

Web Application to Classify and Subcategorize Skin Sensitizers Using Human Data

A. Unnikrishnan¹, K.T. To^{1,2}, M. Herzler³, D. Germolec⁴, E. Reinke¹, N. Kleinstreuer^{4,5}

¹Inotiv, RTP, NC, United States; ²ICF, Reston, VA, United States; ³BfR, Berlin, Germany;

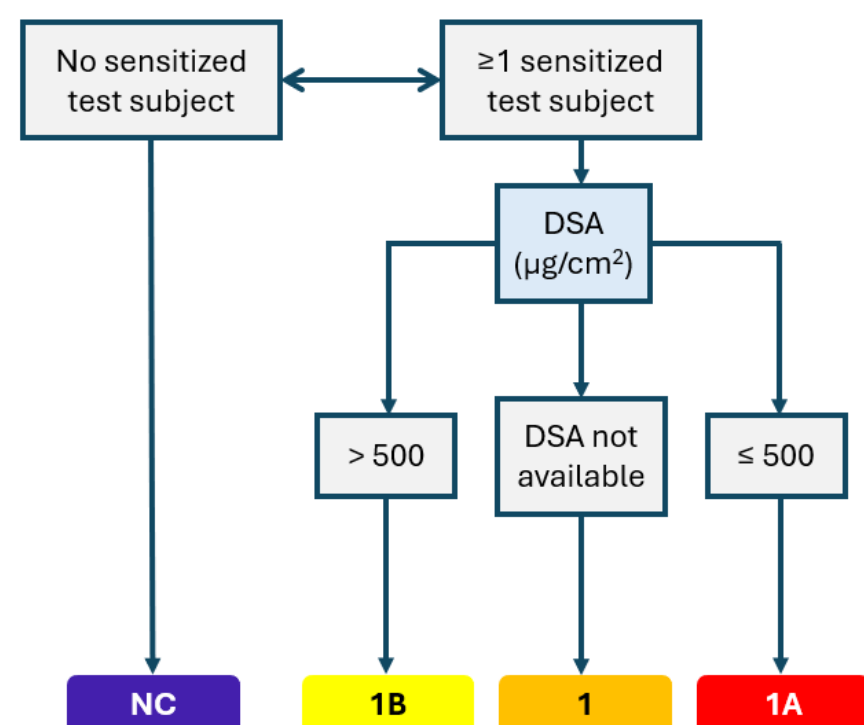
⁴NIH/NIEHS/DTT/NICEATM, RTP, NC, United States; ⁵NIH/OD/DPCPSI, United States

Background

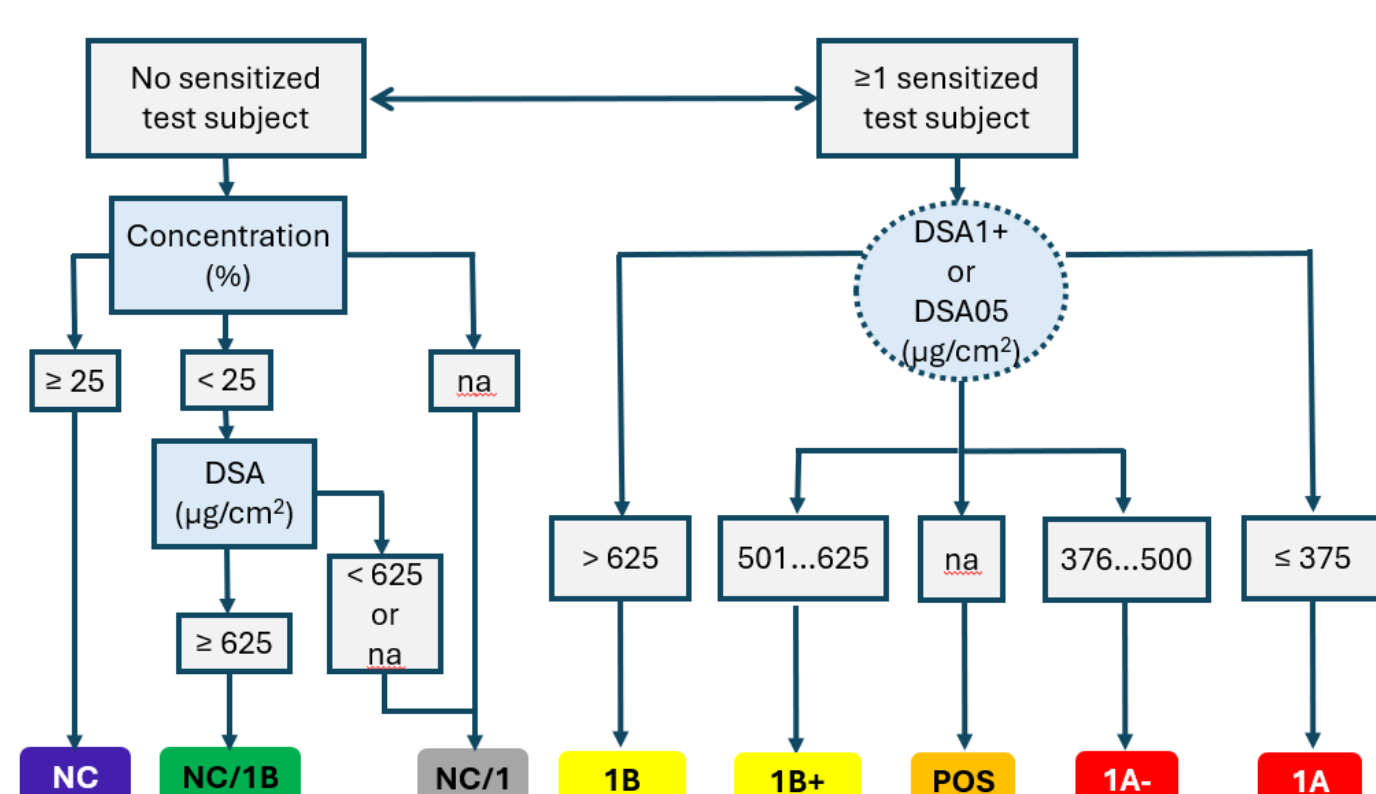
- To support the evaluation of non-animal approaches for assessing skin sensitization hazard, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the German Federal Institute for Risk Assessment (BfR) curated data from two types of human predictive patch tests (HPPT), the human repeat insult patch test and human maximization test (Strickland et al. 2023).
- These data were used to develop a modified approach for assigning skin sensitization potency subcategorizations based on the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS; Herzler et al. 2024).

Figure 1. Classification Approaches for Skin Sensitization Potency

1a. Standard GHS Classification Approach



1b. Modified Classification Approach



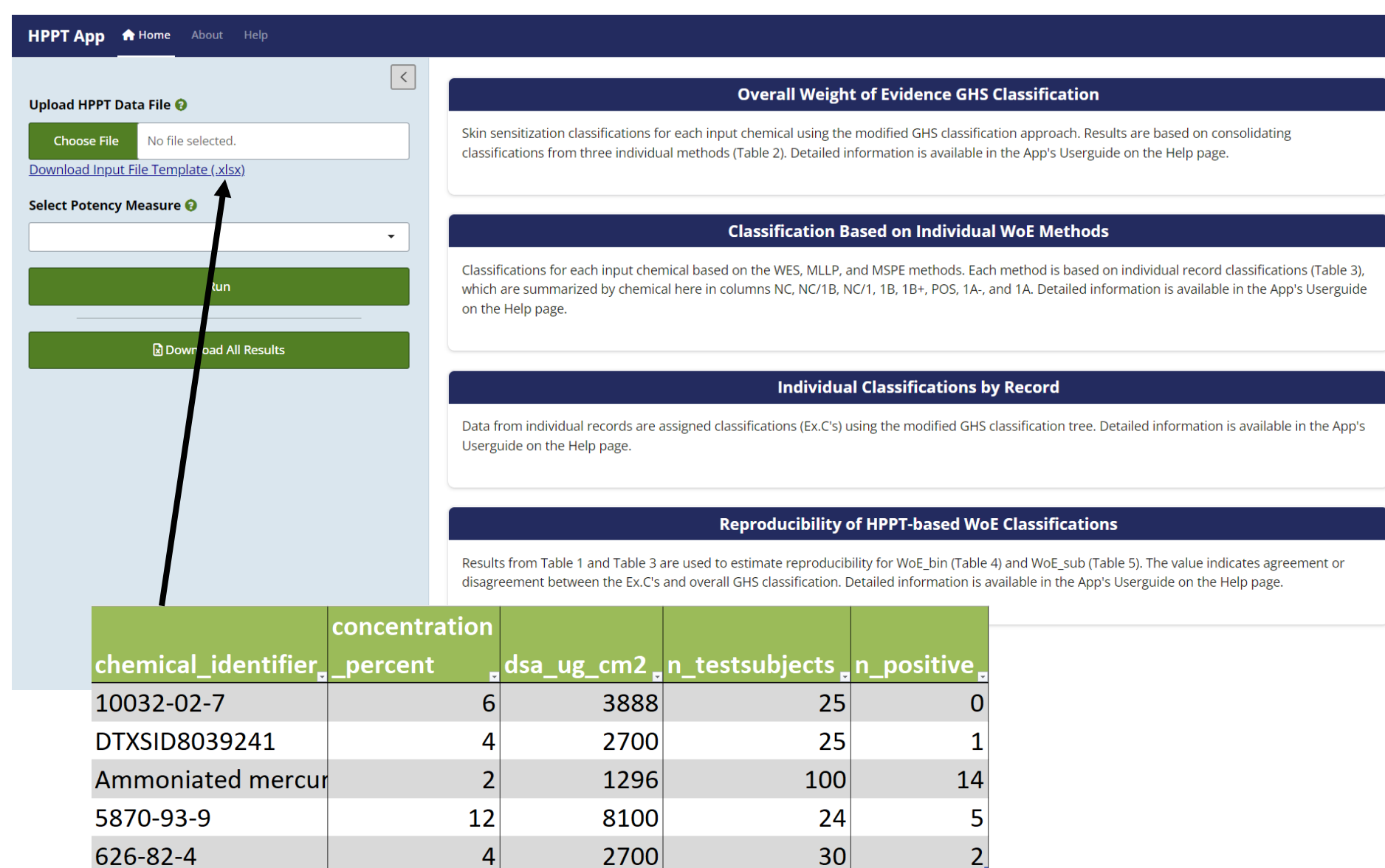
* Figure adapted from Herzler et al. 2024

- Approaches currently used to assign skin sensitizers to GHS potency subcategories do not consider the number of sensitized test subjects, potentially leading to an inaccurate estimation of potency.
- The modified classification approach (Figure 1b) improves upon the standard GHS classification approach by:
 - Incorporating potency using the DSA1+ (the dose that sensitizes one test subject) or DSA05 (the dose that sensitizes 5% of test subjects) dose metric.
 - Introducing ambiguous subcategorizations to address uncertainty.
- The approach also uses a weight-of-evidence (WoE) method developed by the Human Data Subgroup of the Expert Group on Defined Approaches for Skin Sensitization of the Organisation for Economic Co-operation and Development to consolidate multiple HPPT results into a single GHS classification for each chemical.
- To enable users to classify their own HPPT data for skin sensitization potency using the modified classification approach, we have developed an open-source web application, the **HPPT App**.
- The HPPT App offers a reliable and consistent interactive platform allowing users to input data, visualize results, and understand classification outputs.

HPPT App – User Interface

- The HPPT App user interface (UI) was designed to provide an intuitive layout for data input and results display. There is also an “About” page that provides background information and a “Help” page with contact details for questions or feedback.

Figure 2. Application Interface



Data Input & Processing:

The HPPT App UI facilitates data input via file upload using a downloadable template and allows choosing between DSA1+ or DSA05 classification. RShiny scripts then process these data to provide real-time classification and subcategorization of skin sensitizers.

Output Results:

The HPPT App first derives DSA1+ and DSA05 classifications. It then assigns GHS classifications for each record, later consolidating those results into an overall substance classification. Users can then view and download these results in interactive tables.

Classifications Based on Individual Methods

- Substances are first assigned extrapolated classifications (Ex.Cs) for each individual test outcome (Figure 3). The Ex.Cs are then consolidated using three WoE approaches (Herzler et al. 2024; Figure 4):
 - WES** (WoE score) provides a score for each test substance based on individual test outcomes.
 - MLLP** (median-like location parameter) provides the value at the median position of individual test outcomes.
 - MSPE** (median sensitization potency estimate) is a modified version of the MLLP. It aims to be more cautious in its classification by being more selective about the test results included in the median calculation, particularly by excluding potentially ambiguous negative results (NC/1) and including certain positive results that might have been excluded by MLLP.
- The HPPT App classifies substances using three different GHS category modes:
 - GHS_{BIN}**: substance classified in a binary manner either as Category 1 (sensitizer) or NC (not classified).
 - GHS_{SUB}**: substance assigned to one of three classes: Category 1A (strong sensitizer), Category 1B (weak sensitizer), or NC.
 - GHS_{BORDER}**: substance assigned to one of five classes: Category 1A (strong sensitizer), 1* (sensitizer, but subclassification not possible), 1B (weak sensitizer), NC/1B (ambiguous; substance may or may not be a sensitizer, but Category 1A can be ruled out), or NC.

Figure 3. Output Table Showing Ex.C and WES for Each Record

Chemical Identifier	Conc (%)	DSA (ug/cm²)	No. Test Subjects	No. Positive	Call	DSA1+	DSA5%	Ex.C	WES_indiv
All	All	All	All	All	All	All	All	All	All
5870-93-9	12	8100	24	5	Active	1620	1944	1B	1
626-82-4	4	2700	30	2	Active	350	2025	1B	1
Ammoniated mercury	2	1296	100	14	Active	93	463	1A	2
DTXSID8039241	4	2700	25	1	Active				1
10032-02-7	6	3888	25	0	Inactive				0.5

Each field can be sorted. Here the Call field is sorted to show the Active values.

Figure 4. Sections of Output Table with GHS Classifications based on WES, MLLP, and MSPE and Summary of Ex.Cs for Each Chemical

Chemical Identifier	WES	WES_GHS_bin	WES_GHS_sub	WES_GHS_border	MLLP_bin	MLLP_sub	MLLP_GHS_bin	MLLP_GHS_sub	MLLP_GHS_border	MSPE	MSPE_GHS_bin	MSPE_GHS_sub	MSPE_GHS_border
All	All	All	All	All	All	All	All	All	All	All	All	All	All
10032-02-7	0.5	NC	NC/1B	NC/1	NC	NC	NC	NC	NC	NC	NC	NC	NC
5870-93-9	1	1B	1B	1B	1B	1B	1B	1B	1B	1B	1B	1B	1B
626-82-4	1	1B	1B	1B	1B	1B	1B	1B	1B	1B	1B	1B	1B
Ammoniated	2	1A	1A	1A	1A	1A	1A	1A	1A	1A	1A	1A	1A
DTXSID8039241	1	1B	1B	1B	1B	1B	1B	1B	1B	1B	1B	1B	1B

User-provided chemical identifier can be chemical name, CASRN, DTXSID, or custom identifiers.

NC	NC/1B	NC/1	1B	1B+	POS	1A-	1A
All	All	All	All	All	All	All	All
0	1	0	0	0	0	0	0
0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	1
0	0	0	1	0	0	0	0

Each field can be filtered.

Overall WoE GHS Classifications

Figure 5. Output Table with Overall WoE GHS Classifications

Chemical Identifier	WoE_bin	WoE_sub	WoE_border	Total Tests
All	All	All	All	All
10032-02-7	NC	NC/1B	NC/1B	1
5870-93-9	1B	1B	1B	1
626-82-4	1B	1B	1B	1
Ammoniated mercury	1A	1A	1A	1
DTXSID8039241	1B	1B	1B	1

- Each chemical is assigned overall WoE GHS classifications based on the concordant outcome across the MLLP, MSPE, and WES (Figure 5).

Acknowledgements

- This project was supported by the Intramural Research Program (ES# 103386) at the National Institute of Environmental Health Sciences, National Institutes of Health under contract HHSN273201500010C.
- The views expressed above do not necessarily represent the official positions of any Federal agency. Since the poster was written as part of the official duties of the authors, it can be freely copied.
- To receive announcements of NICEATM activities, visit the NIH mailing list page for NICEATM News at <https://list.nih.gov/cgi-bin/wa.exe?SUBED1=niceatm-I&A=1> and click “Subscribe.”



Reproducibility of HPPT-based WoE Classifications

- The HPPT App also estimates reproducibility for chemicals with at least two unambiguous test results by comparing Ex.C to WoE_{BIN} and WoE_{SUB} classifications, indicating agreement or disagreement with overall classification outcome.
- The tables provide reproducibility as a percentage and report the total number of tests it was based on.

Figure 6. Output Tables Showing Classification Reproducibility

6a. WoE_{BIN} Classifications

Chemical Identifier	WoE_bin	WoE_bin Reproducibility	Total Tests (Reproducibility)
All	All	All	All
10124-43-3	1	100	3
10124-48-8	1	100	18
103-16-2	1	100	24

6b. WoE_{SUB} Classifications

Chemical Identifier	WoE_sub	WoE_sub Reproducibility	Total Tests (Reproducibility)
All	All	All	All
10124-43-3	1B	100	3
10124-48-8	1B	77.78	18
103-16-2	1B	62.5	24

Download Results

- Individual Output Tables
 - Tables can be downloaded as-is.
 - Alternatively, tables can be downloaded after applying custom filtering or sorting.
 - Download options include formats like CSV and Excel.
- All Query Results
 - Option to download overall results.
 - Option to download results per record (Figure 7).
 - Field or column descriptions are also provided.

Figure 7. Download File with All Query Results by Record

Discussion and Conclusion

- The modified classification approach based on HPPT data enhances the evaluation of human-based skin sensitization tests by incorporating potency into the GHS classification decision tree and applying WoE methods.
- However, the approach involves complex decision-tree logic that may be challenging for users lacking experience with the relevant analysis methods.
- This user-friendly, open-source R Shiny-based HPPT App was developed to facilitate public use of the modified classification approach and allow users to easily classify their HPPT data in a way that reflects human potency.
- The HPPT App provides individual classifications along with overall WoE results, allowing users to characterize uncertainty in the overall result. This helps users compare individual method classifications, identify inconsistencies, and assess overall result strength.
- Potency classifications generated by the HPPT App can serve as reference data for the evaluation of non-animal skin sensitization tests.
- The HPPT App offers benefits to various stakeholders involved in assessing chemical hazards and risks, particularly concerning skin sensitization. It can also be applied in risk assessment frameworks.

References

- Strickland J et al. A database of human predictive patch test data for skin sensitization. Archives of Toxicology (2023) 97:2825–2837. <https://doi.org/10.1007/s00204-023-03530-3>
- Herzler M et al. Use of human predictive patch test (HPPT) data for the classification of skin sensitization hazard and potency. Archives of Toxicology (2024) 98:1253–1269. <https://doi.org/10.1007/s00204-023-03656-4>