DASS App User Guide

10 February, 2022

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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

To begin, click *Browse* and select your data file. The data must be comma-delimited (.csv), tab-delimited (.txt, .tsv), or the first worksheet in an excel file (.xls, .xlsx). Data should be in a tabular format with rows corresponding to chemicals and columns corresponding to assay endpoints. The first row should contain column names. Each row, except the first, should contain data for only a single chemical (i.e., a single row should not contain data for multiple chemicals). Each assay endpoint that is required for using the DAs should have a column that is formatted as described in Step 2 below.

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 predicts skin sensitization hazard identification (sensitizer or non-sensitizer) based on KEs 1-3. Two concordant results 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.

Integrated Testing Strategy

The Integrated Testing Strategy (ITS) DA predicts skin sensitization hazard identification and potency category based on KEs 1 and 3 and *in silico* predictions from either Derek Nexus or the OECD QSAR Toolbox[13]. Chemicals are scored for each assay result and the summed scores are used to predict chemical hazard identification 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	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 1. Test method scoring scheme for the Integrated Testing Strategy, 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

Table 2. Integrated Testing Strategy 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 identification and potency based on KEs 1 and 3. If the h-CLAT predicts a sensitizer, then the hazard identification and potency categories are determined by the h-CLAT Minimum Induction Threshold (MIT). If the h-CLAT predicts a non-sensitizer, then the hazard identification and potency categories are determined by DPRA results. The KE 3/1 STS scheme is shown in Table 3.

Test Method	Result	Hazard Identification	GHS Potency Category
h-CLAT	MIT $\leq$ 10	Positive	1A
h-CLAT	MIT > 10, $\leq$ 5000	Positive	1B
h-CLAT	MIT Negative	Use DPRA	Use DPRA
DPRA	Positive	Positive	1B
DPRA	Negative	Negative	NC

Table 3. Hazard identification and potency prediction scheme for Key Event 3/1 Sequential Testing Strategy. 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. Dropdown menus under each assay endpoint contain the column names from your data. Use the dropdown menus to select the names of the columns corresponding to each given

assay endpoint. Each column can only be selected once. 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 %-Cysteine (%C) and %-Lysine (%K) depletion values are used in the ITS DA. The columns for %C and %K depletion should contain only numeric values. Numeric values should not have commas. Missing values should be blank or labeled as "NA." Any invalid values will be treated as missing and will not be used to predict skin sensitization hazard.

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.

Hazard Identification

DPRA hazard identification is used in the 2o3 and KE 3/1 STS DAs. The column for hazard identification 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. The values are not case sensitive. Missing values should be blank or labeled as "NA." Any invalid values will be treated as missing and will not be used to predict skin sensitization hazard.

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

h-CLAT

Hazard Identification

H-CLAT hazard identification is used in the 2o3 DA. The column for h-CLAT hazard identification 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. The values are not case sensitive. Missing values should be blank or labeled as "NA." Any invalid values will be treated as missing and will not be used to predict skin sensitization hazard.

Minimum Induction Threshold

The h-CLAT minimum induction threshold (MIT) is used in the ITS and KE 3/1 STS DAs. The column for h-CLAT MIT must contain either positive numeric values for positive outcomes or "n," "neg," "negative," or "Inf" to indicate negative outcomes. The values are not case sensitive. Missing values should be blank or labeled as "NA." Any invalid values will be treated as missing and will not be used to predict skin sensitization hazard.

KeratinoSens™ Hazard Identification

KeratinoSens™ (KS) hazard identification is used in the 2o3 DA. The column for KS hazard identification 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. The values are not case sensitive. Missing values should be blank or labeled as "NA." Any invalid values will be treated as missing and will not be used to predict skin sensitization hazard.

Alternatively, iMax values can be provided and evaluated for hazard identification. The column corresponding to KS iMax should only contain numeric values. Chemicals with KS iMax values ≥ 1.5 are labeled as positive and chemicals with KS iMax values <1.5 are labeled as negative.

In Silico Prediction

Hazard Identification

In silico predictions should be derived from either Derek Nexus or the OECD QSAR Toolbox[13]. The column for *in silico* hazard identification 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. The values are not case sensitive. Missing values should be blank or labeled as "NA." Any invalid values will be treated as missing and will not be used to predict skin sensitization hazard.

Applicability Domain

The applicability domain (AD) from the user's chosen *in silico* tool should be an indicator for whether the chemical is in the AD of the tool'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 *in silico* prediction will not be used to predict skin sensitization hazard. The values are not case sensitive. Missing values should be blank or labeled as "NA." Any invalid values will be treated as missing and will not be used to predict skin sensitization hazard.

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 data column that was selected for the assay endpoint. Verify that the selected columns are 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. Review the format of any flagged columns. 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.

Step 4: Results

The *Step 4* tab will display a table with the DASS predictions appended to the user's data. 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. 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. Data should be transformed as:

Column Name	Description
DPRA Hazard Id. Input	
DPRA DPRA DPRA Mean (Calculated)	
DPRA Hazard Id. Input (Calculated)	
h-CLAT Hazard Id. Input	
h-CLAT MIT Input	
h-CLAT MIT Input (Numeric Only)	
Keratinosens(TM) iMax Input	
Keratinosens(TM) Hazard Id. Input	
Keratinosens(TM) Hazard Id. Input (Calculated)	
In Silico Hazard Id. Input	
In Silico Applicability Domain Input	

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

References

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