DASS App User Guide

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About

This app uses the Defined Approaches for Skin Sensitization (DASS) outlined in the OECD DASS Guideline No. 497[1]. The Defined Approaches (DAs) integrate results from *in vitro* and *in silico* test methods to predict chemical hazard potential.

System Requirements

This app works on Windows. This app requires R. When first launching the app locally, the *renv*[2] package will be installed. While launching, the data.table[3], DT[4], shiny[5], shinyBS[6], and shinyjs[7] packages will be installed.

This app was developed and tested on Windows 10 using R v.4.1.2[8] and Google Chrome v.91.

Installation

If installing from Bitbucket, clone the repository using the "Clone" button at the top of the repository. If installing from zip file, unzip the folder.

Open the downloaded folder. To launch the app, click the run_app.bat file.

Data Import

Once the app is launched, to begin, click *Browse* to upload your data. The data file must be comma-delimited (.csv) or tab-delimited (.tsv, .txt). The first row should contain column names. Columns must be formatted as described in Step 2. Once you select a file, the data can be viewed by clicking on the *View Data* tab.

Step 1: Select Defined Approaches

After uploading your data, the *Step 1* tab will open. Select the DAs you want to apply and click Done. The DAs are based on the first 3 key events (KEs) in the Adverse Outcome Pathway (AOP) for Skin Sensitization Initiated by Covalent Binding to Proteins[9]. Each KE is represented by a validated OECD test method:

1. The direct peptide reactivity assay (DPRA)[10] maps to the first KE, protein binding.
2. The KeratinoSens™[11] assay maps to the second KE, keratinocyte activation.
3. The human cell line activation test (h-CLAT)[12] maps to the third KE, dendritic cell activation.

2 out of 3

The 2 out of 3 (2o3) DA is a sequential testing strategy that identifies skin sensitization hazard based on KEs 1-3. Two concordant results from DPRA, KeratinoSens™, or h-CLAT determine the final classification 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 evaluate potency.

Integrated Testing Strategy

This app implements version 2 of the Integrated Testing Strategy (ITSv2) DA. ITSv2 predicts skin sensitization hazard potential and potency category based on KEs 1 and 3 and *in silico* predictions from the OECD QSAR Toolbox[13]. Chemicals are scored for each assay result and the summed scores are used to predict chemical hazard and potency using the scoring schemes in Tables 1 and 2.

Score	h-CLAT MIT (μg/mL)	DPRA mean Cysteine and Lysine % depletion	DPRA Cysteine % depletion	OECD QSAR Toolbox
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 1. Test method scoring scheme for version 2 of the Integrated Testing Strategy defined approach, adapted from [1].

Combined Score	DPRA + h-CLAT + OECD QSAR TB	DPRA + h-CLAT	DPRA + OECD QSAR TB or h-CLAT + OECD QSAR TB
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

Table 2. Potency predictions for combined scores from available information sources. 1* indicates conclusive for hazard, inconclusive for potency. Adapted from [1].

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 potency category is determined by the h-CLAT Minimum Induction Threshold (MIT). If the h-CLAT predicts a non-sensitizer, then the DPRA result defines both hazard and potency. The KE 3/1 STS scheme is shown in Table 3.

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

Table 3. Hazard and potency prediction scheme. Adapted from [14] and [15].

Step 2: Select Data Columns for Predictions

After clicking "Done," the panel for column selection will expand. All assay endpoints that are needed to apply the selected DAs will be shown. Use the dropdown menus to select the names of the columns corresponding to each given assay result. Click "Done" to evaluate the values in the column for proper formatting.

Columns must be formatted correctly to ensure an accurate prediction. Descriptions of the column requirements are given below.

DPRA

% Depletion

DPRA % Depletion is used in ITSv2. The columns for % Cysteine (%C) and % Lysine (%K) depletion should contain only numeric values or "NA" if the data do not exist for a given chemical.

The mean of %C and %K depletion for each chemical is used to score the chemical using the scoring scheme shown in Table 1. Any negative %C or %K depletion values are set to 0 when calculating the mean, as specified in OECD Test Guideline 442c[10]. If there is no %C depletion value for a given chemical, then the DPRA results can't be used for that chemical. If only the value for %K depletion is missing, then the %C depletion values are used for scoring, with a different scoring scheme as shown in Table 1.

Call

DPRA call should be an indicator for a positive or negative outcome from DPRA. Positive outcomes must be indicated by "p," "pos," "positive," or 1.Negative outcomes must be indicated by "n," "neg," "negative," or 0. Any other values will not be used to predict skin sensitization hazard.

Alternatively, the %C and %K depletion values can be provided and the app will define the chemical call as outlined in OECD Test Guideline 442c [10].

h-CLAT

Call

h-CLAT call should be an indicator for a positive or negative outcome from h-CLAT. Positive outcomes must be indicated by "p," "pos," "positive," or 1. Negative outcomes must be indicated by "n," "neg," "negative," or 0. Any other values will not be used to predict skin sensitization hazard.

Minimum Induction Threshold

h-CLAT minimum induction threshold (MIT) must contain either positive numeric values for positive outcomes or "n," "neg," "negative," or "Inf" to indicate a negative outcome. Any other values, including "NA," will not be used to predict skin sensitization potency.

KeratinoSens™ Call

KeratinoSens™ (KS) call should be an indicator for a positive or negative outcome from the KS assay. Positive outcomes must be indicated by "p," "pos," "positive," or 1. Negative outcomes must be indicated by "n," "neg," "negative," or 0. Any other values will not be used to predict skin sensitization hazard.

OECD QSAR Toolbox

Call

The OECD QSAR Toolbox (TB) call should be an indicator for a positive or negative prediction where positive predictions are indicated by "p," "pos," "positive," or 1. Negative predictions must be indicated by "n," "neg," "negative," or 0. Any other values will not be used to predict skin sensitization hazard.

Applicability Domain

The OECD QSAR Toolbox (TB) applicability domain (AD) should be an indicator for whether the chemical is in the AD of the toolbox's models. A value of "In" or 1 indicates that the chemical is in the AD. A value of "Out" or 0 indicates that the chemical is outside the AD and the OECD QSAR TB prediction will not be used to predict skin sensitization potency.

Step 3: Review Selection

After clicking "Done" in *Step 2*, the selected columns will be evaluated for proper formatting. The *Step 3* tab will display a table with 3 columns. The "Variable" column contains the name of the assay endpoint the app requested. The "Selected Column" column has the name of the column selected for the assay endpoint. Verify that the selected column is correct. If needed, return to *Step 2*, update the selected columns and click "Done" to update the table in *Step 3*. The "Flag" column will contain text describing the formatting requirement that was violated. Updates to the data format must be made externally, and the data will need to be re-uploaded.

After reviewing the selections, 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 and will not be used to predict skin sensitization hazard and potency.

Step 4: Results

The *Step 4* tab will show a table with the original data with DASS prediction columns appended to the end. Values calculated by the app and used in predictions will also be appended to the data. Prior to running the predictions, the selected columns are reformatted to ensure the values are properly formatted. The reformatted columns are appended to the data, showing exactly how the input data were processed and what values were used for each prediction.

Click the *Download Results* button to download a file with the results table.

References

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