# DASS App User Guide

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## About

The DASS App is a user-friendly web tool for applying defined approaches on skin sensitization (DASS) that are described in Guideline No. 497 from the Organisation for Economic Co-operation and Development (OECD) (OECD 2021a) and the U.S. Environmental Protection Agency (EPA) Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing (EPA). The DASS App predicts both skin sensitization hazard (either a sensitizer or non-sensitizer) and potency for chemicals of interest. For assigning potency predictions to query chemicals, the DASS App uses categories established by the United Nations Globally Harmonized System for Classification and Labelling of Chemicals (GHS).

The defined approaches (DAs) integrate data from in vitro assays that represent key events (KEs) in the Adverse Outcome Pathway (AOP) for Skin Sensitisation Initiated by Covalent Binding to Proteins (OECD 2014) and in silico hazard predictions. Each KE is represented by a validated OECD test method:

1. The direct peptide reactivity assay (DPRA)(OECD 2021b) maps to KE 1, protein binding.

2. The KeratinoSens™ assay (OECD 2018a) maps to KE 2, keratinocyte activation.

3. The human cell line activation test (h-CLAT) (OECD 2018b) maps to KE 3, dendritic cell activation.

In addition, one of the DAs, the Integrated Testing Strategy (ITS) method, integrates an in silico prediction of skin sensitization potential generated by either Derek Nexus or the OECD QSAR Toolbox (Yordanova et al. 2019).

The app runs the DA analysis using data from a file you provide; data templates in Excel and text format are provided to help you correctly format your data. No data is provided by or retained by the app, and it can thus be used to analyze data from substances in development.

This app was developed by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). Version 1.0 was tested on Windows 10 using R v.4.2.2(R Core Team 2022) and Google Chrome v.91. The DASS App is available at https://ntp.niehs.nih.gov/go/952311.

For more information or to report a problem with the app, please contact NICEATM at ICE-support@niehs.nih.gov.

## Step 1: Select Defined Approaches

In Step 1, select the DAs to be applied. Click the green information buttons next to the DA name to view a description of the DA and the test methods required to implement the DA.

## 2 out of 3 DA

The 2 out of 3 (2o3) DA predicts skin sensitization hazard based on KEs 1-3. The prediction is based on results from the DPRA, KeratinoSens, and h-CLAT, with at least two concordant results from the three assays determining the final prediction as a sensitizer or non-sensitizer

(Figure 1). If there are only results from two assays and the results are discordant, the chemical can't be classified and the evaluation will return an "Inconclusive" result.
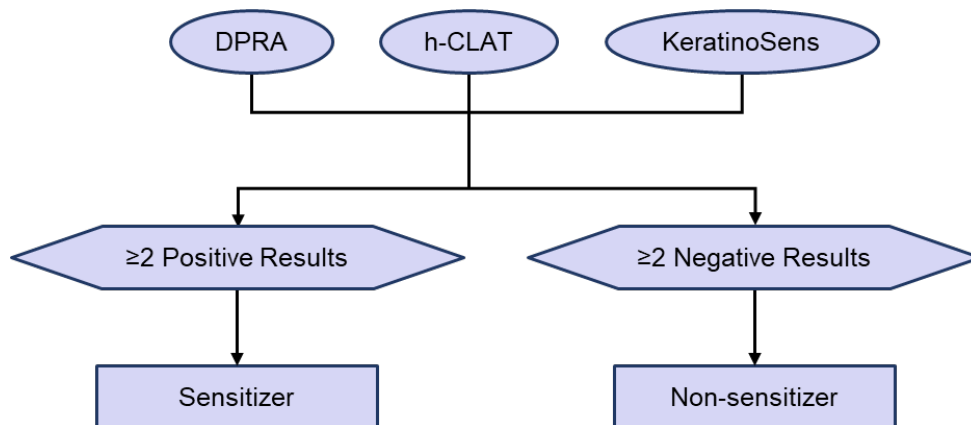
2o3 does not predict GHS potency category.



**Figure 1. Diagram of the 2o3 DA data interpretation procedure**

## Integrated Testing Strategy DA

The 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 (Yordanova et al. 2019). The summed scores are used to predict chemical hazard and potency (Figure 2).

**DPRA**

**Only %-Cys**

| %-Cys Depletion | Score |
|---|---|
| x ≥ 98.24 | 3 |
| 23.09 ≤ x < 98.24 | 2 |
| 13.89 ≤ x < 23.09 | 1 |
| x < 13.89 | 0 |

**Both %-Cys and %-Lys**

| Mean %-Cys and %-Lys Depletion | Score |
|---|---|
| x ≥ 42.47 | 3 |
| 22.62 ≤ x < 42.47 | 2 |
| 6.38 ≤ x < 22.62 | 1 |
| x < 6.38 | 0 |

**h-CLAT**

| Minimum Induction Threshold | Score |
|---|---|
| x ≤ 10 | 3 |
| 10 < x ≤ 150 | 2 |
| 150 < x ≤ 5000 | 1 |
| Negative | 0 |

**In Silico Prediction**

| Prediction | Score |
|---|---|
| Positive | 1 |
| Negative | 0 |

**Sum scores**

**DPRA + h-CLAT + In Silico Prediction**

| Combined Score | Hazard | Potency |
|---|---|---|
| 6-7 | Sensitizer | 1A |
| 2-5 | | 1B |
| 0-1 | Non-sensitizer | NC |

**DPRA + h-CLAT Only**

| Combined Score | Hazard | Potency |
|---|---|---|
| 6 | | 1A |
| 5 | Sensitizer | Inconclusive |
| 2-4 | | 1B |
| 1 | Inconclusive | Inconclusive |
| 0 | Non-sensitizer | NC |

**DPRA or h-CLAT + In Silico Prediction**

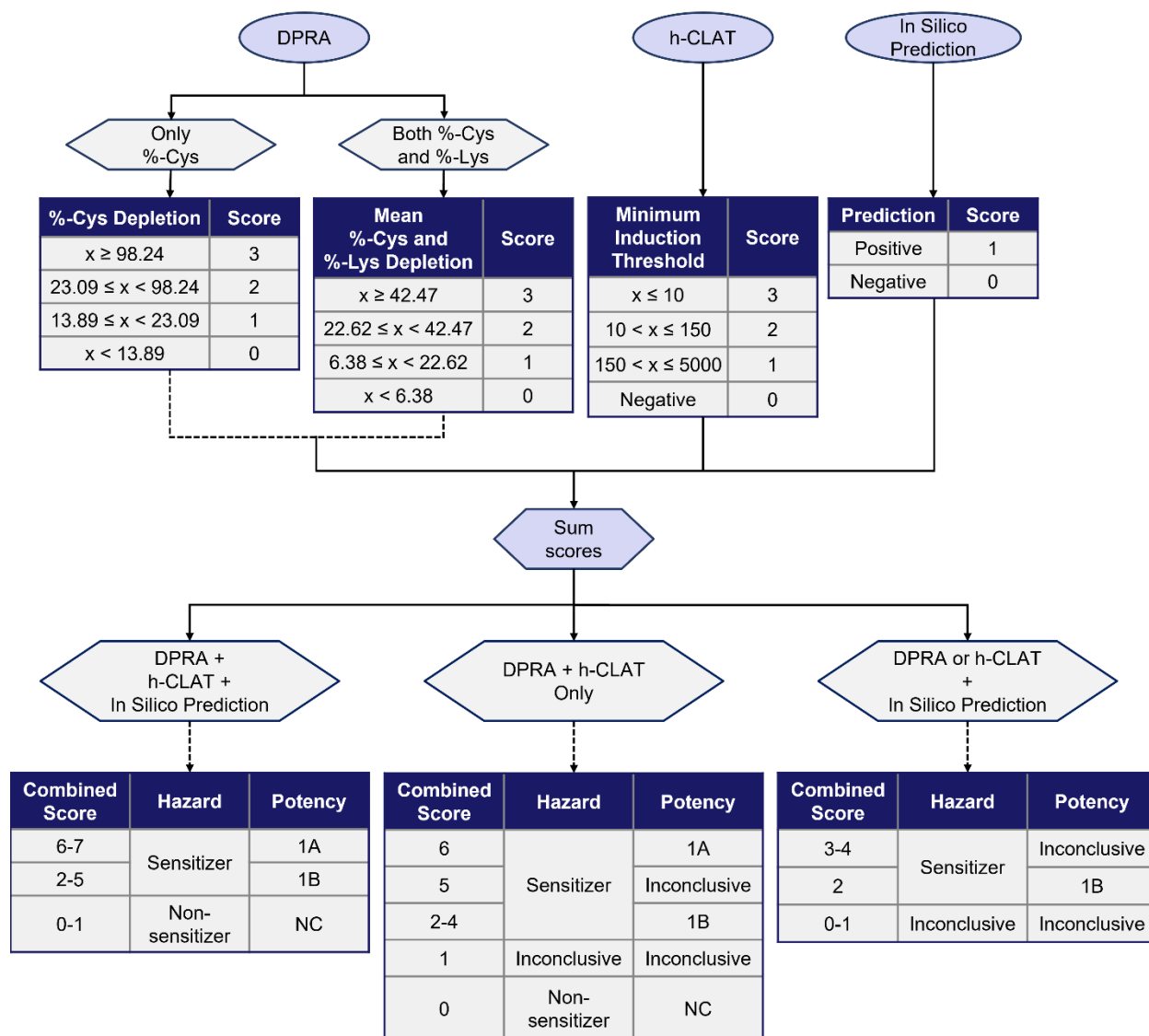| Combined Score | Hazard | Potency |
|---|---|---|
| 3-4 | Sensitizer | Inconclusive |
| 2 | | 1B |
| 0-1 | Inconclusive | Inconclusive |

**Figure 2. Diagram of ITS DA data interpretation procedure**

# Key Event 3/1 Sequential Testing Strategy DA

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 that a chemical will be a sensitizer, then the hazard and potency categories are determined by the h-CLAT MIT. If the h-CLAT predicts the chemical to be a non-sensitizer, then the hazard and potency categories are determined by DPRA results (Figure 3).
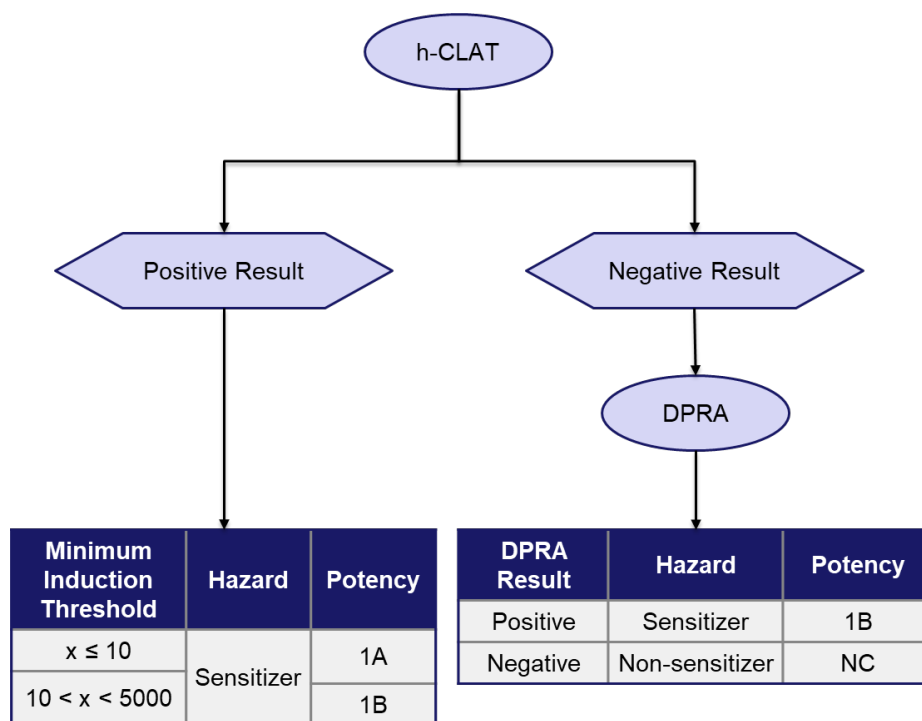
**Figure 3. Diagram of KE 3/1 STS DA data interpretation procedure**

## Step 2: Upload Data

To upload the data, first click "Browse" and select your file using the dialog box that opens. Then click "Upload" to load the data into the app. 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.

3. The first row should contain column names. Column names must be unique.

4. 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 1. Values that do not meet the assay endpoint requirements will be treated as missing data and will not be used to derive predictions.

**Table 1. Data format requirements for assay endpoints.**

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

[Columns 4-6 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.]

# Data Template

A downloadable data template is provided in tab-delimited and Excel format. The template is filled with example data that must 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.

Use of the template is not required.

## Complete Data Upload

If you have uploaded an Excel file, select the appropriate worksheet from the dropdown menu. The data will be displayed for your review. Use the scroll bars on the right side and bottom of the display window to view your entire data set. Once you have reviewed your data, click "Continue" to proceed to the next step.

To clear your data and DA selections and start over, click "Reload App". A dialog box will appear to confirm if you want to do this; click "Reload App" to confirm or "Cancel" to keep your current data and DA selections.

## Step 3: Select Data Columns for Predictions

After clicking "Continue" in Step 2, the Step 3 section will expand, allowing you to select data to be used for the DA analysis. All assay endpoints required by the selected DAs will be shown. Dropdown lists under each assay endpoint contain all of the column names from the uploaded data. Use the dropdown lists to select the name of the column corresponding to each assay endpoint. Each column should only be selected once. The app will automatically select a column for an endpoint if that column name matches the corresponding column name in the data template.

Click the green information buttons next to the assay endpoint names to view information about the endpoints and column formatting requirements.

When you are done selecting columns, click "Done" to proceed to the next step. The app will not allow you to proceed unless you have specified a column from your uploaded data for each required input.

## Step 4: Review Selection

After clicking "Done" in Step 3, the selected columns will be evaluated against the data and formatting requirements. A table with three columns will be displayed allowing you to review your inputs. 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, a warning message will appear at the top of the section, and the third column of the table, "Flag", will contain text noting any requirements for the specific variable that your data have violated. A warning message will also appear at the top of the section if a unique column was selected for more than one assay endpoint.

Verify that the selected columns are correct. If needed, return to Step 3 to change column selections. Clicking "Done" in Step 3 will update the table.

Rows corresponding to a flagged user column will contain orange text. If these appear, first review your selection in Step 3 to verify whether the flag was triggered by an incorrect column selection. If the flag was triggered by incorrect formatting in the source data:

1.  Open your Excel or text file providing the source data and review the formatting of the column corresponding to the flagged data.

2.  Make the needed corrections to the data in the source file.

3.  Return to DASS App Step 2, use the "Reload App" button to clear your current data upload, and upload your corrected data file.

After reviewing the warnings and flags, click "Run" to generate the DASS predictions.

The DASS App will allow you to proceed with generating predictions if your data have 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. Use the scroll bars on the right side and bottom of the display window to view the entire results table. You can also use the Download Results dropdown menu to download the results table as a tab-delimited or Excel file, which may allow easier viewing of the entire table.

- The data columns that were selected in Step 3 are highlighted in yellow. These data columns are reformatted for use in the DAs.

- The reformatted columns are appended to the original data columns and highlighted in pink.

- The DASS predictions are appended to the uploaded data and are highlighted in blue. If the ITS DA was selected, then the individual ITS scores are also appended and highlighted in blue. The DASS prediction columns are described in Table 2.

**Table 2. Descriptions of prediction columns in the results table.**

| Column Name | Description |
|---|---|
| DA ITS h-CLAT Score | ITS score for the h-CLAT MIT outcomes. |
| DA ITS DPRA Score | ITS score for the DPRA %-cysteine and %-lysine outcomes. |
| DA ITS in Silico Score | ITS score for the in silico outcome. |
| DA ITS Total Score | Final score from the ITS DA 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 3 contains a description of the input columns and how they were reformatted. For example, if you selected only the 2o3 DA, then the column "h-CLAT Call Input" would be added to the results, but the column "h-CLAT MIT Input" would not be added because it is not a required endpoint for the 2o3 DA.

**Table 3. 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) Call Input | Positive outcomes are set to 1 and negative outcomes are set to 0. Any invalid values are set to NA. |
| 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

EPA. Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing Draft for Public Comment. EPA's Office of Chemical Safety and Pollution Prevention.

OECD. 2021a. Guideline No. 497: Defined Approaches on Skin Sensitisation.

OECD. 2021b. Test No. 442C: In Chemico Skin Sensitisation.

OECD. 2018a. Test No. 442D: In Vitro Skin Sensitisation.

OECD. 2018b. Test No. 442E: In Vitro Skin Sensitisation.

OECD. 2014. The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins.

R Core Team. 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing:Vienna, Austria.

Yordanova D, Schultz TW, Kuseva C, Tankova K, Ivanova H, Dermen I, et al. 2019. Automated and standardized workflows in the OECD QSAR Toolbox. Comput Toxicol 10:89–104; doi:https://doi.org/10.1016/j.comtox.2019.01.006.