National Institute of Environmental Health Sciences
*Division of the National Toxicology Program*

# Variability of Human Reference Data for Skin Sensitization


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## Introduction

- Characterizing the variability of data from reference test methods is important to enable the use of these data to establish the performance of and confidence in new approach methodologies (NAMs).
- We compiled a data set of human predictive patch test results using human maximization tests (HMTs) and human repeated insult patch tests (HRIPTs) published in the scientific literature.
- We evaluated the test results based on the completeness of the study information and identified 2255 tests that were sufficiently reliable for analysis. This project attempts to identify the characteristics responsible for the variability of these data.

## Human Predictive Patch Tests

- Both HMT and HRIPT involve the application of repeated exposures of potential skin sensitizers over several weeks, followed by a rest period and then a reapplication of chemical to determine whether an allergic response can be elicited.
- The HMT tests a smaller number of subjects and incorporates pre-treatment with an irritant to enhance skin penetration of non-irritating test substances.
- Only one dose is tested; the result is positive if at least one subject has a sensitization response.
- These tests are different from diagnostic patch tests which are performed on patients to identify the chemical source of allergic contact dermatitis.

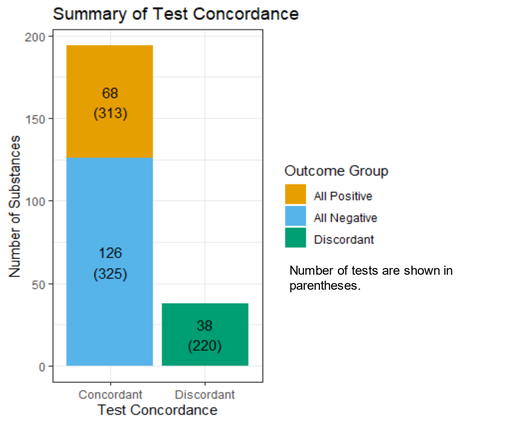
## Table 1. Variables for Analysis

| Name | Description |
|---|---|
| Test type | HMT or HRIPT |
| Skin patch area | Size of the skin patch used for application of the induction dose |
| Sample size | Number of subjects tested |
| Physicochemical properties | Molecular weight, boiling point, Henry's Law constant, melting point, acid dissociation constant, octanol-air partition coefficient, octanol-water distribution coefficient, octanol-water partition coefficient, vapor pressure, water solubility |
| Dose | Dose applied per skin area (DSA) or concentration applied |

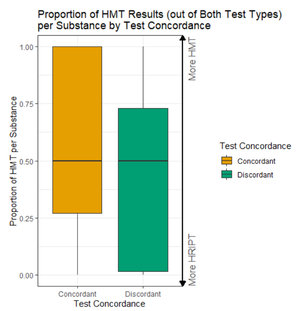
## Data Curation and Analysis

- A data set of 858 tests of 232 substances was compiled for analysis of variability by removing:
  - Data for substances with only one test.
  - Tests for a given substance if the results were negative and the test doses were less than the median dose from the positive tests for the substance.
- Our variability evaluation was based on binary test outcomes: positive or negative.
- We grouped the 232 substances into concordant and discordant categories (**Figure 1**). Concordant substances were further subcategorized into positive and negative groups.
- We determined whether the concordance groups were different for the variables in **Table 1** using the Mann-Whitney U test. For each variable, tests were removed for substances that had fewer than two reported values for the given variable.

## Figure 1. Test Concordance Groups

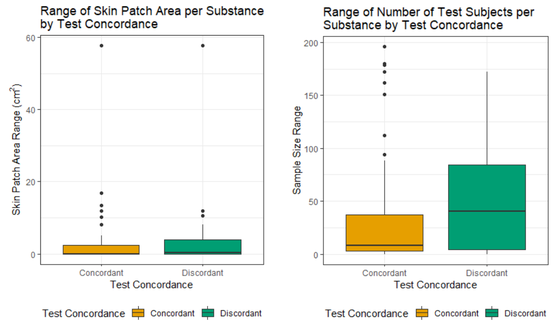



## Figure 2. Variability by Test Type




- The proportion of HMT results of the total number of HMT and HRIPT results for each substance was calculated.
  - Values closer to 1 indicate that a substance had more HMT results than HRIPT.
  - Values closer to 0 indicate that a substance had more HRIPT results than HMT.
- There was no significant difference (p>0.05) between the concordant and discordant groups for the proportion of HMT and HRIPT results per chemical.

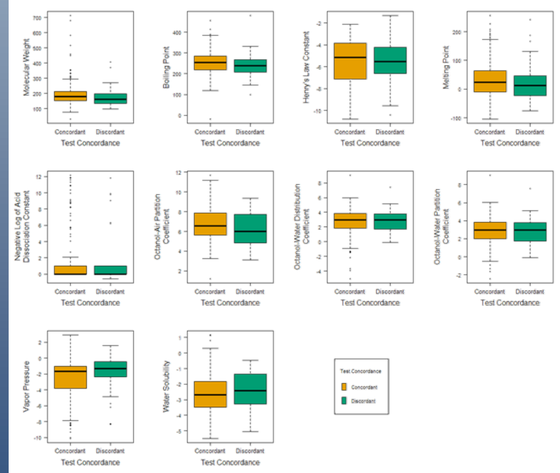
## Figure 3. Variability by Skin Patch Size and Sample Size




Bold black lines show the median values. Whiskers extend from the first and third quartiles by 1.5x the interquartile range. Points outside the whiskers are outliers.

- There was no significant difference (p>0.05) between the concordant and discordant groups for skin patch area or sample size.

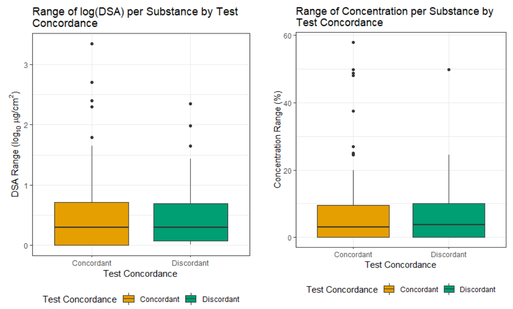
## Figure 4. Variability by Physicochemical Property




Bold black lines show the median values. Whiskers extend from the first and third quartiles by 1.5x the interquartile range. Points outside the whiskers are outliers.

- There was no significant difference (p>0.05) between the concordant and discordant groups for any physicochemical property.

## Figure 5. Variability by Dose




Bold black lines show the median values. Whiskers extend from the first and third quartiles by 1.5x the interquartile range. Points outside the whiskers are outliers.

- There was no significant difference (p>0.05) between the concordant and discordant groups for dose or concentration.

## Summary and Future Directions

- These analyses showed that none of the protocol variables or physicochemical properties evaluated was associated with the variability of test results with respect to concordance of positive or negative outcomes.
  - Vehicle data could not be analyzed because one vehicle (petrolatum) was used in the vast majority of tests (74.1% [617/833]).
- These analyses suggest that HMT and HRIPT tests can be considered equivalent.
- Future work will examine the variability of potency estimates, measured as the dose per skin area that sensitizes one subject.

## Acknowledgements


This project was funded in whole or in part with federal funds from the National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN273201500010C.

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