

# Variability in the Rabbit Skin Irritation Assay

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## Highlights

- Skin irritation testing has historically been conducted in rabbits. Results of these studies are the benchmark against which NAMs are compared.
- Chemicals classified as **mild or moderate** irritants in one test were approximately **50% or less** likely to be replicated when tested again.
- **Variability** in the in vivo assay should be taken into **consideration** when **evaluating the performance** of NAMs.

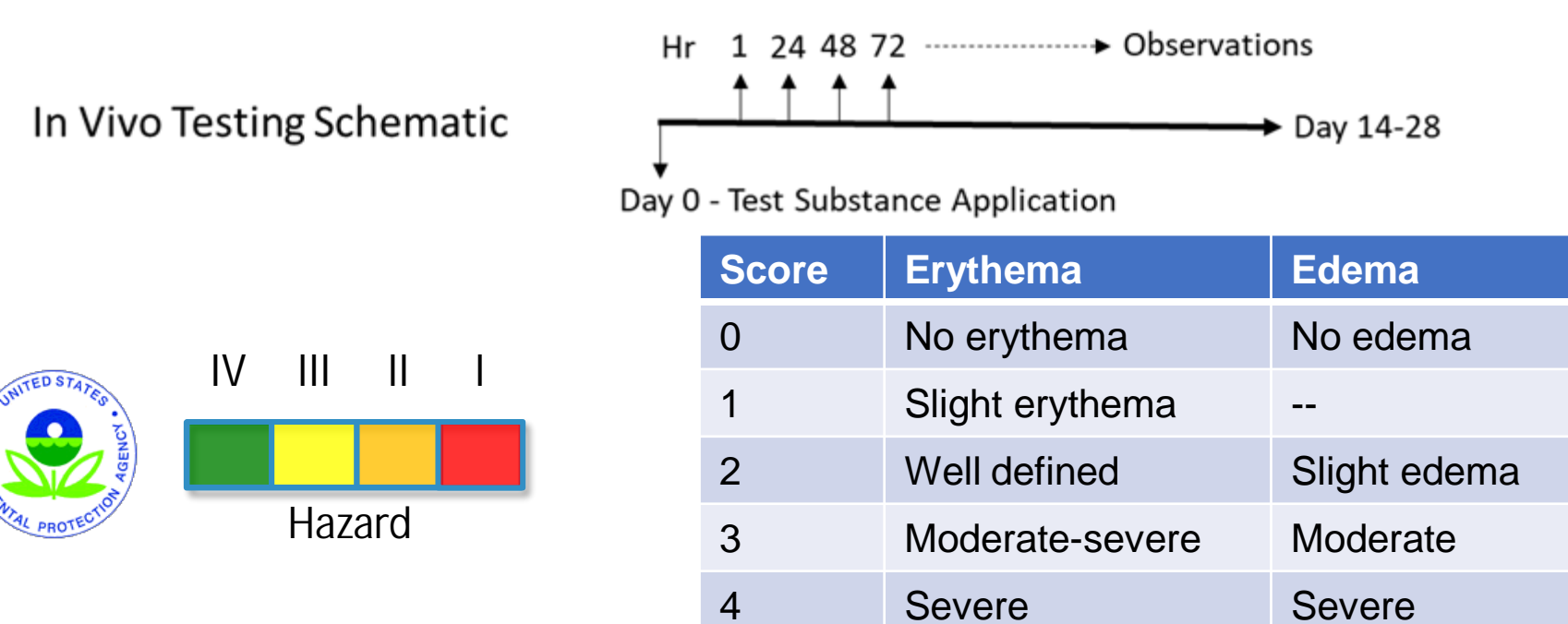
## Conditional Probability Calculations

Conditional probabilities, conducted iteratively for each category, were used to evaluate the reproducibility of the rabbit skin irritation test for identification of severe, moderate, mild, and non-irritants.

$$P(T_2 = 1 | T_1 = 1) = \frac{P(T_2 = 1 \cap T_1 = 1)}{P(T_1 = 1)}$$

- Data subsets were created for each EPA hazard classification category (I, II, III, IV) defined by the chemicals classified in the category by at least one test.
  - Frequency of classification for each category (Ci), given the total number of ESRs in that data subset (A), was determined.
- Probability of repeat testing was calculated for each category by dividing the frequency of each category by the frequency of all categories (total number of assays) in that data subset.
  - $P = C_i/A$

## Background: EPA Skin Irritation Classification



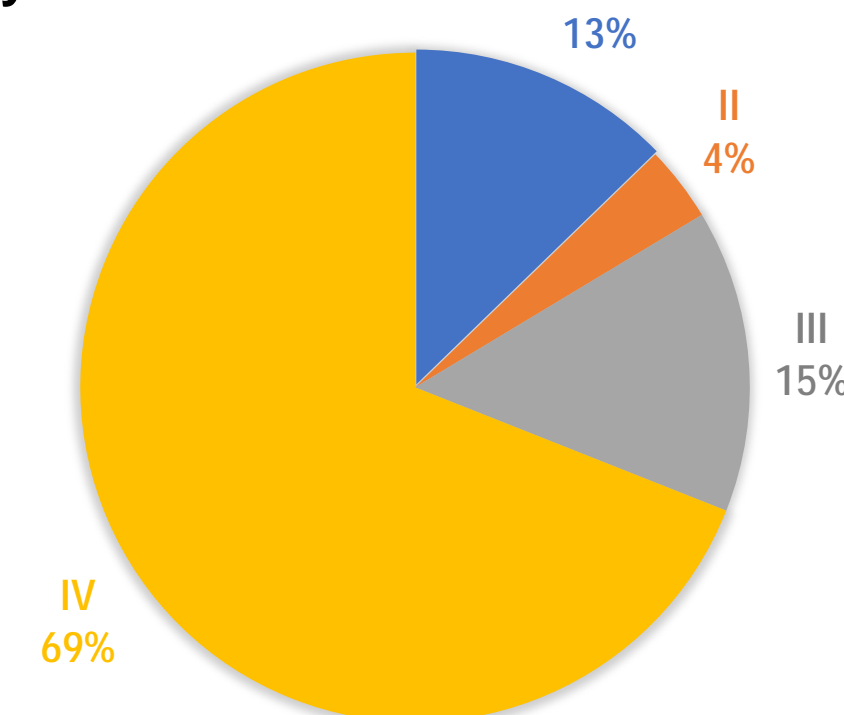
$$PDII = \frac{\text{Sum erythema (all time points)} + \text{Sum edema (all time points)}}{\text{number of intervals} \times \text{number of animals}}$$

US-EPA	Category I	Category II	Category III	Category IV
PDII	Corrosive	>5.0	2.1 - 5.0	0 - 2.0
Signal Word (Color)	DANGER (Red)	WARNING (Orange)	CAUTION (Yellow)	CAUTION (Green)
PPE Required	Coveralls worn over long-sleeved shirt and long pants	Coveralls worn over short-sleeved shirt and short pants	Long-sleeved shirt and long pants	Long-sleeved shirt and long pants
	Socks	Socks	Socks	Socks
	Chemical-resistant footwear	Chemical-resistant footwear	Shoes	Shoes
	Waterproof or chemical resistant gloves	Waterproof or chemical resistant gloves	Waterproof or chemical resistant gloves	No minimum

**Abbreviations:**  
**ESR** = Endpoint Study Report      **NAMs** – New Approach Methodologies  
**PDII** – Primary Dermal Irritation Index      **EPA** = U.S. Environmental Protection Agency

## Dataset Summary and Conditional Probabilities

### Analysis 1: Full Dataset



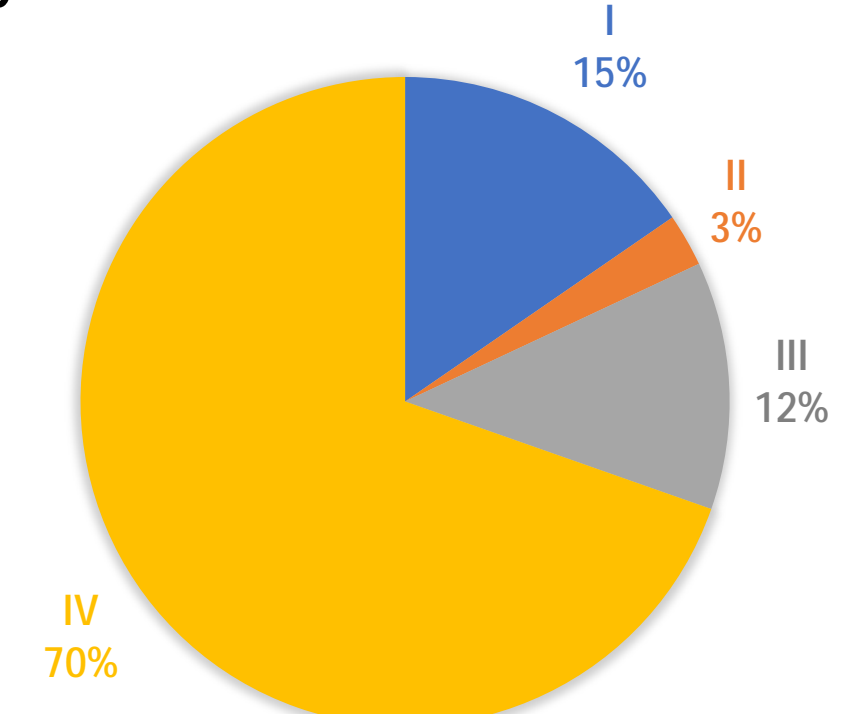
- Includes all study results with sufficient data to generate a PDII.
- No exclusion of studies for methodological concern (see Flags table).
- **Results based on 2431 total study reports including 797 individual chemicals.**

### Conditional Probabilities

Prior result	I	II	III	IV	N
<b>I</b>	76.0%	8.0%	8.5%	7.5%	313
<b>II</b>	12.0%	<b>28.1%</b>	35.3%	24.6%	89
<b>III</b>	5.8%	5.0%	<b>43.5%</b>	45.7%	357
<b>IV</b>	2.2%	1.9%	11.6%	<b>84.4%</b>	1672

Table describes the conditional probabilities for the **full** dataset for receiving the same categorical classification when a chemical is tested multiple times.

### Analysis 2: Curated Dataset



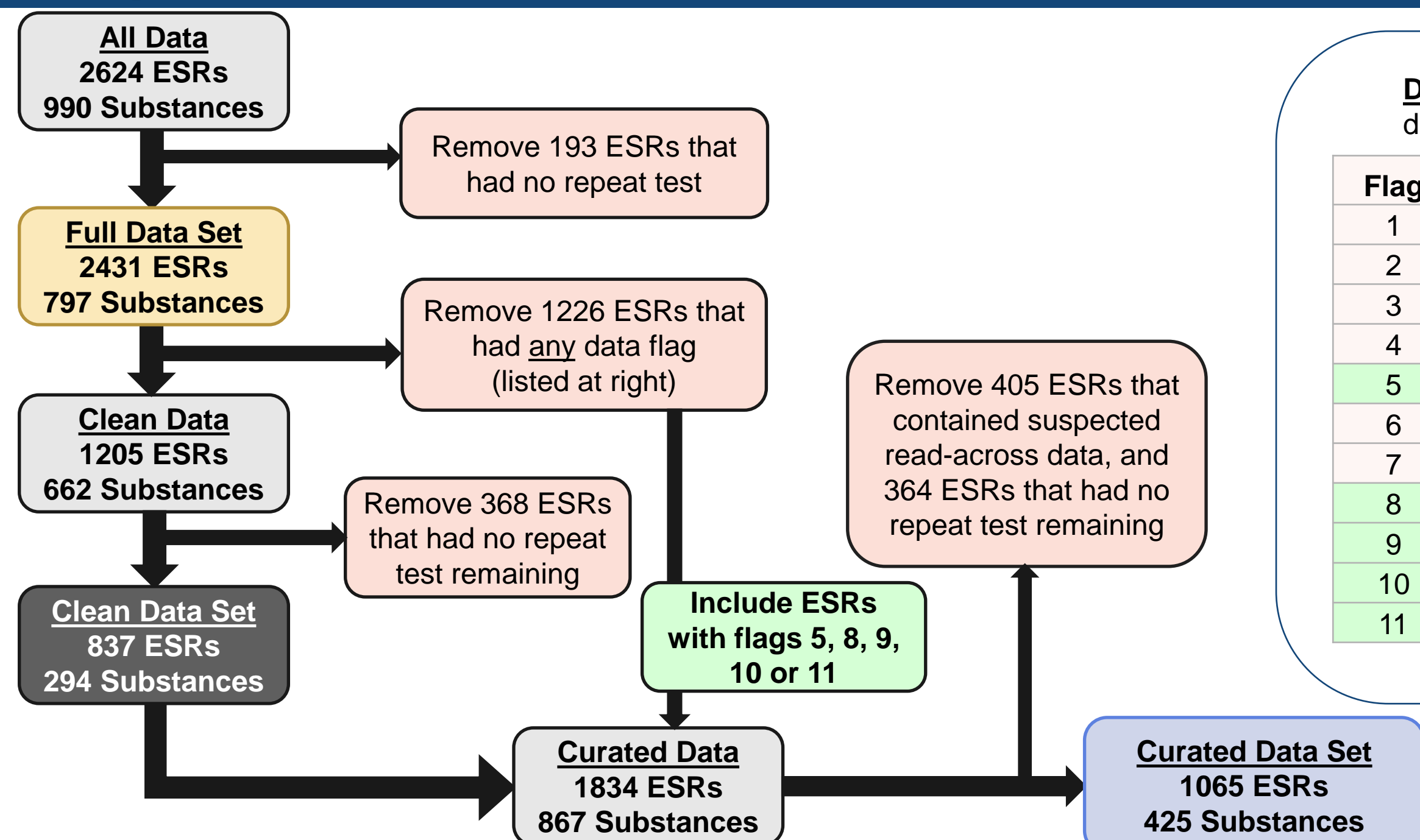
- The full dataset was curated to exclude studies with methodological deviations/limitations
- **Results based on 1065 total study reports including 425 individual chemicals.**

### Conditional Probabilities

Prior result	I	II	III	IV	N
<b>I</b>	86.3%	4.2%	7.1%	2.5%	207
<b>II</b>	14.1%	<b>44.9%</b>	20.5%	20.5%	35
<b>III</b>	6.9%	5.2%	<b>53.6%</b>	34.3%	133
<b>IV</b>	0.9%	2.0%	9.1%	<b>88.0%</b>	690

Table describes the conditional probabilities for the **curated** dataset for receiving the same categorical classification when a chemical is tested multiple times.

## Data Preparation Workflow



**Data Flags:** Studies were flagged for methodological deviations from study guidelines.

Flag	Description
1	Only tested on abraded skin
2	Scores reported from combined intact and abraded skin
3	Concentration tested was less than 90%
4	Exposure duration <4 hours and substance was not corrosive
5	Exposure duration >4 hours
6	Tested on unknown number of animals
7	Tested on <3 animals and substance was not corrosive
8	Scoring at incomplete time points (24 hr, 48 hr, 72 hr)
9	Parameter reported other than erythema/edema/PDII
10	Reported and calculated PDII do not match
11	Reported scores are a range

## Conclusions

- Chemicals classified as moderate irritants at least once are just as likely to be classified as mild irritants or non-irritants when tested again.
- Variability present in the in vivo assay should be taken into consideration when evaluating performance of NAMs
- These analyses help provide much needed context both to assess “gold standard” reference test methods and to aid in setting expectations for NAM performance.

## More Information

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• Data will be available at: <https://ice.ntp.niehs.nih.gov/DATASETDESCRIPTION>  
 • More information on this topic at: <https://ntp.niehs.nih.gov/go/irr-data>

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