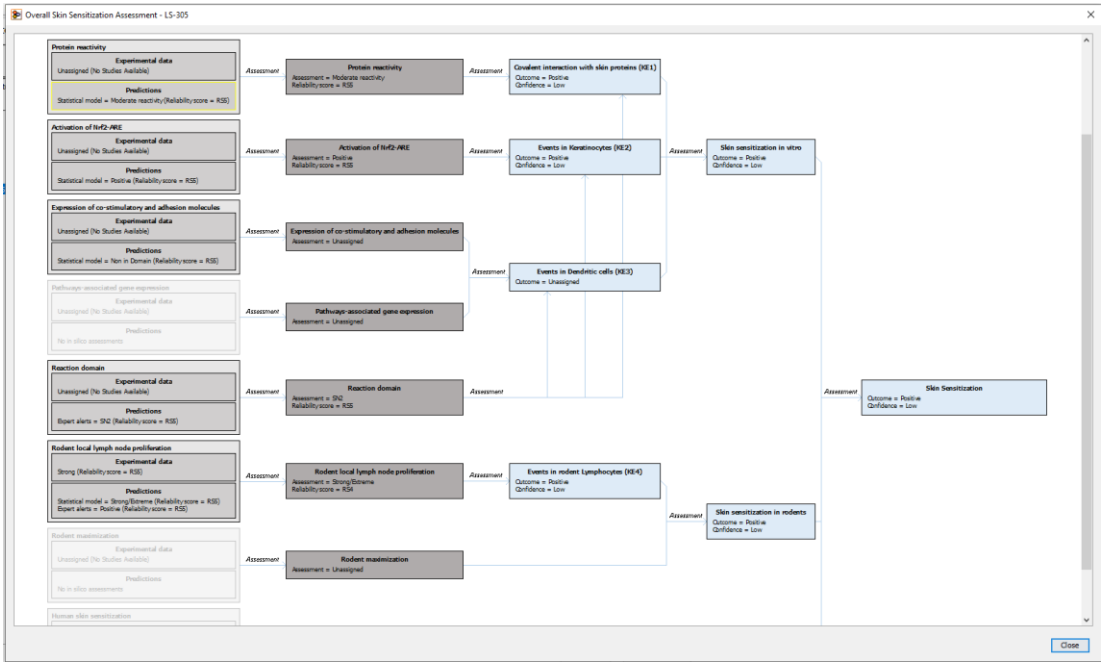


Figure 16: Skin sensitization hazard assessment framework

4. Mechanism/effect

4.1. Overview

Figures 17 and 18 highlight a single effect/mechanism.

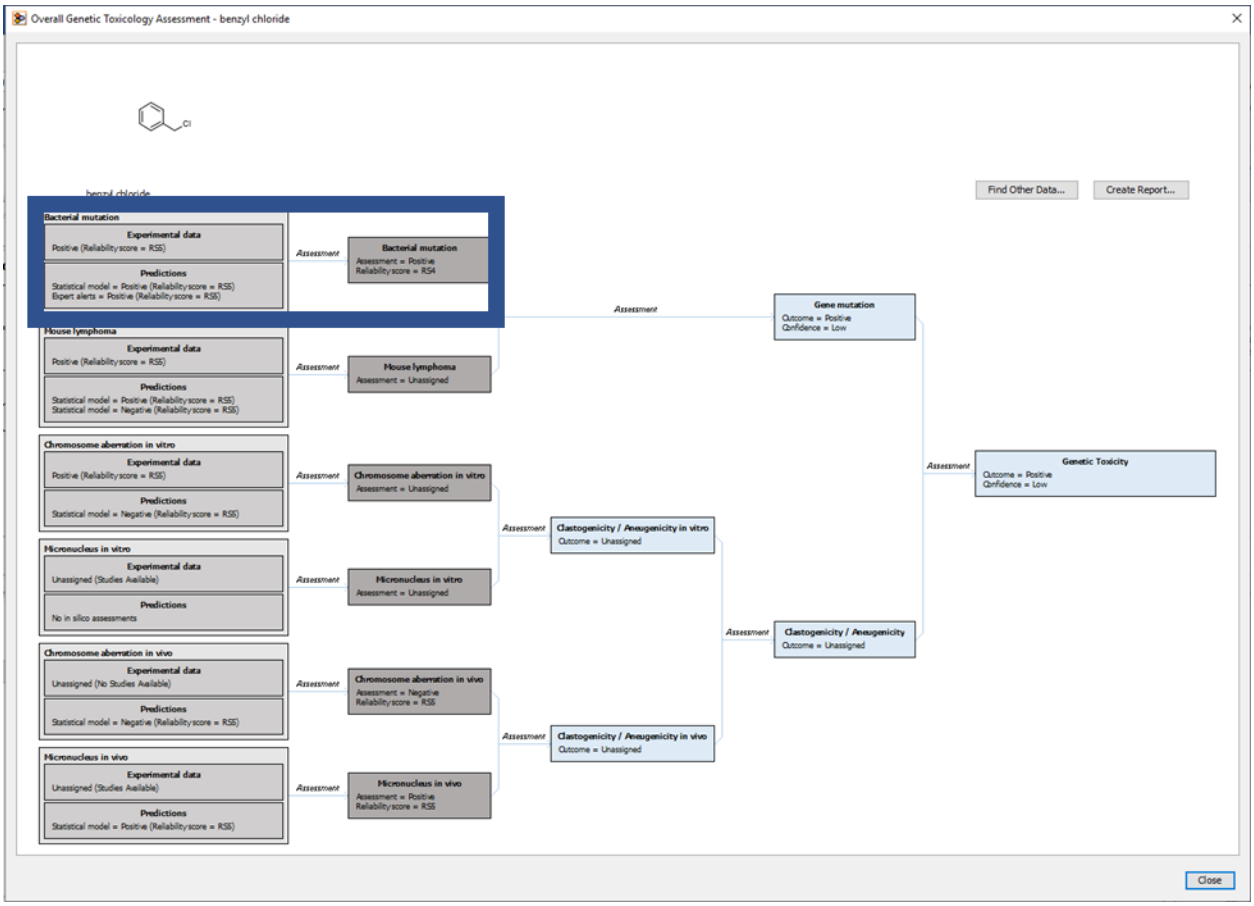


Figure 17: A hazard assessment framework with a single effect/mechanism highlighted

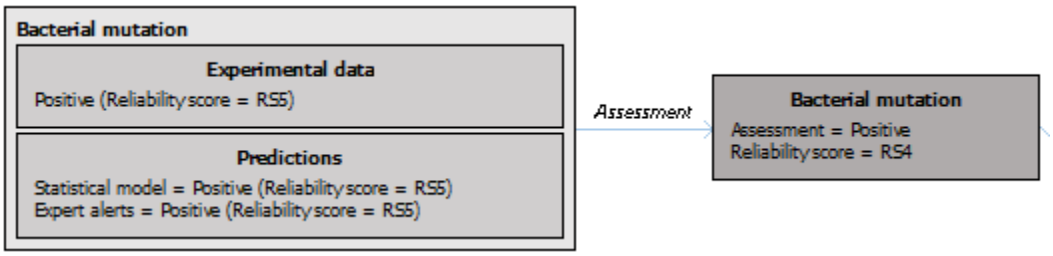


Figure 18: A single effect/mechanism

Each individual effect/mechanism in the hazard assessment framework is represented by two gray boxes (as shown in Figure 18). The first box (on the left) represents experimental data and/or prediction

results for the effect/mechanism alongside a reliability score for the individual element. The second box (on the right) represents the overall assessment for the effect/mechanism and a reliability score for the whole assessment. All items are calculated by default based on the principles outlined in the framework paper and any individual criteria outlined in the endpoint-specific protocol. The details behind each element can be interrogated and expert review provided and documented. By hovering over the different element, a yellow outline should appear and be clicking in any of the boxes (Experimental data, Predictions, and Bacterial mutation) will expose more details.

4.2. Experimental data

By clicking on the experimental data box, a dialog will be shown, as shown in Figure 19. The overall assessment is set based on the overall value assigned in the database. The reliability is set to the default value of RS5 (since no review of the study quality has been made). The data assessment and reliability score can be modified based on any expert review and a comments box is provided to document why any changes were made. In this case the Positive value was not changed but the reliability score was updated to RS1. In addition, the dialog lists a summary of all the studies identified for the chemical as well as a link to view the full study record. It is possible to select any study or studies to include in the assessment as well as comment on why the study was included. It is also possible to remove any study from the list by clicking on the "Remove" button above each of the study summaries. The dialog also includes a "Redo Database Study Search" button as a way to be able to get the original list of studies since you can remove any of the studies.

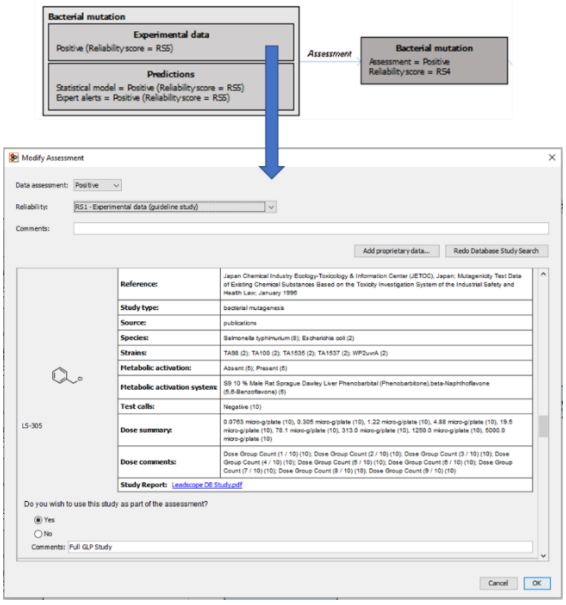


Figure 19: Displaying experiment study data related to a single effect/mechanism

As shown in Figure 20, it is also possible to include and document proprietary experimental data to use as part of the assessment. By clicking on the "Add proprietary data" button, a dialog will be displayed

where you can add a summary of the findings as well as attach a study report or link to a report already uploaded.

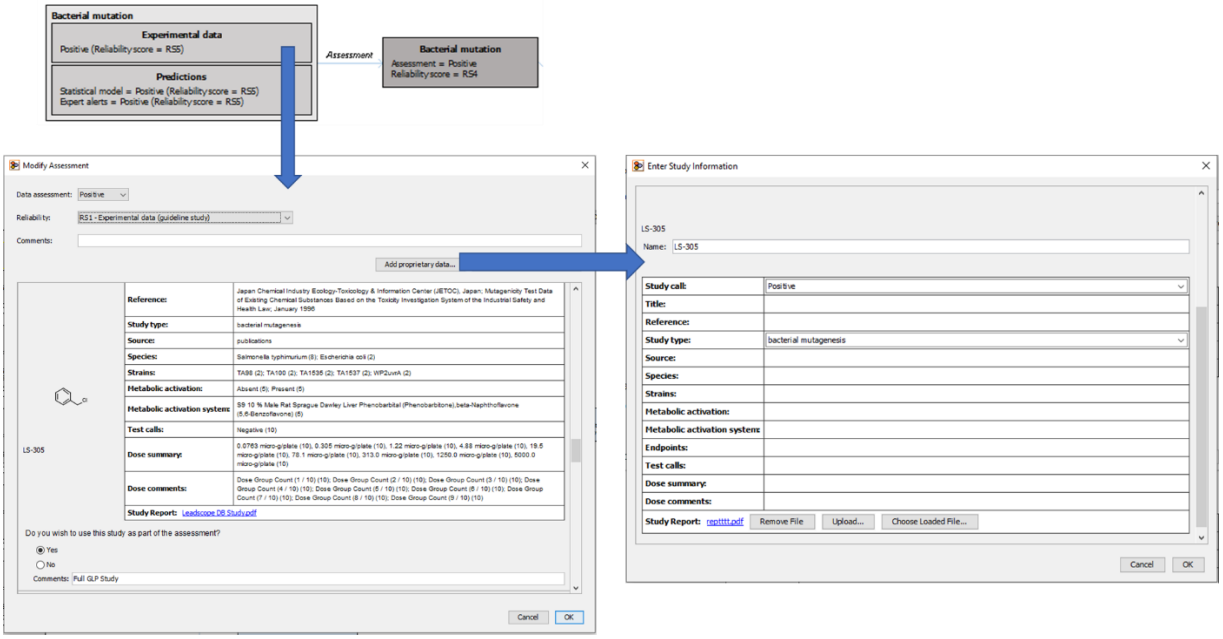


Figure 20: Adding proprietary study information

Once any changes have been made, the hazard assessment framework diagram is updated, as shown in Figure 21. In this example, the experimental data is now assigned a reliability score of RS1 which in turn updates the reliability score (shown in the right box) reflecting all information (experimental data and *in silico* models) collected for this chemical. This change could also change other endpoints in the framework.

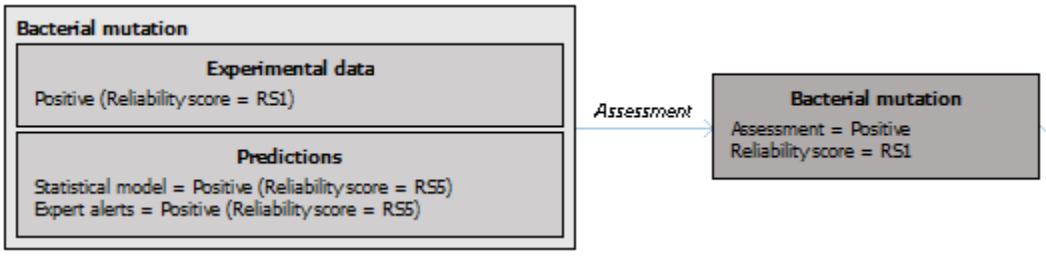


Figure 21: A single effect/mechanism with an updated reliability score (RS1)

4.3. In silico result

The hazard assessment framework window includes a summary of the prediction results. By clicking on the Prediction box (as shown in Figure 22), more details are available to inspect. It is also possible to generate an expert review as well as modify the results based on this review.

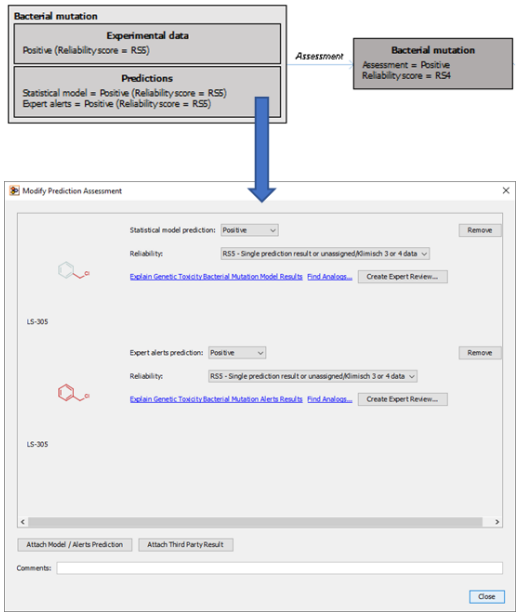


Figure 22: Detailed view of the prediction results

For each model result, it is possible to inspect how the result was calculated using the standard Leadscope explain, as shown in Figure 23. For statistical-based models that predict binary results, such as a positive/negative value, the explain will present the model features and weighting and allow you to inspect the underlying chemicals. For statistical-based models that predict a category, such as low/medium/high, a decision tree is presented showing how a series of models are used to generate a final category (it is also possible to inspect the individual features and weighting of each model). For expert alert based models, the alerts that match the chemical are presented along with supporting information.

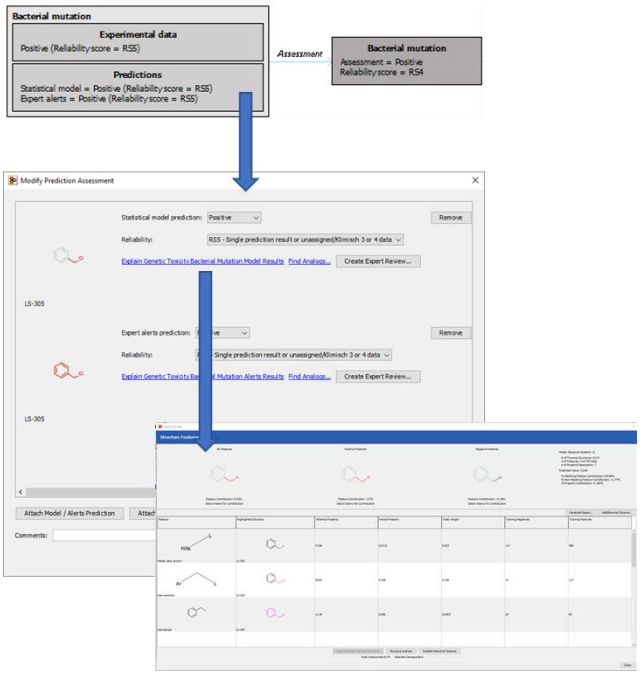


Figure 23: Viewing an explanation of how the in silico model calculated the prediction

In addition, it is possible to view close analogs to the test chemical by clicking on the “Find analogs” button, as shown in Figure 24. A new window will show the test chemical in the first row followed by a list of analogs from the database. The analogs are ordered based on their structural similarity to the test chemical and include experimental data results. It is also possible to run the prediction model over these analogs (“Add predictions”) as well as the other operations shown.

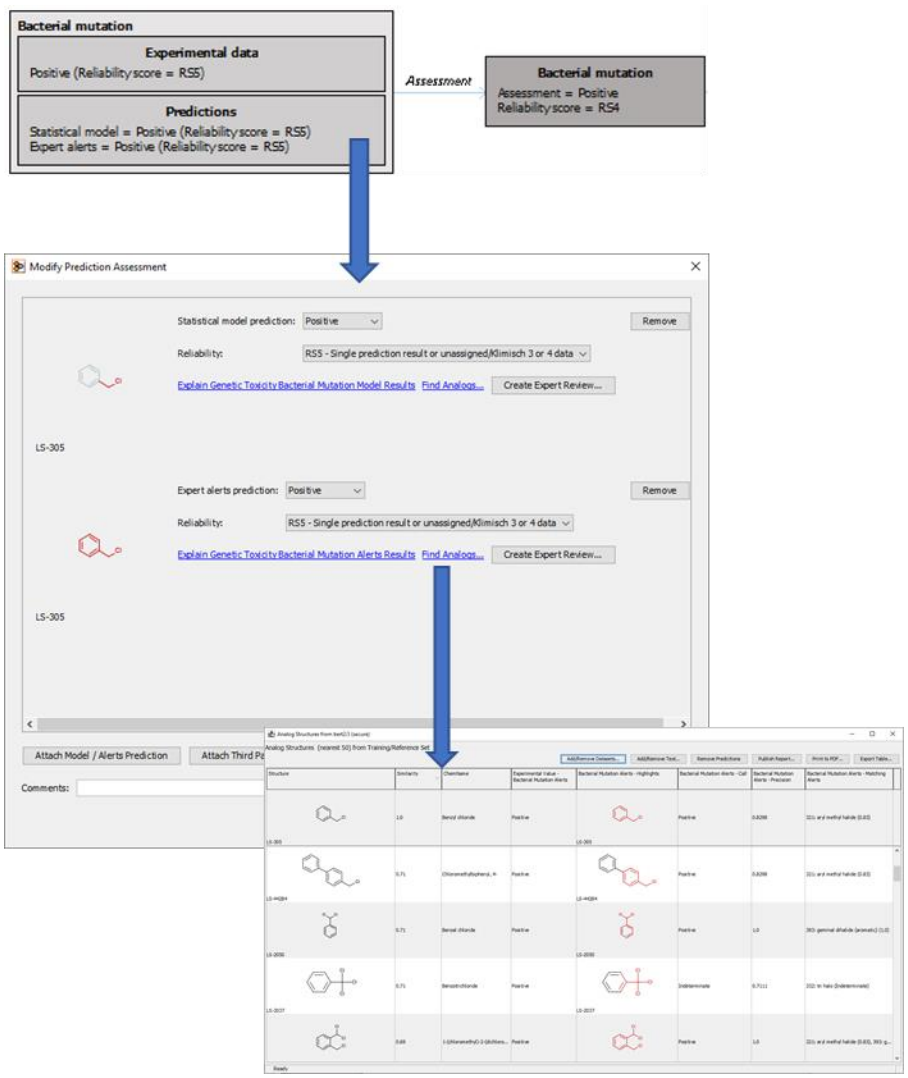


Figure 24: Viewing analogs of the test chemical

The software automatically links the different effects/mechanisms to Leadscope's prediction models. In addition, it is possible to add prediction results from other custom (proprietary) statistical-based or expert alert-based models that are available on the Leadscope database (the Leadscope predictive data miner module is required to build these custom models). This is achieved by clicking on the "Attached Model / Alert Prediction" button and linking it to another model or alert. This option is only available when the Leadscope predictive data miner is licensed. It is also possible to add results from other tools by clicking on the "Attach Third Party Result". After identifying the type of methodology used (i.e., statistical model, expert alerts, read-across, other) a summary of the results should be provided, and any associated report uploaded or alternatively linked to the record (if previously uploaded).

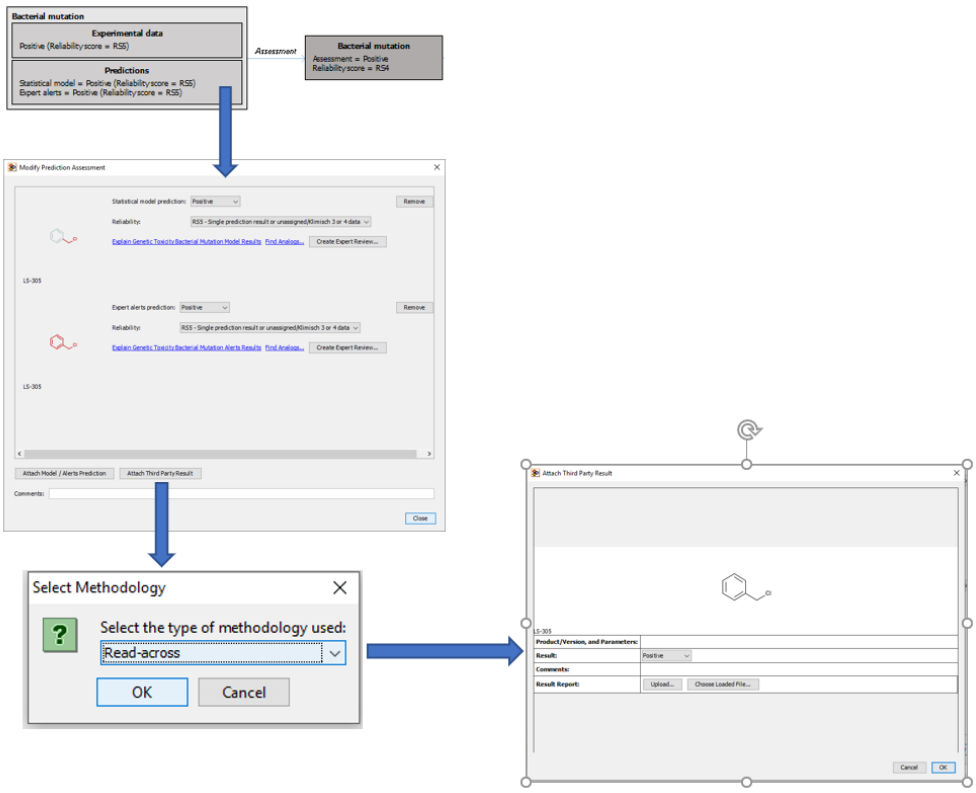


Figure 25: Including other in silico results in the analysis

The assessment of any effect/mechanism is based on the rules and principles outlined in the framework paper as well as modification that take into consideration the specific protocol. However, it is possible to manually override any calculated assessment and reliability score by clicking on the overall assessment box (such as the Bacterial mutation box shown below). To override the default assessment, click on the "Manually override calculated value" check box, make any changes to the outcome and reliability score and provide comments explaining why you made these changes.

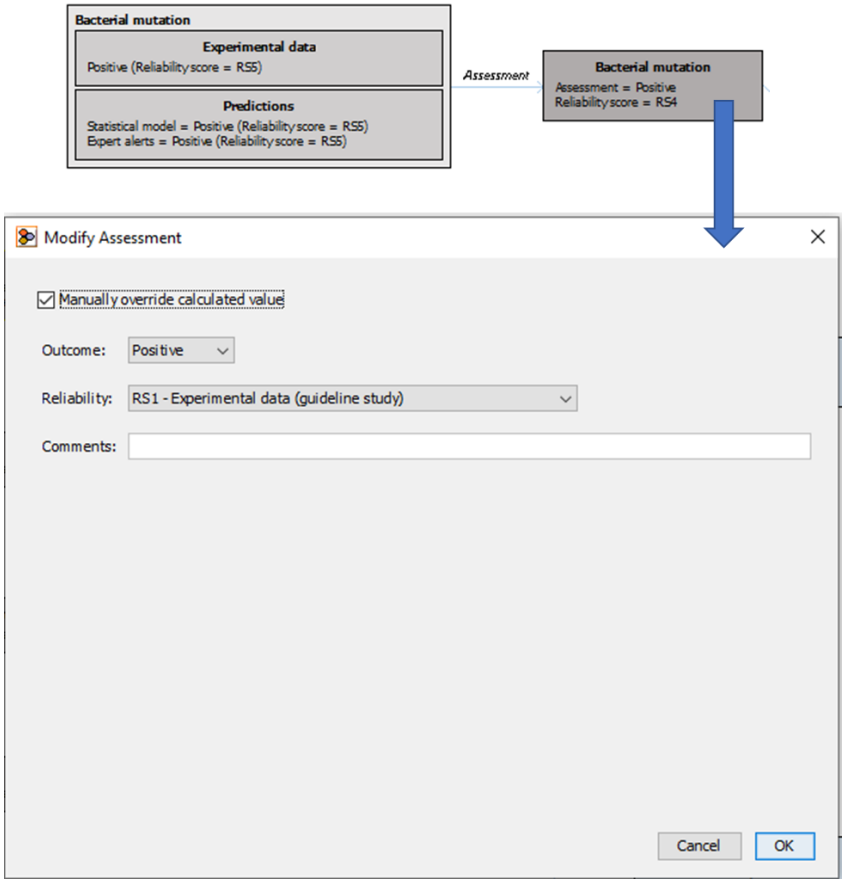


Figure 26: Examining and modifying the results for an individual effect/mechanism assessment

4.4. In silico expert review

It is possible to add an expert review to any individual *in silico* model result. To provide an expert review, click on the "Create Expert Review…" button. This will display a dialog mirroring the guideline of an expert review[7] from Myatt et al., 2018. By clicking on each "Edit response" it is possible to make an assessment as to whether a review of the context sensitive information (1) increases the prediction reliability, (2) does not increase the prediction reliability or (3) refutes the prediction, or (4) has not been concluded. In addition, comments on why the selection was made can be provided. Answering the checklist of questions supports an overall assessment which should be separately made. If the reliability is increased, an option will be provided to update the reliability score to RS3, which in turn may change the value of this assessment as well as any related (sub)endpoints.

[7] Described in Tables 2 and 3 in Myatt et al., 2018

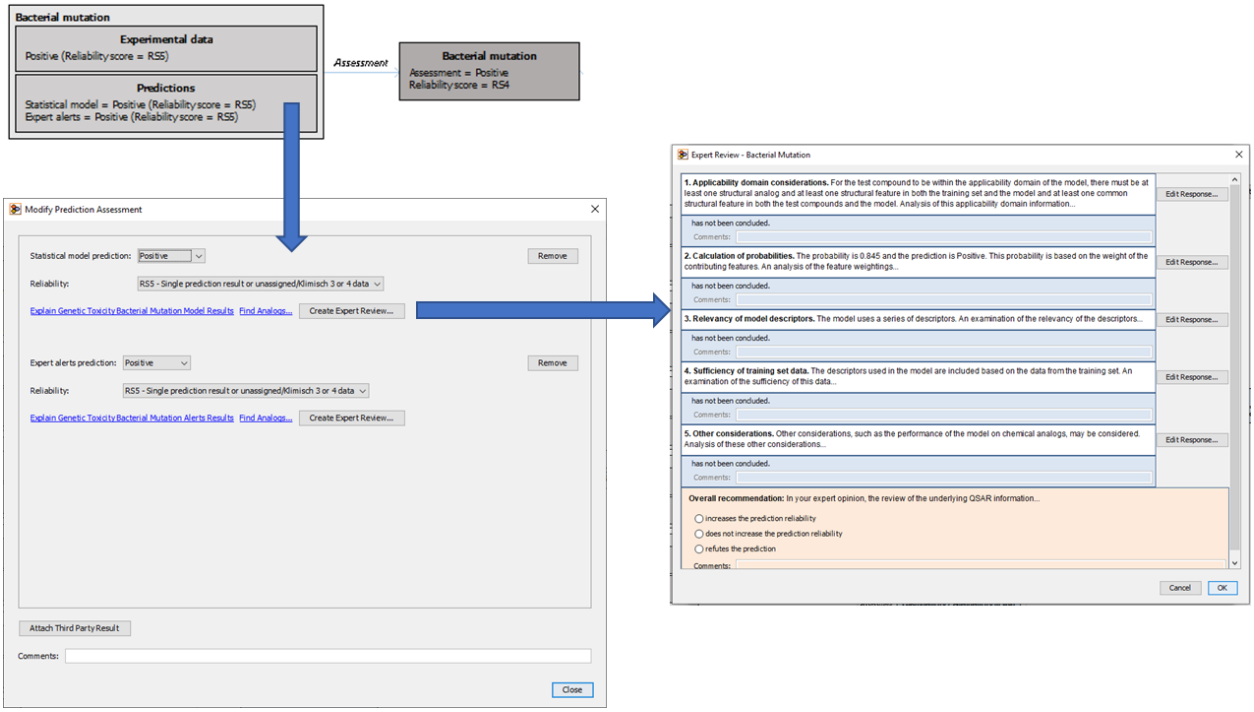


Figure 27: Performing an expert review of the in silico result based on the framework guidelines

Figure 28 illustrates the type of contextual information provided under each "Edit response…". As an example, by clicking on "Edit response" of item "1. Applicability domain consideration", the user may consider chemical analogs as well as statistical-based model features to assess the applicability domain of the model. Each checklist item presents different information.

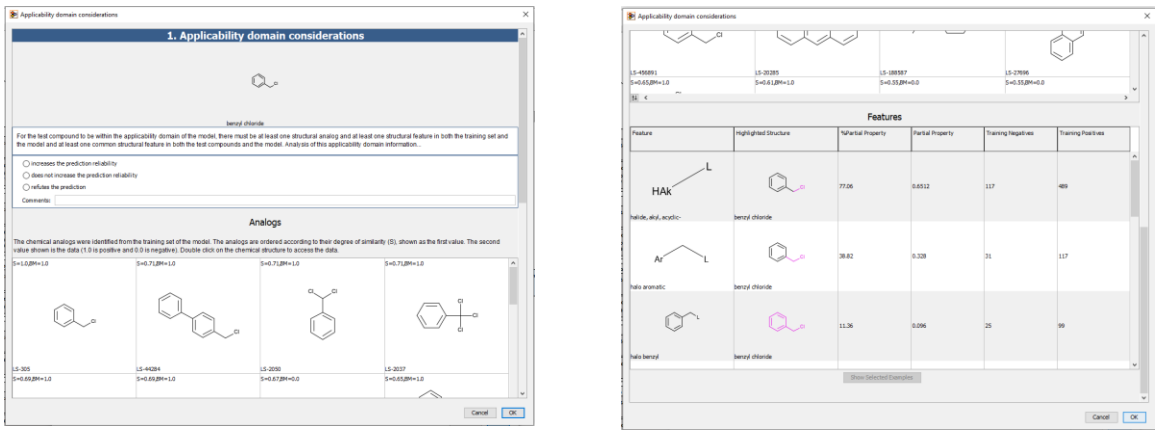


Figure 28: Contextual information supporting an expert review of the in silico results

Tables 1 and 2 summarizes the available expert review options, the information presented to support any response and options to support a customizable report.

Table 1: Summary of expert review of statistical model guideline dialog and options

Expert review options	Supporting information	Customizable options
Applicability domain considerations	• Analogs • Model features	It is possible to customize the analogs and features to include in the final report
Calculation of probabilities	• Model features and weighting	It is possible to customize the features to include in the final report
Relevancy of model descriptors	• Model features and weighting	It is possible to customize the features to include in the final report
Sufficiency of training set data	• Analogs • Model features	It is possible to customize the analogs and features to include in the final report
Potentially reactive features [only for binary models]	• Listing of possible reactive features showing the number of positive and negative matching examples, the mean value (e.g., the proportion of positives) and a z-score (the number of standard deviations from the mean, with positive z-score values above the mean and negative z-score values below the mean)	It is possible to add (or remove) custom substructure features as well as customize the analogs and features to include in the final report
Comparison with drug substance or related compound [only for binary models]	• Side-by-side comparison of the test chemical and physico-chemical properties with a comparison chemical (e.g., API or related chemical)	It is possible to enter a substance to compare the test chemical to, along with experimental data and study report.
Other considerations	• Listing of chemical analogs, including experimental and prediction results	It is possible to customize the analogs to include in the final report

Table 2: Summary of expert review of expert rule-based model guideline dialog and options

Expert review options	Supporting response information	Customizable options
Applicability domain considerations	• Analogs	It is possible to customize the analogs to include in the final report
Calculation of precision	• Summary of the alert with access to the alert definition and matching chemicals	
Sufficiency of data	• Analogs • Matching alert	It is possible to customize the analogs to include in the final report
Potentially reactive features	• Listing of possible reactive features showing the number of positive and negative matching examples, the mean value (e.g., the proportion of positives) and a z-score (the number of standard deviations from the mean, with positive z-score values above the mean and negative z-score values below the mean)	It is possible to add (or remove) custom substructure features as well as customize the analogs and features to include in the final report
Shared alert with known negative (ICH M7 class 4) [Note only available for the bacterial mutagenicity alerts when there are positive or indeterminate results otherwise the item is "Comparison with drug substance or related compound"]	• Side-by-side comparison of the test chemical and physico-chemical properties with a comparison chemical (e.g., API or related chemical)	It is possible to enter a substance to compare the test chemical to, along with experimental data and study report.
Other considerations	• Listing of chemical analogs, including experimental and prediction results	It is possible to customize the analogs to include in the final report

5. Sub-endpoint

The software calculates an assessment for each endpoint (shown as blue boxes) as well as a confidence score based on the rules and principles discussed in the protocol using the items shown as inputs to the endpoint (assessments, reliability scores, and in some situations confidence score). The confidence scores also take into consideration the completeness of the information available. If any of these inputs (e.g., assessment and reliability score associated with any effect/mechanism) change, then other endpoints in the framework may also be modified. An inspection of any individual endpoint can be made by clicking on the blue box, as shown in Figure 29. This shows an explanation of how the rule was calculated. It also provides an option to perform an expert review on the endpoint. To add such a review, the check box "Manually override calculated value" should be selected. This will then make the outcome and confidence pull-downs and comments box editable and changes can be made and documented.

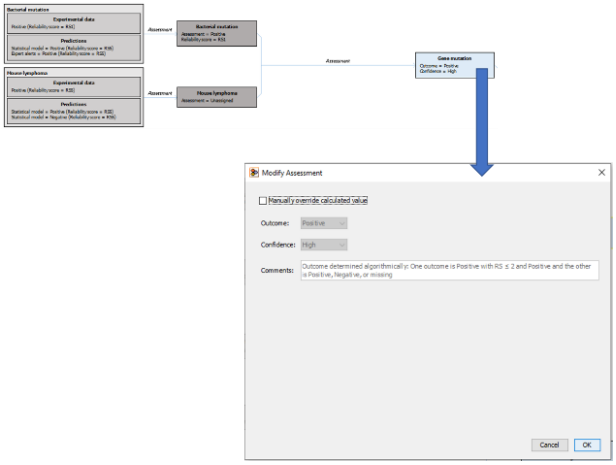


Figure 29: Inspection of an endpoint assessment

6. Major endpoint

As background to the overall assessment it is possible to look up additional data in the Leadscope database. Clicking on the “Find data” button will initiate a search (family structure search) to identify whether other data exists in the Leadscope database.

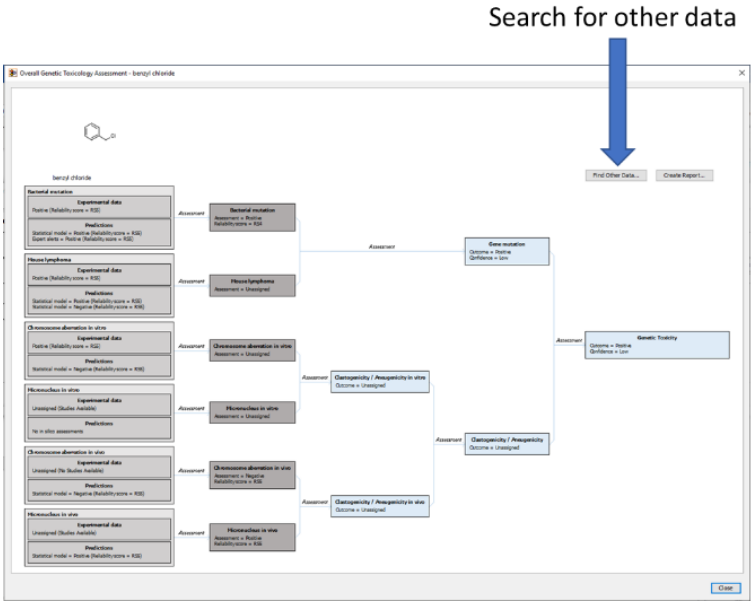


Figure 30: Search other experimental data to support an overall assessment

Since a family search has been initiated, multiple chemical structures may be displayed (as shown in Figure 31); however, only one should be selected. All experimental data available for this chemical will be displayed that may support an expert review of the information.

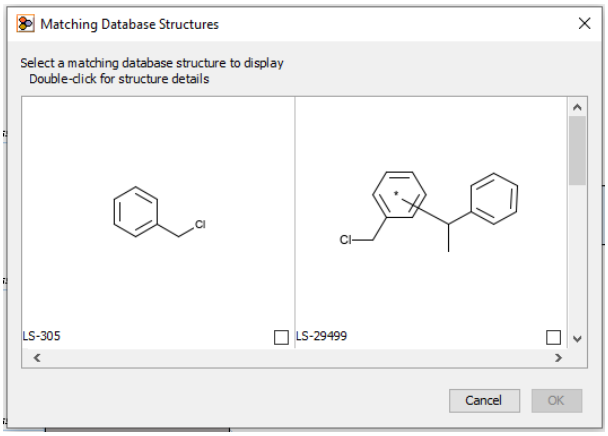


Figure 31: Selecting a chemical to display data from

The standard Leadscope data view is shown in Figure 32 with tabs for the different study types. In addition, for many chemicals a link to the ECHA database is available to support any assessment.

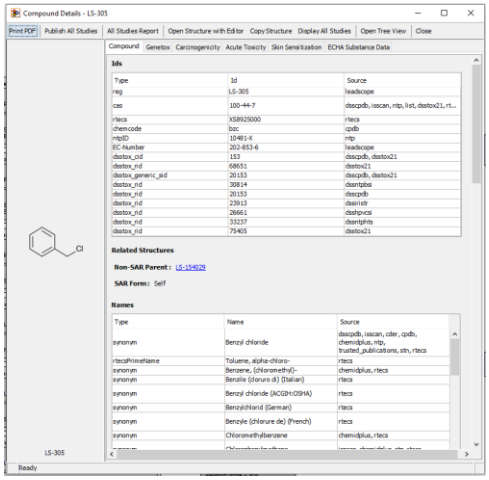


Figure 32: Retrieving data on the selected chemical

Again, the final assessment is automatically calculated based on the protocol rules and principles using information shown in the hazard assessment framework. It is possible to inspect the underlying information by clicking on the blue box, as shown in Figure 33. This provides details on the rules used to calculate the overall endpoint (shown in the Comments field). It is possible to override the results by checking the box “Manually override calculated value” and then making modifications along with comments justifying the changes.

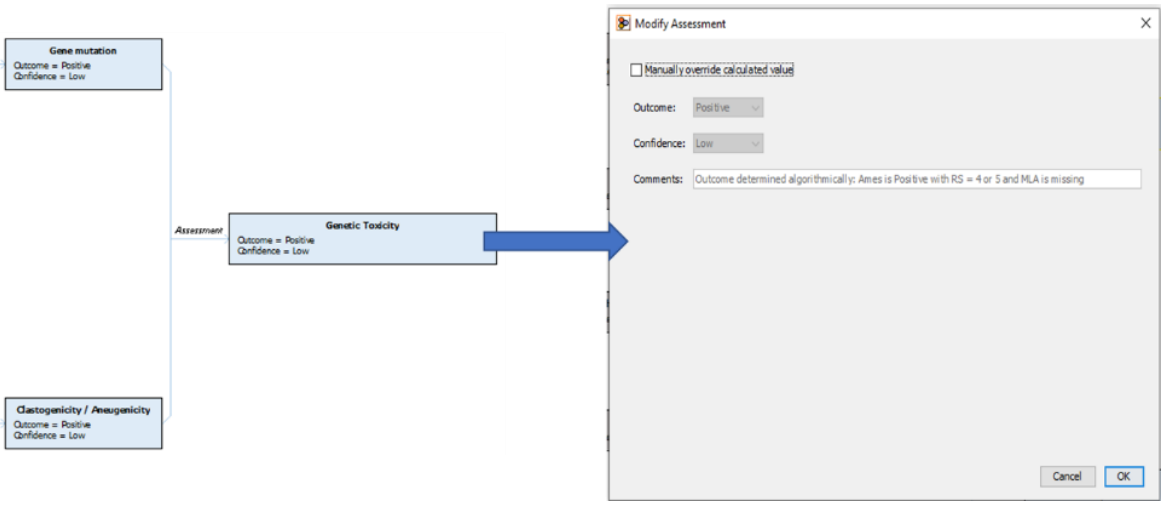


Figure 33: Inspecting information on the endpoint

7. Documentation

A report summarizing the results alongside any expert reviews performed is provided by clicking on the button “Create report…” as shown in Figure 34.

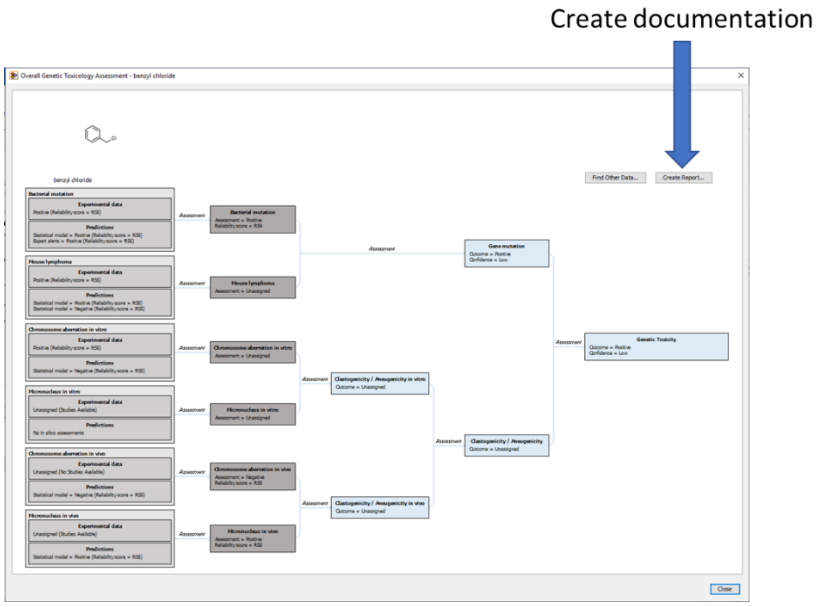


Figure 34: Creating a report on the assessment and expert review

A zip file with a series of folders for each chemical is generated. Within each folder, a single (PDF or Word/RTF) report is included containing the following information:

- An editable title page
- An executive summary including
    - the materials and methods used for the prediction of each effect/mechanism
    - any rules/principles used to combine the information
    - results for the individual effects/mechanisms and associated reliability scores
    - results for the endpoints and associated confidence scores
- The hazard assessment framework view (broken down into a series of graphs) and any expert review comments (and selected supporting information) included in the assessment

Each folder contains additional information including full study and *in silico* reports.

8. ICH M7 protocol implementation

An assessment of genotoxic impurities based on the ICH M7 guideline[8] is performed using the databases and (Q)SAR models shown in Table 3.

Table 3. Databases and prediction methodologies used in the ICH M7 protocol implementation

Expert rule-based methodology and parameters:	Leadscope Bacterial Mutation expert alerts; the domain assessment was turned on; Carcinogenicity Cohorts of Concern Alerts
Statistical-based methodology and parameters:	Leadscope Bacterial Mutation statistical-based QSAR model; the domain assessment was turned on
Genetic toxicity database used for searching:	Leadscope SAR genetox
Rodent carcinogenicity database used for searching:	Leadscope SAR carcinogenicity

Once one or more chemicals have been selected, the tool allows you to initially assign a chemical as an API (Active Pharmaceutical Ingredient) and then assign the remaining chemicals as impurities to process. The tool will then display a table ("Integrated hazard assessment") summarizing the results from applying the models and database searched, as shown in Figure 35.

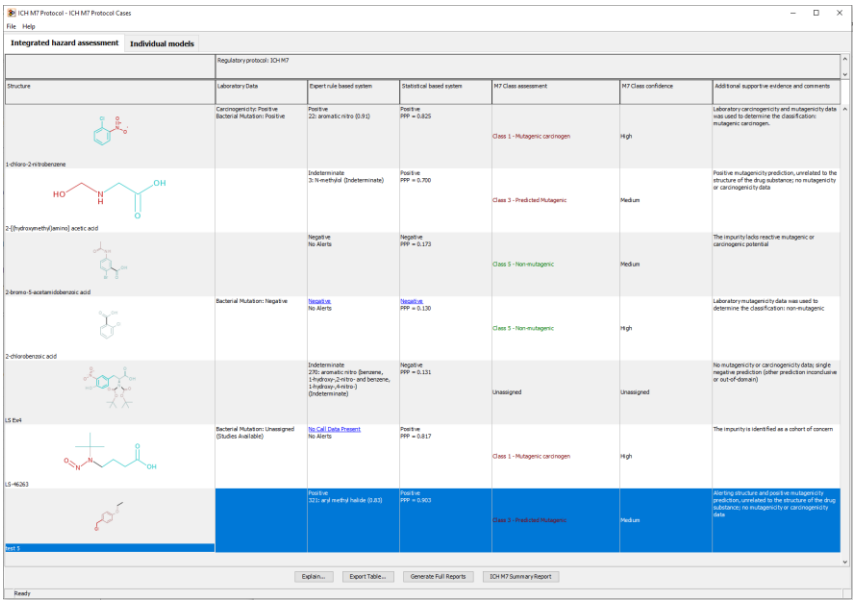


Figure 35: ICH M7 Integrated Hazard Assessment

If no API is selected, the table includes the following columns:

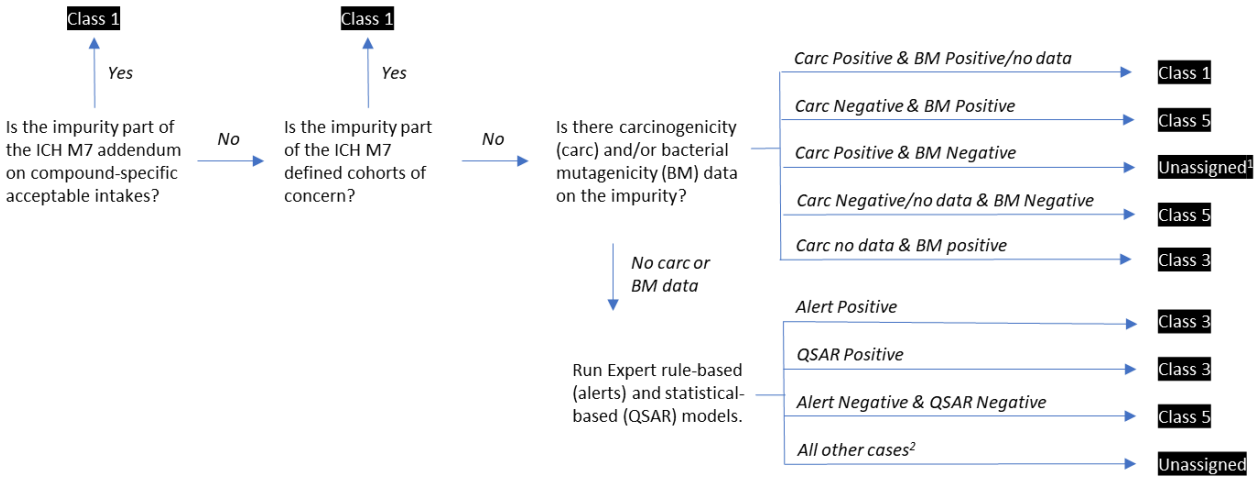
[8] https://database.ich.org/sites/default/files/M7_R1_Guideline.pdf

- **Structure**: A depiction of the chemicals structure with any alerting fragments highlighted in red, deactivating fragments highlighted in blue/green, neutral fragments shown in gray and fragments not covered by the model shown in black.
- **Laboratory Data**: A summary of the laboratory results is displayed.
- **Expert rule-based system**: A summary of the results from running the chemicals through the expert rule-based methodology is displayed, including the call as well as any alerts that fired.
- **Statistical-based system**: A summary of the results from the statistical-based QSAR model are shown alongside the positive prediction probability (PPP) value.
- **M7 Classification**: The impurities are classified in accordance with the classification scheme defined in the ICH M7 guideline (ICH M7 classification table is reproduced in Table 4). The default decision tree to generate this classification is shown in Figure 36.
- **M7 Class confidence**: Generally, results based on experimental data, based on an expert review of the (Q)SAR results or assigned to a cohort of concern are assigned a high confidence whereas results based on a (Q)SAR only assessment are assigned a medium confidence.
- **Additional supportive evidence and comments:** These comments include information supporting the ICH M7 classification.

If an API was selected, this will appear on the first row (no QSAR assessment is performed).

Table 4: ICH M7 classification definitions

Class	Definition
1	Known mutagenic carcinogens
2	Known mutagens with unknown carcinogenic potential (bacterial mutagenicity positive, * no rodent carcinogenicity data)
3	Alerting structure, unrelated to the structure of the drug substance; no mutagenicity data
4	Alerting structure, same alert in drug substance or compounds related to the drug substance (e.g., process intermediates) which have been tested and are non-mutagenic
5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity
*Or other relevant positive mutagenicity data indicative of DNA-reactivity-related induction of gene mutations (e.g., positive findings in in vivo gene mutation studies)	



Notes

1. Likely non-genotoxic carcinogen to be controlled according to ICH Q3C (Permissible Daily Exposure [PDE]). Carcinogens that are negative in the bacterial reverse mutation assay do not have a DNA reactive mechanism of carcinogenicity and therefore are not in scope of the ICH M7 guidance (e.g., acetamide and hydroxylamine). (Q&A 2 July 2020)
2. Single negative prediction (other prediction inconclusive or out-of-domain) or both predictions are inconclusive or out-of-domain

Figure 36: Default rules for deriving the ICH M7 classification

Another view of the results is displayed in the "Individual models" view where is it possible to customize a summary of all the models ran, as well as a more detail view of the specific model results (a separate tab is provided for each model). The summarized view shown in Figure 37 includes a number of columns that can be modified through the "Add/Remove Column" button. One of these columns can also include a "Expert Review" of the individual models which will launch a separate expert review tool as shown in Figure 38.

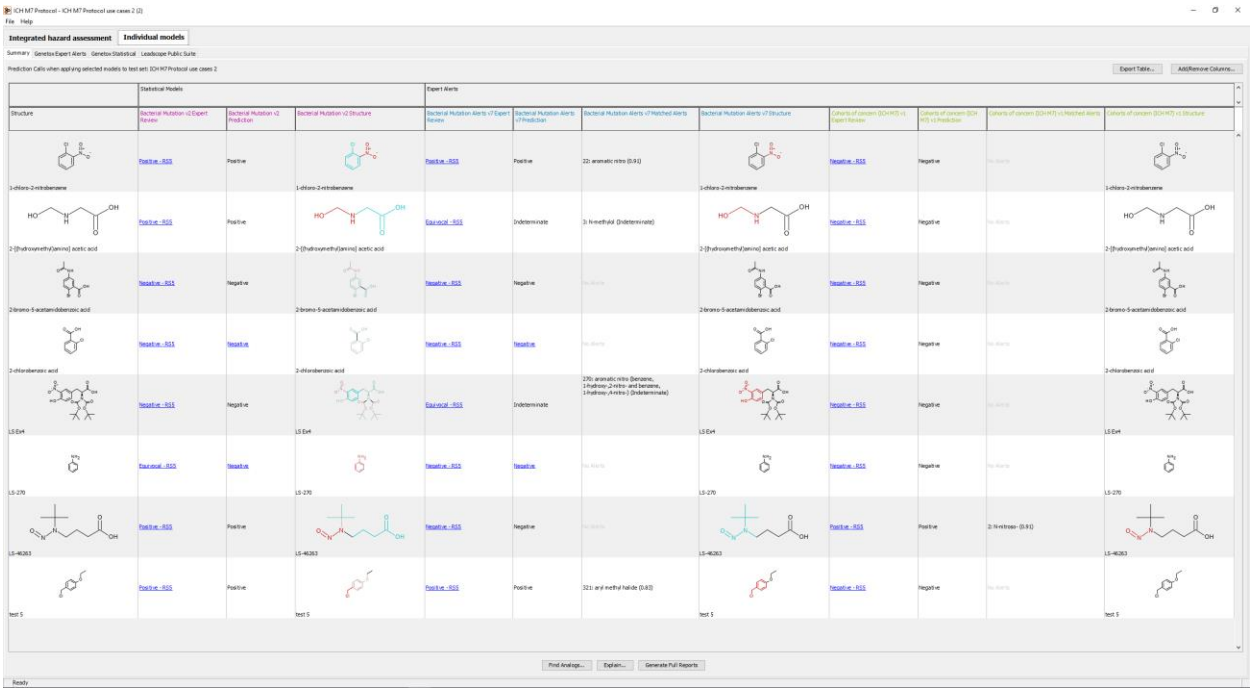


Figure 37: Customizable table showing the results from the individual models

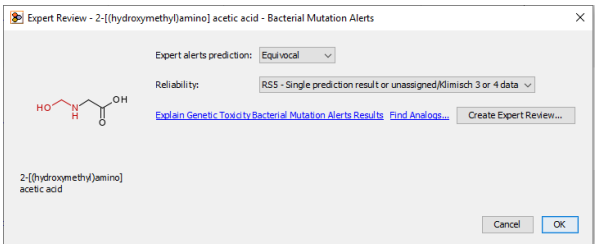


Figure 38: Expert review dialog for an individual result

From the “Integrated hazard assessment” view, the standard hazard assessment window will be displayed by either double clicking on a row or selecting a row and clicking on the “Explain” button, as shown in Figure 39. This window provides the capability to inspect the decision-making process, including any experimental data (bacterial mutagenesis and carcinogenesis data), the two (Q)SAR methodologies to support the prediction of bacterial mutagenicity, as well as the result of the cohort of concern profiler. A guided expert review (as discussed in the previous sections) can be performed on the experimental data and the individual model results, which will be automatically documented. An expert review of how the information was combined is also possible, including manually changing the conclusions and documenting the rationale for these changes.

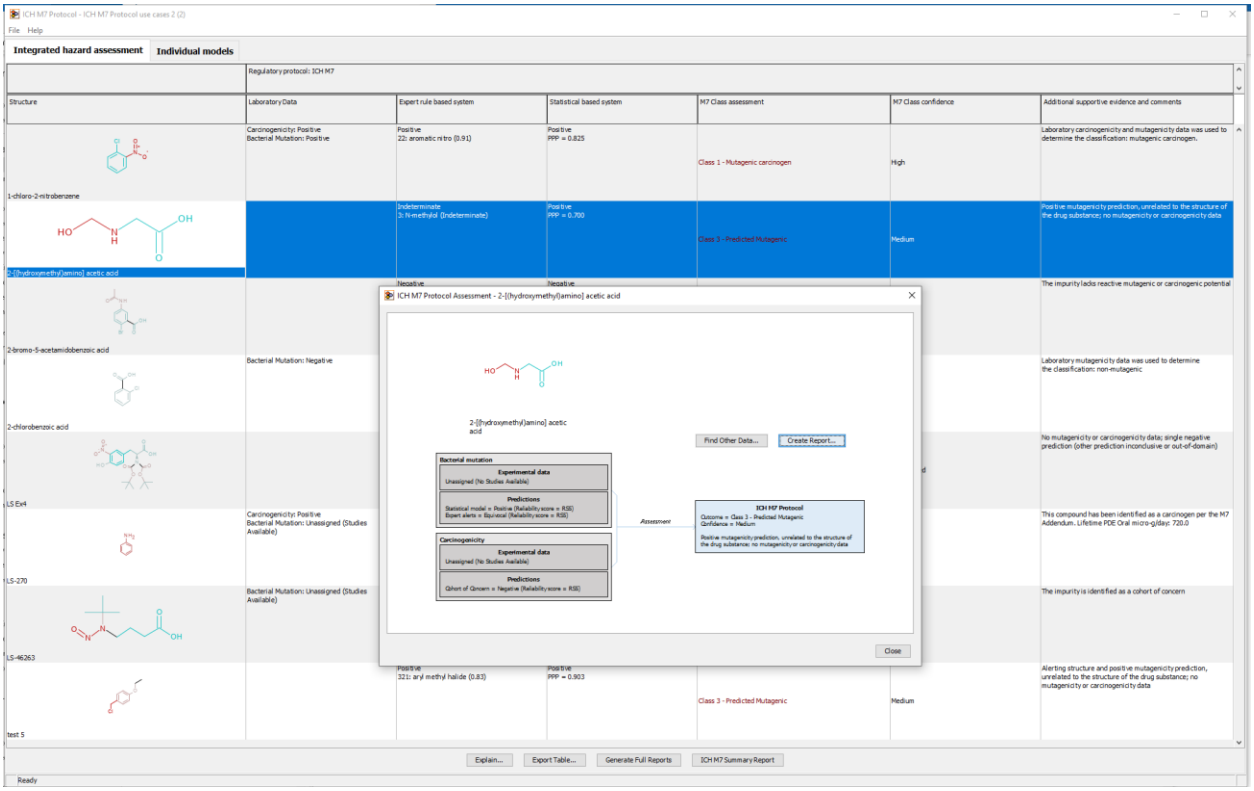


Figure 39: Explaining the result for a chemical analyzed

All comments and modification will be automatically saved and incorporated into any reports. If any changes to the experimental results, the M7 class assignment, the M7 class confidence as well as the M7 class comments will be shown in the “Integrated hazard assessment” table.

A full report can be generated as a zip file containing

- **Summary report (Word RTF format)**: Includes a title page, a description of the materials and methods used, a summary of the report context, a table with a summary of the results which is almost identical to the table shown in the “Integrated hazard assessment” tab, documentation of any expert review of the results as well as references
- **Executive Summary (Excel format):** An excel spreadsheet with the “Integrated hazard assessment” table
- **Folder for each chemical analyzed:** Contains reports for any experimental data and full *in silico* results

An ICH M7 summary report will generate a single summary report as described earlier.

9. Genetic toxicology *in silico* protocols

An assessment of the genetic toxicity hazard was performed for the following toxicological effects and mechanisms, using the methodologies indicated in Table 5.

Table 5. The toxicological effects / mechanisms and endpoints that were predicted and the methodologies used.

Materials and Methods			
Effects or mechanisms	**Methodologies**	**Description**	**Endpoints**
Bacterial Mutation	Database study types: bacterial mutagenesis Statistical model, Expert alerts	Database sources: ccris, dsscpdb, epa-genetox, ntp, publications, trusted_publications Genetic Toxicity Bacterial Mutation Model[9], Genetic Toxicity Bacterial Mutation Alerts	Gene Mutation
Mouse Lymphoma	Database study types: in vitro mammalian mutagenesis Statistical model	Database sources: fda, ntp Genetic Toxicity MLA Activated Model, Genetic Toxicity MLA Unactivated Model	Gene Mutation
Chromosome Aberration *In Vitro*	Database study types: in vitro chromosome aberration Statistical model	Database sources: ccris, ntp Genetic Toxicity CA CHL Model	Clastogenicity / Aneugenicity *In Vitro*
Micronucleus *In Vitro*	Database study types: in vitro micronucleus	Database sources: ccris	Clastogenicity / Aneugenicity *In Vitro*
Chromosome Aberration *In Vivo*	Statistical model	Genetic Toxicity In Vivo CA Model	Clastogenicity / Aneugenicity *In Vivo*
Micronucleus *In Vivo*	Database study types: in vivo micronucleusStatistical model	Database sources: trusted_publications Genetic Toxicity In Vivo Micronucleus Mouse Model[10]	Clastogenicity / Aneugenicity *In Vivo*

Endpoints derived through rules and principles		
Endpoint	**Rules / principles**	**Reference**
Genetic Toxicity	Outlined in the supplemental material of Hasselgren et al., 2019: https://ars.els-cdn.com/content/image/1-s2.0-S0273230019301655-mmc1.docx	Hasselgren, C., et al., Genetic toxicology *in silico* protocol, REGULATORY TOXICOLOGY AND PHARMACOLOGY, 107, 2019, p. 1-21

[9] Landry C, Kim MT, Kruhlak NL, et al. Transitioning to composite bacterial mutagenicity models in ICH M7 (Q)SAR analyses. *Regul Toxicol Pharmacol*. 2019;109:104488. doi:10.1016/j.yrtph.2019.104488

[10] Yoo JW, Kruhlak NL, Landry C, Cross KP, Sedykh A, Stavitskaya L. Development of improved QSAR models for predicting the outcome of the in vivo micronucleus genetic toxicity assay. *Regul Toxicol Pharmacol*. 2020;113:104620. doi:10.1016/j.yrtph.2020.104620

Endpoints derived through rules and principles		
Endpoint	**Rules / principles**	**Reference**
Gene Mutation	Outlined in the supplemental material of Hasselgren et al., 2019: https://ars.els-cdn.com/content/image/1-s2.0-S0273230019301655-mmc1.docx	Hasselgren, C., et al., Genetic toxicology *in silico* protocol, REGULATORY TOXICOLOGY AND PHARMACOLOGY, 107, 2019, p. 1-21
Clastogenicity / Aneugenicity	Outlined in the supplemental material of Hasselgren et al., 2019: https://ars.els-cdn.com/content/image/1-s2.0-S0273230019301655-mmc1.docx	Hasselgren, C., et al., Genetic toxicology *in silico* protocol, REGULATORY TOXICOLOGY AND PHARMACOLOGY, 107, 2019, p. 1-21

In silico models were developed according to the general principles outlined by OECD.

10. Skin sensitization *in silico* protocol

An assessment of the skin sensitization hazard will be performed for the following toxicological effects and mechanisms, using the methodologies indicated in Table 6.

Table 6. The toxicological effects / mechanisms and endpoints that were predicted and the methodologies used.

Materials and Methods			
Effects or mechanisms	**Methodologies**	**Description**	**Endpoints**
Reaction domain	Expert alerts	Skin Sensitization Reaction Domain Alerts	Covalent interaction with skin proteins (KE 1), Events in Keratinocytes (KE2)
Protein reactivity	Statistical model	Database sources: Publications Skin Sensitization DPRA Model	Covalent interaction with skin proteins (KE 1)
Activation of Nrf2-ARE	Statistical model	Database sources: Publications Skin Sensitization KeratinoSens Model	Events in Keratinocytes (KE2)
Expression of co-stimulatory and adhesion molecules	Statistical model	Database sources: Publications Skin Sensitization h-CLAT Model	Events in Dendritic cells (KE3)
Rodent local lymph node proliferation	Database study types: local lymph node assay Statistical model, Expert alerts	Database sources: NICEATM, ECHA records compliant with ECHA license agreement, Publications Skin Sensitization LLNA Model , Skin Sensitization LLNA Alerts	Events in rodent Lymphocytes (KE4)

Materials and Methods			
Effects or mechanisms	**Methodologies**	**Description**	**Endpoints**
Rodent maximization	Database study types: Guinea Pig Maximization Test	Database sources: ICCVAM	Skin Sensitization in Rodents
Human skin sensitization	Database study types: Human Repeat Insult Patch Test and Human Maximization Test	Database sources: Publications	Skin Sensitization in Humans

Endpoints derived through rules and principles		
Endpoint	**Rules / principles**	**Reference**
Skin sensitization *in vitro*	AOP '2 out of 3'	Urbisch D, et al. Assessing skin sensitization hazard in mice and men using non-animal test methods. *Regul Toxicol Pharmacol.* 2015;71(2):337-351
Skin sensitization in humans	Weight of evidence: Rules and principles outlined in publication (CTE)	Johnson C et al., Skin sensitization *in silico* protocol, *Regul Toxicol Pharmacol.* 2020

In silico models were developed according to the general principles outlined by OECD.