EVALUATION OF THE VALIDATED *IN VITRO* SKIN IRRITATION TEST (OECD TG 439) FOR THE ASSIGNMENT OF EPA HAZARD CATEGORIES

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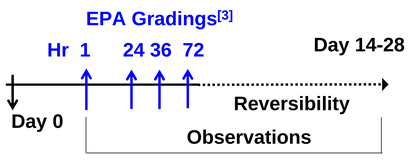
ABSTRACT

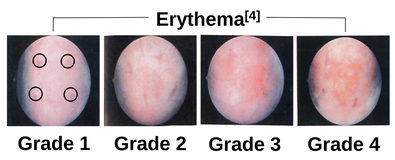
Historically dermal safety assessment of chemicals has been conducted using the Draize rabbit test. However progress within the *in vitro* toxicology field has made available testing platforms based on reconstructed tissue models that are validated to address the skin corrosion and irritation endpoints. While the validated *in vitro* assays can be used for the hazard identification of chemicals irritant to skin in accordance with the United Nations (UN) Globally Harmonized System (GHS) for Classification and Labeling of Chemicals, they were not calibrated to address the classification system used by the United States Environmental Protection Agency (US EPA). The validated *in vitro* Skin Irritation Test (SIT – OECD TG439) can be used to discriminate between skin irritants (GHS Category 2) and non-irritants (No Category) based on a single exposure time (60 minutes, using the EpiDerm™ model from MatTek Corporation, Ashland, MA, USA) followed by a 42 hours post-exposure period. A single cut-off value of 50% tissue viability separates Category 2 from the No Category prediction. To determine if these same parameters could be used to determine EPA labeling information, we performed a retrospective analysis of paired *in vivo-in vitro* data for 41 chemicals used for the validation of SIT. This analysis revealed an over-prediction of some EPA Category III and Category IV chemicals. We also conducted a preliminary testing of a sub-set of 13 chemicals from the group of 41 using a modified protocol with a 15 minutes exposure followed by a 24- or 42 hours post-exposure period in addition to the validated method. The results were analyzed based on a new prediction model: 15- and 60 minutes exposure, 24- and 42 hours post-exposure, and a revised cut-off value of the % viability endpoint (20%) which improves the prediction of the EPA Category III and IV chemicals. Its performance characteristics are similar to those found in the validation of the SIT. We are currently investigating a larger set of chemicals with already assigned EPA skin hazard categories to assess the validity of this new prediction model for EPA labeling.

INTRODUCTION

US EPA Registration of Products – Workflow Using the Rabbit Draize Test

The *In Vivo* Test for Acute Dermal Corrosion and Irritation Assessment (OECD 404) [1,2]





Erythema and eschar formation	Oedema formation	
No erythema	No oedema	0
Very slight erythema (barely perceptible)		1
Well defined erythema	Slight oedema (edges or area well defined by definite raising)	2
Moderate to severe erythema	Moderate oedema (raised approximately 1 mm)	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Chemicals Hazard Classification and Labeling

Primary Dermal Irritation Index (PDII)

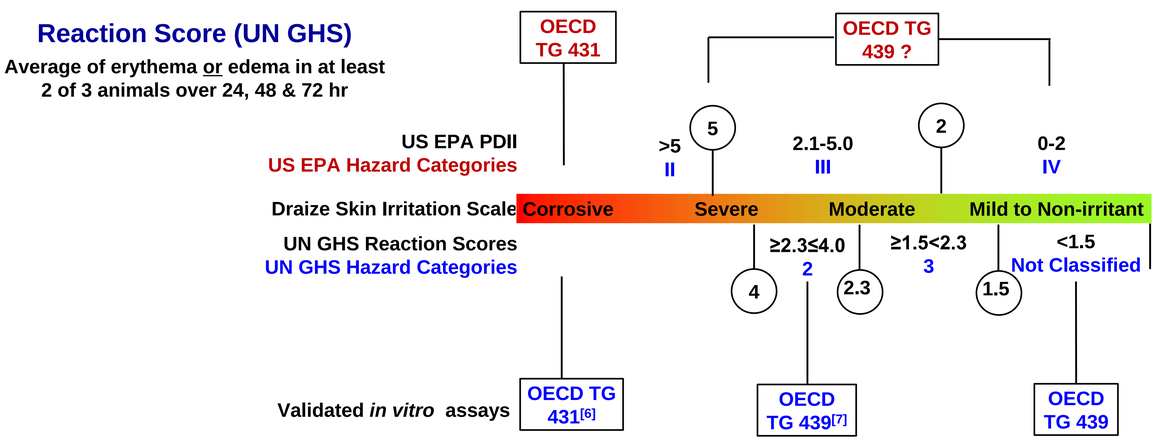
$$\frac{\text{Sum erythema (1/24/48/72 hr) + Sum oedema (1/24/48/72 hr)}}{\text{4 intervals (1/24/48/72 hr) x no. of animals}}$$

US EPA	Hazard Category[5]			
	I	II	III	IV
PDII	Corrosive	>5.0	2.1-5.0	0-2
Signal Word[5]	DANGER	WARNING	CAUTION	CAUTION

US EPA Registration of Products - Assessment of Workflow Using OECD Validated *In Vitro* Tests

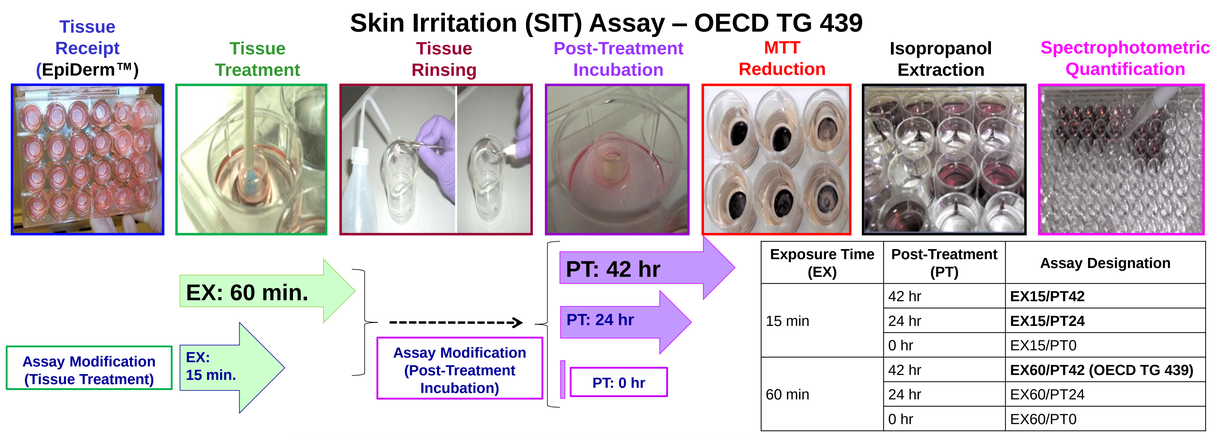
Reaction Score (UN GHS)

Average of erythema or edema in at least 2 of 3 animals over 24, 48 & 72 hr



MATERIALS & METHODS

Skin Irritation (SIT) Assay – OECD TG 439



Exposure Time (EX)	Post-Treatment (PT)	Assay Designation
15 min	42 hr	**EX15/PT42**
	24 hr	**EX15/PT24**
	0 hr	EX15/PT0
60 min	42 hr	**EX60/PT42 (OECD TG 439)**
	24 hr	EX60/PT24
	0 hr	EX60/PT0

REFERENCES

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RESULTS

Chemical Identification	*In vivo* Results[8] PDII	EPA Category per *in vivo* PM	*In vitro* Results[9,10] EpiDerm™ Tissue Viability (%) EX60/PE42	EPA Category per UN GHS PM	*In vitro* Results[11, 12] EpiDerm™ Tissue Viability (%) EX15/PE42	EPA Category per UN GHS PM	EPA Category per Proposed US EPA PM1	EPA Category per Proposed US EPA PM2
1,1,1-trichloroethane	5.50	II	16.8%	II / III	16.2%	II / III	II	
heptanal	5.53	II	6.0%	II / III	10.8%	II / III	II	
sodium dodecyl sulphate (20%)	5.83	II	4.2%	II / III	13.2%	II / III	II	
benzylalcohol*	2.25	III	5.0%	II / III	89.2%	IV	III	
eugenol	2.66	III	5.3%	II / III	11.8%	II / III	II	
di-n-propyl disulphide	2.75	III	83.0%	IV	96.2%	IV	IV	
1-decanol	2.84	III	5.0%	II / III	9.8%	II / III	II	
linalol*	3.08	III	5.0%	II / III	73.1%	IV	III	
hexyl salicylate	3.17	III	99.0%	IV	93.2%	IV	IV	
linalyl acetate	3.50	III	79.6%	IV	95.0%	IV	IV	
1-bromohexane	3.58	III	20.0%	II / III	95.5%	IV	III	
cinnamaldehyde#	4.22	III	5.0%	II / III	11.4%	II / III	II	
cyclamen aldehyde#	4.25	III	11.0%	II / III	18.8%	II / III	II	III
1-bromopentane	4.42	III	5.8%	II / III	81.7%	IV	II	
potassium hydroxide (5%)	4.83	III	4.3%	II / III	10.5%	II / III	II	
tetrachloroethylene#	5.00	III	4.8%	II / III	14.0%	II / III	II	III
1-bromo-4-chlorobutane#	0	IV	6.0%	II / III	55.5%	IV	III	IV
3,3'-dithiodipropionic acid	0	IV	98.0%	IV	95.7%	IV	IV	
sodium bicarbonate	0.08	IV	90.9%	IV	100.6%	IV	IV	
4-amino-1,2,4-triazole	0.08	IV	91.0%	IV	92.1%	IV	IV	
diethyl phtalate	0.17	IV	94.0%	IV	100.1%	IV	IV	
4,4-methylene bis-(2,6-ditert-butyl) phenol	0.17	IV	96.9%	IV	94.4%	IV	IV	
erucamide	0.25	IV	102.7%	IV	103.6%	IV	IV	
benzyl benzoate	0.25	IV	93.4%	IV	100.1%	IV	IV	
dipropylene glycol	0.33	IV	97.0%	IV	102.3%	IV	IV	
allyl phenoxyacetate	0.38	IV	96.0%	IV	100.2%	IV	IV	
lauric acid	0.58	IV	20.2%	II / III	101.4%	IV	IV	
sodium bisulphite	0.75	IV	56.1%	IV	93.5%	IV	IV	
isopropanol	0.83	IV	83.0%	IV	96.1%	IV	IV	
4-(methylthio)benzaldehyde	0.92	IV	7.0%	II / III	79.1%	IV	III	
benzyl salicylate	0.92	IV	89.5%	IV	97.3%	IV	IV	
isopropyl myristate	1.17	IV	97.5%	IV	100.8%	IV	IV	
isopropyl palmitate	1.17	IV	92.9%	IV	107.8%	IV	IV	
n-butyl propionate	1.25	IV	20.8%	IV	108.7%	IV	IV	
hydroxycitronellal#	1.33	IV	14.7%	II / III	72.5%	IV	III	IV
2-ethoxy ethyl methacrylate	1.33	IV	6.9%	II / III	99.0%	IV	III	
benzylalcohol*	1.33	IV	5.0%	II / III	89.2%	IV	III	
heptyl butyrate	1.50	IV	97.0%	IV	97.1%	IV	IV	
methyl stearate	1.50	IV	101.0%	IV	100.9%	IV	IV	
3-chloro-4-fluoronitrobenzene#	1.63	IV	7.4%	II / III	93.3%	IV	III	IV
linalol*	1.75	IV	5.0%	II / III	73.1%	IV	III	
benzyl acetate	1.92	IV	74.4%	IV	102.8%	IV	IV	
allyl heptanoate	1.94	IV	100.0%	IV	100.1%	IV	IV	

- Data Set: 41 chemicals with paired *in vivo-in vitro* data (averaged EpiDerm™-based results available for both EX15 and EX60)
- *Chemicals with two sets of *in vivo* data (EPA Category predicted differently by the animal studies)
- #Chemicals tested for the proposed US EPA PM2
- In black: chemicals included in the IIVS R&D set: 13 chemicals
  - chlorinated solvents (2)
  - aldehydes (3)
  - esters (2)
  - brominated derivatives (1)
  - alcohols (1)
  - surfactants (1)
  - S-containing compounds (1)
  - alkalis (1)
  - halogenated aromatic (1)

2 of EPA Category II
6 of EPA Category III
5 of EPA Category IV

EPA Category Predicted by:	PREDICTION MODELS (PM)				Adjusted EPA Predicted Category
	In vivo PM	UN GHS PM	Proposed US EPA PMs 1	Proposed US EPA PMs 2	
Used endpoint:	PDII	% Tissue Viability			
III	>5.0	≤ 50	< 20% (EX15/PT42)		II
			NA	and > 20% (EX15/PT24)	III
IV	2.1-5.0		≥ 20% (EX15/PT42) and ≤ 20% (EX60/PT42)		III
			NA	and > 20% (EX15/PT24)	IV

CONCLUSIONS AND FUTURE PLANS

US EPA Category Determined per *in vivo* PM	US EPA Category Determined *in vitro* per Proposed PM1 II		Proposed PM1 III		Proposed PM2 IV		Total	Concordance (%)		Toxicity Over Predicted (%)		Toxicity Under Predicted (%)
II	3		0		0		3	100		0		0
III	6	4	4	6	3		13	30.8	46.2	46.2	30.8	23.0
IV	0		7	4	20	23	27	74.1	85.2	25.9	14.8	0
Total	9	7	11	10	23	26	43	62.8	74.4			
Predictivity (%)	33.3	42.9	36.4	60.0	87.0	88.5						
Category Under Predicted (%)	0		0		13.0	11.5						
Category Over Predicted (%)	66.6	57.1	63.6	40.0	0							

We propose to further investigate the US EPA Proposed PM2 by increasing the number of chemicals tested following the proposed strategy and by expanding the chemical class range.

1. **Our retrospective data analysis (based on published *in vivo* and *in vivo* results for skin irritation) showed that the UN GHS PM could not discriminate between US EPA Hazard Categories II and III.**
2. **The proposed US EPA PM1 based on combined EX15/PT42 and EX60/PT42 assays separated the US EPA Hazard Categories II and III, with 100% concordance for Category II chemicals. However, US EPA Category III chemicals were both under and over-predicted by this PM1.**
3. **The addition of the EX15/PT24 assay to the PM1 decreased the over-prediction rate for US EPA Category III and IV.**