DASS App User Guide

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About

The DASS App predicts skin sensitization hazard (sensitizer or non-sensitizer) and potency by applying Defined Approaches on Skin Sensitisation (DASS) that are outlined in OECD Guideline No. 497 [1] and the U.S. EPA's Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing [2]. The defined approaches (DA) integrate data from in vitro assays that represent key events in the Adverse Outcome Pathway (AOP) for Skin Sensitisation Initiated by Covalent Binding to Proteins [3] and in silico hazard predictions.

This app was developed and tested on Windows 10 using R v.4.0.3[4] and Google Chrome v.91.

Step 1: Select Defined Approaches

In the Step 1 tab, select the DAs to be applied. The DAs address the first 3 key events (KEs) in the skin sensitization AOP. Each KE is represented by a validated OECD test method:

1. The direct peptide reactivity assay (DPRA)[5] maps to the first KE, protein binding.
2. The KeratinoSens™ assay [6] maps to the second KE, keratinocyte activation.
3. The human cell line activation test (h-CLAT) [7] maps to the third KE, dendritic cell activation.

In addition, the ITS method integrates an in silico prediction.

Click the green question buttons next to the DA to show a description of the DA and the test methods required to implement the DA. Additional details about the DAs are provided here.

DA: 2 out of 3

The 2 out of 3 (2o3) DA predicts skin sensitization hazard based on KEs 1-3. Two concordant results each from DPRA, KeratinoSens™, or h-CLAT determine the final prediction as a sensitizer or non-sensitizer. If there are only results from two assays and the results are discordant, the chemical can't be classified and will return an "Inconclusive" result.

2o3 does not predict GHS potency category.

DA: Integrated Testing Strategy

The Integrated Testing Strategy (ITS) DA predicts skin sensitization hazard and GHS potency category based on KEs 1 and 3 and *in silico* predictions. Chemicals are scored using the minimum induction threshold (MIT) from the h-CLAT, the mean percent depletion for both Cysteine and Lysine in the DPRA, and hazard predictions from either Derek Nexus or the OECD QSAR Toolbox [8]. The summed scores are used to predict chemical hazard and potency using the scoring schemes in Table 1 and Table 2.

Table 1. Test method scoring scheme for the Integrated Testing Strategy, adapted from [1].

Score	h-CLAT MIT (µg/mL)	DPRA mean %-Cysteine and %-Lysine depletion	DPRA %-Cysteine depletion	In Silico Prediction
3	≤10	≥42.47	≥98.24	
2	>10, ≤150	≥ 22.62, < 42.47	≥ 23.09, < 98.24	
1	>150, ≤5000	≥ 6.38, < 22.62	≥ 13.89, < 23.09	Positive
0	Negative (Not Calculated)	< 6.38	< 13.89	Negative

Table 2. Integrated Testing Strategy potency predictions for combined scores from available information sources. 1 indicates conclusive for hazard and inconclusive for potency. Adapted from [1].*

Combined Score	DPRA + h-CLAT + In Silico Prediction	DPRA + h-CLAT	DPRA or h-CLAT + In Silico Prediction
7	UN GHS 1A	-	-
6	UN GHS 1A	UN GHS 1A	-
5	UN GHS 1B	UN GHS 1*	-
4	UN GHS 1B	UN GHS 1B	UN GHS 1*
3	UN GHS 1B	UN GHS 1B	UN GHS 1*
2	UN GHS 1B	UN GHS 1B	UN GHS 1B
1	NC	Inconclusive	Inconclusive
0	NC	NC	Inconclusive

DA: Key Event 3/1 Sequential Testing Strategy

The KE 3/1 Sequential Testing Strategy (STS) predicts skin sensitization hazard and potency based on KEs 1 and 3. If the h-CLAT predicts a sensitizer, then the hazard and potency categories are determined by the h-CLAT MIT. If the h-CLAT predicts a non-sensitizer, then the hazard and potency categories are determined by DPRA results. The KE 3/1 STS scheme is shown in Table 3.

Table 3. Hazard identification and potency prediction scheme for Key Event 3/1 Sequential Testing Strategy. Adapted from [9], [10].

Test Method	Result	Hazard	GHS Potency Category
h-CLAT	MIT ≤ 10	Sensitizer	1A
h-CLAT	MIT > 10, < 5000	Sensitizer	1B
h-CLAT	MIT Negative	Use DPRA	Use DPRA
DPRA	Positive	Non-sensitizer	1B
DPRA	Negative	Non-sensitizer	NC

Step 2: Upload Data

To upload the data, first click *Browse* and select your file. Then click *Upload* to load the data into the app. If you have uploaded an Excel file, select the appropriate worksheet from the dropdown menu. The data will be displayed for your review. Once you have selected the DAs and reviewed your data, click *Continue* to proceed to the next step.

Prior to uploading data, ensure that the data meet the Data and Formatting Requirements.

Data and Formatting Requirements

General

1. File can be comma-delimited (.csv), tab-delimited (.tsv, .txt), or a Microsoft Excel workbook (.xls, .xlsx).
2. Data should be in a tabular format with each row corresponding to a single substance and a column for each required assay endpoint.
    - The first row should contain column names.
3. Missing values should be indicated by a blank cell or as 'NA' (without quotes).

Assay Endpoints

Each assay endpoint that is required for implementing the DAs should have a column that is formatted according to the formatting requirements shown in Table 4. Values that do not meet the assay endpoint requirements will be treated as missing data and will not be used to derive predictions.

Table 4. Data format requirements for assay endpoints. Columns 1-3 indicate the DAs that require a given endpoint. Requirements are not case sensitive. X: the DA requires the endpoint. O: This endpoint can be used to derive a required call endpoint.

2o3	ITS	STS	Assay	Endpoint	Format Requirements
X		X	DPRA	Call	• Positive outcomes should be indicated by 'sensitizer', 'sensitiser', 'active', 'a', 'positive', 'pos', 'p', or '1'. • Negative outcomes should be indicated by 'non-sensitizer', 'non-sensitiser', 'inactive', 'i', 'negative', 'neg', 'n', or '0'.
O	X	O	DPRA	%-Cysteine Depletion	• Numeric values only. • No symbols.
O	X	O	DPRA	%-Lysine Depletion	• Numeric values only. • No symbols.
X			h-CLAT	Call	• Positive outcomes should be indicated by 'sensitizer', 'sensitiser', 'active', 'a', 'positive', 'pos', 'p', or '1'. • Negative outcomes should be indicated by 'non-sensitizer', 'non-sensitiser', 'inactive', 'i', 'negative', 'neg', 'n', or '0'.
	X	X	h-CLAT	Minimum Induction Threshold	• For positive h-CLAT outcomes, numeric values only. No symbols. • Indicate negative h-CLAT outcomes with 'non-sensitizer', 'non-sensitiser', 'Inf', 'i', 'inactive', 'n', 'neg', or 'negative'
X			KeratinoSens™	Call	• Positive outcomes should be indicated by 'sensitizer', 'sensitiser', 'active', 'a', 'positive', 'pos', 'p', or '1'. • Negative outcomes should be indicated by 'non-sensitizer', 'non-sensitiser', 'inactive', 'i', 'negative', 'neg', 'n', or '0'.
O			KeratinoSens™	Imax	• Numeric values only. • No symbols.
	X		In Silico Prediction	Call	• Positive outcomes should be indicated by 'sensitizer', 'sensitiser', 'active', 'a', 'positive', 'pos', 'p', or '1'. • Negative outcomes should be indicated by 'non-sensitizer', 'non-sensitiser', 'inactive', 'i', 'negative', 'neg', 'n', or '0'.
	X		In Silico Prediction	Applicability Domain	• Predictions within the applicability domain should be indicated by '1' or 'In'. • Predictions outside the applicability domain should be indicated by '0' or 'Out'. These will be omitted from analysis.

Data Template

A downloadable data template is provided in tab-delimited and Excel format. The template is filled with example data that should be deleted before entering your data. The template contains a column for CASRNs and columns for every possible assay endpoint. Additional columns (e.g., other chemical identifiers) can be added to the file if needed. If an assay endpoint will not be used, the corresponding column can be deleted but that is not required.

Using the template is not required.

Step 3: Select Data Columns for Predictions

After clicking *Continue*, the panel for column selection will expand. All assay endpoints required by the selected DAs will be shown. Dropdown menus under each assay endpoint contain the column names from the uploaded data. Use the dropdown menus to select the names of the columns corresponding to each given assay endpoint. Each column should only be selected once. The app will attempt to pre-fill the selections by matching column names with those in the data template.

Click the green question buttons next to the assay endpoint names to show information about the given endpoint.

When you are done selecting columns, click *Done* to proceed to the next step.

Step 4: Review Selection

After clicking *Done*, the selected columns will be evaluated against the data and formatting requirements. A table with 3 columns will be displayed. The first column, Variable contains the name of the assay endpoint. The second column, Selected Column contains the name of the data column that was selected in the previous step. If the data column contains any invalid values, the third column, Flag will contain text describing the requirements that were violated.

Verify that the selected columns are correct. If a unique column was selected for more than one assay endpoint, a warning will be displayed. If needed, return to the previous step to change the selected column, and click *Done* to update the table.

Rows corresponding to a flagged user column will contain orange text. Review the formatting of the flagged user column. Updates to the data formatting must be made externally and the data will need to be re-uploaded.

After reviewing the warnings and flags, click *Run* to run the DASS predictions using the columns shown in Selected Column. If there are any unresolved flags, any invalid values will be marked as missing data and will not be used for predictions.

Step 5: Results

After the DAs have been applied, a table with the DASS predictions will be displayed in the Results panel. The DASS predictions are appended to the uploaded data and are highlighted in blue. If ITS was selected, then the individual ITS scores are also appended and highlighted in blue. The DASS prediction columns are described in Table 5. The data columns that were selected in Step 2 are

highlighted in yellow. These data columns are reformatted for use in the DAs. The reformatted columns are appended to the data and highlighted in pink.

Table 6 contains a description of the input columns and how they were reformatted. It may be useful to compare the selected columns and their transformations to ensure that data were properly interpreted, especially if the DAs were run with flagged data.

Use the Download Results dropdown menu to download the results table as a tab-delimited or Excel file.

Table 5. Descriptions of prediction columns in the results table.

Column Name	Description
DA ITS h-CLAT Score	The ITS score for the h-CLAT MIT outcomes
DA ITS DPRA Score	The ITS score for the DPRA %-Cysteine and %-Lysine outcomes
DA ITS in Silico Score	The ITS score for the in Silico outcome
DA ITS Total Score	The final score from the ITS DA that was used to predict call and potency
DA ITS Call	Hazard prediction from the ITS DA
DA ITS Potency	Potency prediction from the ITS DA
DA 2o3 Call	Hazard prediction from the 2o3 DA
DA KE 3/1 STS Call	Hazard prediction from the KE 3/1 STS DA
DA KE 3/1 STS Potency	Potency prediction from the KE 3/1 STS DA

Table 6. Descriptions of columns in the results that contain reformatted user column data.

Column Name	Description
DPRA Call Input	Positive outcomes are set to 1 and negative outcomes are set to 0. Any invalid values are set to NA.
DPRA %-C Depletion Input	Only numeric values are retained. Any invalid values are set to NA.
DPRA %-K Depletion Input	Only numeric values are retained. Any invalid values are set to NA.
DPRA Mean (Calculated)	The calculated mean of DPRA %-C and %-K depletion values, where any negative values are set to 0 when calculating the mean. DPRA mean is used when the ITS DA is selected or when DPRA hazard is derived from depletion values.
DPRA Call Input (Calculated)	The outcome derived from DPRA Mean (Calculated) when 2o3 or KE 3/1 STS DAs are selected, and the user provides DPRA %-C and %-K depletion columns instead of a DPRA hazard column.
h-CLAT Call Input	Positive outcomes are set to 1 and negative outcomes are set to 0. Any invalid values are set to NA.
h-CLAT MIT Input	Numeric values for positive h-CLAT outcomes are retained. In addition, negative h-CLAT outcomes are set to Inf. Any invalid values are set to NA.

Keratinosens(TM) Imax Input	Only numeric values are retained. Any invalid values are set to NA.
Keratinosens(TM) Call Input	Positive outcomes are set to 1 and negative outcomes are set to 0. Any invalid values are set to NA.
Keratinosens(TM) Call Input (Calculated)	The outcome derived from KeratinoSens(TM) Imax Input.
In Silico Call Input	Positive outcomes are set to 1 and negative outcomes are set to 0. Any invalid values are set to NA.
In Silico Applicability Domain Input	Predictions in the applicability domain are set to 1 and predictions outside the applicability domain are set to 0. Any invalid values are set to NA.

References

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[3] OECD, *The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins*. 2014. doi: https://doi.org/https://doi.org/10.1787/9789264221444-en.

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[9] Y. Nukada, M. Miyazawa, S. Kazutoshi, H. Sakaguchi, and N. Nishiyama, "Data integration of non-animal tests for the development of a test battery to predict the skin sensitizing potential and potency of chemicals," *Toxicol. In Vitro*, vol. 27, no. 2, pp. 609–618, 2013, doi: https://doi.org/10.1016/j.tiv.2012.11.006.

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