Evaluation of Skin Sensitization Classification Rules to Reflect Human Potency

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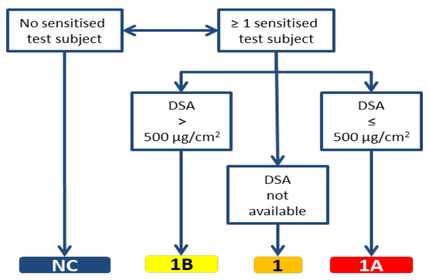
Introduction

- To support the development of Guideline 497 on Defined Approaches for Skin Sensitization published by the Organisation for Economic Co-operation and Development (OECD; OECD 2021), we collected historical human predictive patch test (HPPT) data to be used as reference data.
- We deemed data from 2255 HPPTs, representing 1366 different substances, to be sufficiently reliable to assign skin sensitization potency classifications according to the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2021) (Fig. 1a).
- Approaches currently used to assign skin sensitizers to GHS potency subcategories consider only the dose inducing the skin sensitization response and not the frequency of induced sensitization in human subjects. Variations in conduct of assays may also introduce uncertainty into otherwise valid data.
- To address these limitations, we developed a modified approach to GHS classification (Fig. 1b) that incorporates a frequency metric into potency classification and also addresses uncertainty in assay results.
- We also developed a strategy for using these classifications in a weight-of-evidence (WoE) approach with animal reference data, when classifications do not agree, to develop an overall classification.

Figure 1. Standard and Modified GHS Classification Decision Trees

a) Standard GHS Classification Approach



b) Modified GHS Classification Approach

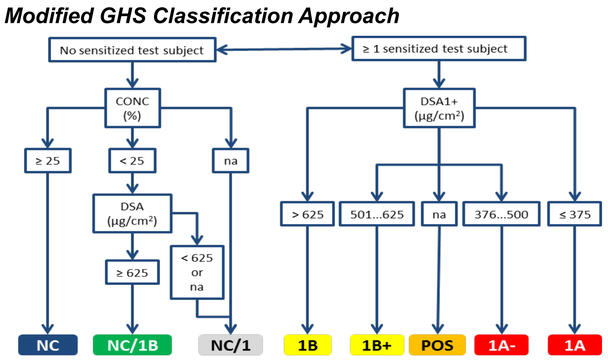


Figure 1a represents the standard GHS classification approach. The modified approach. Fig. 1b incorporates sensitization incidence as well as ambiguous/borderline cases. Two dose metrics were applied to this approach: DSA1+ or DSA05 (not shown). Derivation of the dose metrics is explained below. DSA = dose per skin area.

GHS Classification of Human Predictive Patch Test Results

- The dose metric for assessing potency of a skin sensitizer in humans is the dose per skin area (DSA), in µg/cm², required to induce an allergic reaction.
- In the standard GHS classification system (Fig. 1a), a substance is classified as a skin sensitizer (Category 1) if at least one subject is sensitized.
  - A positive result at DSA ≤ 500 µg/cm² results in a classification as a 1A (strong) sensitizer.
  - A positive result at DSA > 500 µg/cm² typically indicates a 1B (other) sensitizer, but 1A cannot be ruled out because a lower dose could produce a positive result.
- Chemicals that test negative are assigned a GHS designation of Not Classified (NC). However, NC classifications can sometimes by ambiguous because of uncertainties of testing chemicals at concentrations that may simply be too low to produce a positive result.
- To resolve these uncertainties, we derived a borderline range of 375 to 625 µg/cm² (± 25% around the 500 µg/cm² cut-off) (Fig. 1b) and established a test concentration cut-off of at least 25% (the 99th percentile of the top concentrations of negative tests) to classify negative tests as NC. Under this proposed modification:
  - Chemicals testing negative at concentrations < 25% with DSA ≥ 625 µg/cm² were classified as NC/1B, an outcome that, while ambiguous, enables exclusion of a strong skin sensitization potential.
  - Chemicals testing negative at concentrations < 25% with DSA < 625 µg/cm² were classified as NC/1, an ambiguous classification that provides no information on the skin sensitization potential.
- GHS classification does not account for the number of sensitized individuals contributing to a positive result. To incorporate this measure into classification, we examined two additional dose metrics:
  - DSA1+, the hypothetical DSA producing one sensitized test subject.
  - DSA05, the hypothetical DSA that sensitizes 5% of the test subjects.

Evaluation of Substances with Multiple Discordant Tests

- After classification of each of the 2255 HPPTs using the Modified GHS Classification Approach (Fig. 1b), substances with discordant tests were classified by combining the multiple results using three weight-of-evidence approaches:
  - WoE score: average of individual test data scores.
  - Median-like location parameter (MLLP) (adapted from Hoffmann et al. 2018).
  - Median sensitization potency estimate (MSPE), a slightly modified version of the MLLP.
- Substances were classified using three different modes based on GHS categories:
  - GHS_{BIN}: substance classified in a binary manner as Category 1 (sensitizer) or NC.
  - GHS_{SUB}: substance assigned to one of three classes: 1A sensitizer, 1B sensitizer, or NC.
  - GHS_{BORDER}: substance assigned to one of five classes:
    - the three classes used in GHS_{SUB} with different criteria (except NC);
    - 1 (sensitizer, but subclassification not possible); and
    - NC/1B (substance may or may not be a sensitizer, but 1A can be ruled out).
- Table 1 compares the DSA1+ and DSA05 metrics to one another for the GHS_{BIN} and GHS_{SUB} classification modes, which are broadly accepted.

Table 1. Comparison of Classifications Using DSA1+ and DSA05

GHS_{BIN}

		DSA05			Total
		1	NC	NA	
DSA1+	1	234	0	0	234
	NC	0	53	0	53
	NA	1	0	1078	1079
Total		235	53	1078	1366

GHS_{SUB}

		DSA05				Total
		1A	1B	NC	NA	
DSA1+	1A	55	9	0	0	64
	1B	7	150	0	0	157
	NC	0	0	53	0	53
	NA	0	3	0	1089	1092
Total		62	162	53	1089	1366

Table 1 shows number of substances. NA = insufficient data to support a classification for this approach. Gray shading shows matching classifications for DSA1+ and DSA05.

Table 2. Reproducibility of Test Classifications

	Number of test results	Number of substances		Reproducibility (%) Mean (SD)	
		DSA1+	DSA05	DSA1+	DSA05
GHS_{BIN}	> 1	97	98	99.4 (3.6)	99.1 (4.9)
	> 2	53	54	98.9 (4.9)	98.3 (6.5)
	> 3	37	37	98.5 (5.8)	98.5 (5.8)
	> 4	27	27	99.8 (1.1)	99.8 (1.1)
GHS_{SUB}	> 1	96	97	82.5 (22.3)	84.2 (22.6)
	> 2	53	57	79.7 (21.7)	79.2 (23.6)
	> 3	40	39	77.2 (21.5)	77.3 (22.9)
	> 4	28	28	76.4 (21.0)	77.3 (23.0)

Table 2 shows reproducibility results for classifications of substances with at least two test results relevant to binary (GHS_{BIN}) or subcategory (GHS_{SUB}) classifications using both DSA1+ and DSA05 dose metrics.

Conclusions

- We conclude that using a modified GHS approach to classifying HPPT data provided good reproducibility and concordance with animal reference data while considering potency and uncertainty.
- DSA1+ or DSA05 may be a more relevant dose descriptor for potency determination.
- We developed a WoE assessment strategy that uses both DSA1+ and DSA05 from HPPT data with LLNA data to determine GHS skin sensitization classifications.

WoE Concordance with LLNA-Based Reference Classification

- To further explore the utility of our proposed classification approach, we examined the concordance of HPPT-based reference classifications with those obtained using LLNA data.
  - For GHS_{BIN}, 56/196 OECD reference chemicals had classifications based on both data types. Concordance of HPPT with LLNA was 82% for both DSA1+ or DSA05 outcomes.
  - For GHS_{SUB}, 47/196 OECD reference chemicals had classifications based on both data types. Concordance of HPPT with LLNA was 60% for DSA1+ and 58% for DSA05 outcomes.
- We then developed a strategy to integrate HPPT-based reference classifications using DSA1+ or DSA05 with those obtained using LLNA data to develop an overall WoE classification (Fig. 2). The concordance of LLNA, DSA1+, and DSA05 classifications with overall WoE classifications are shown in Table 3.

Table 3. Comparison of LLNA, DSA1+, DSA05, and Overall WoE Outcomes

		Overall WoE	
		1	NC
LLNA	1	49	2
	NC	3	2
DSA1+	1	47	0
	NC	5	4
DSA05	1	47	0
	NC	5	4

		Overall WoE		
		1A	1B	NC
LLNA	1A	12	0	0
	1B	3	25	2
	NC	0	3	2
DSA1+	1A	12	4	0
	1B	3	20	0
	NC	0	4	4
DSA05	1A	10	3	0
	1B	5	21	0
	NC	0	4	4

Confusion matrices show the relative performance of DSA1+, DSA05, and LLNA vs overall WoE outcomes for the GHS_{BIN} and GHS_{SUB} classification modes for those OECD reference substances with both LLNA- and HPPT-based reference classifications.

Figure 2. WoE Assessment of HPPT and LLNA Reference Classifications

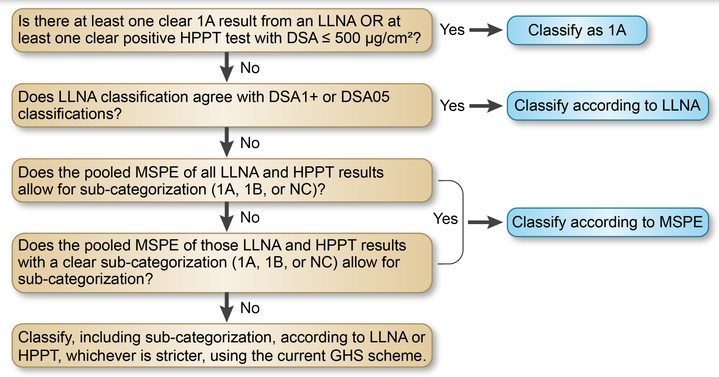


Figure 2. Decision scheme for obtaining an overall classification based on all available LLNA and HPPT data, in cases where the classifications based on LLNA, DSA1+, and DSA05 do not fully agree.

Summary

- We collected a large data set of historical HPPT studies from the scientific literature to use as reference data for development of OECD Guideline 497.
- We developed a new approach for hazard and potency classification of these tests based on GHS categories. The modified approach accounts for uncertain or borderline results and considers the number of sensitized subjects as a measure of potency using DSA1+ and DSA05 dose metrics (Fig. 1b).
- Use of borderline ranges around the 1A/1B cutoff value identified ambiguous subclassifications (Fig. 1b).
- A test concentration cut-off of 25% was used to define the minimum concentration at which a negative test result would be accepted to provide more certainty for negative results (Fig. 1b).
- Both DSA1+ and DSA05 (Table 2) provided reproducible results when used with three different WoE approaches for combining multiple discordant results for single substances.
- Substance classifications based on HPPT results were consistent with LLNA classifications. We developed a strategy to use HPPT and LLNA in a WoE approach to classify substances for skin sensitization potential (Fig. 2).

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References and Acknowledgements

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