Addressing the Assignment of US EPA Hazard Categories for Dermal Safety by a Revised Prediction Model of the Validated *In Vitro* Skin Irritation Test (OECD TG 439)

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ABSTRACT

One of the current goals of the United States Environmental Protection Agency Office of Pesticide Programs (US EPA OPP) is to replace by non-animal testing methods as many of the endpoints of the battery of acute toxicity tests known as the "six-pack" as possible. One of the "six-pack" tests is the Draize rabbit test for dermal irritation. We investigated whether the validated *in vitro* Skin Irritation Test (SIT, OECD TG 439) can be used to determine US EPA OPP dermal hazard category assignment. The SIT discriminates 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-treatment period. A single cut-off value of 50% tissue viability separates GHS Category 2 from the GHS No Category prediction. First we performed a retrospective analysis of paired *in vivo-in vitro* data from 41 chemicals, which indicated that the prediction model (for GHS hazard categories) used in TG 439 did not adequately predict EPA hazard categories. We then modified the validated test method protocol and developed a revised prediction model for EPA hazard categories. Preliminary testing of a sub-set of chemicals from the group of 41 was conducted using a modified protocol based on a 15 minute exposure followed by a 24- or 42 hours post-treatment period in addition to the validated method. The results were analyzed using a new prediction model: 15- and 60 minutes exposure, 24- and 42 hours post-exposure, and a revised cut-off value of the 20% viability endpoint which improved the prediction of the EPA hazard categories. We are currently investigating a larger and diverse set of chemicals with already assigned EPA skin hazard categories to assess the validity of this new protocol and prediction model for EPA hazard 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]

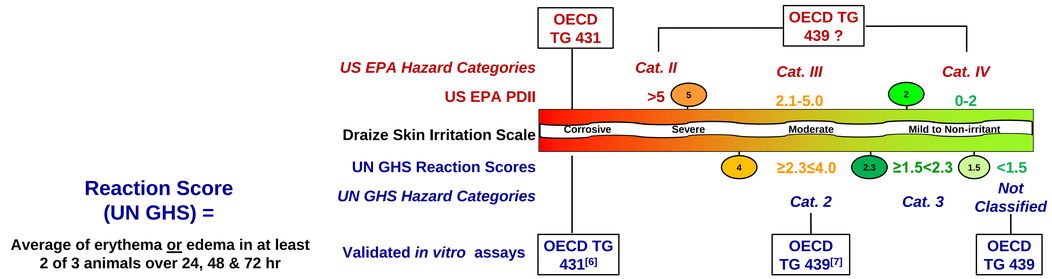
Erythema and eschar formation	Oedema formation	
No erythema	No oedema	0
Very slight erythema (barely perceptible)		1
Well defined erythema	Slight oedema (edges or area well defined by definite raising)	2
Moderate to severe erythema	Moderate oedema (raised approximately 1 mm)	3
Severe erythema (beef redness)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

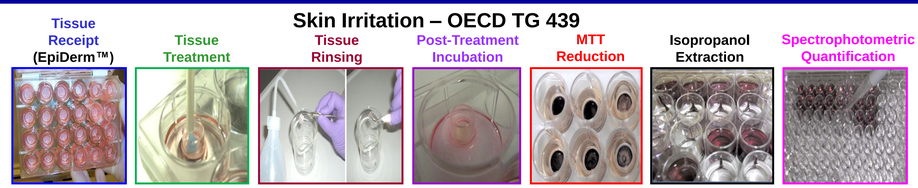


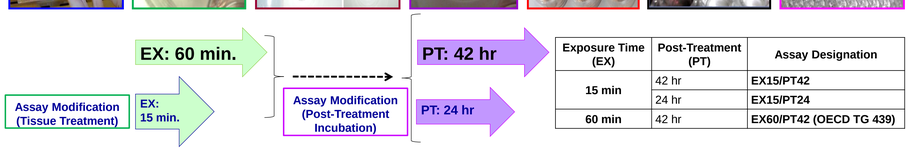
Reaction Score (UN GHS) =

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

MATERIALS & METHODS

Skin Irritation – OECD TG 439





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

RESULTS

Chemical Identification		*In vivo* Results PDII	EPA Category per *in vivo* Assay	EPA Category per UN GHS PM [EX60/PT42]		EPA Category per UN GHS PM [EX15/PT42]		EPA Category determined based on [EX15/PT42] *and* [EX60/PT42]	EPA Category determined based on [EX15/PT42] *and* [EX60/PT42]; *and if* [EX15/PT24]
SLS (20%)	#	5.83	II	II	III	II	III	II	II
Heptanal		5.53	II	II	III	II	III	II	II
1,1,1-Trichloroethane	#	5.5	II	II	III	II	III	II	II
Tetrachloroethylene	#	5	III	II	III	II	III	II	III
Potassium hydroxide(5%)	^	4.83	III	II	III	II	III	II	II
1-Bromopentane		4.42	III	II	III	IV		III	III
Cyclamen aldehyde	#	4.25	III	II	III	II	III	II	III
Cinnamaldehyde	^	4.22	III	II	III	II	III	II	II
1-bromohexane		3.58	III	II	III	IV		III	III
Linalyl acetate		3.5	III	IV		IV		IV	IV
Hexyl salicylate	+	3.17	III	IV		IV		IV	IV
Linalol	*	3.08	III	II	III	IV		III	III
1-decanol		2.84	III	II	III	II	III	II	II
di-n-propyl disulphide		2.75	III	IV		IV		IV	IV
Eugenol		2.66	III	II	III	II	III	II	II
Benzylalcohol	*	2.25	III	II	III	IV		III	III
Allyl heptanoate		1.94	IV	IV		IV		IV	IV
Benzyl acetate		1.92	IV	IV		IV		IV	IV
Linalol	*	1.75	IV	II	III	IV		III	III
Heptyl butyrate		1.5	IV	IV		IV		IV	IV
Methyl stearate		1.5	IV	IV		IV		IV	IV
Hydroxycitronellal	#	1.33	IV	II	III	IV		III	III
2-ethoxy ethyl methacrylate	#	1.33	IV	II	III	IV		III	III
Benzylalcohol	*	1.33	IV	II	III	IV		III	III
n-butyl propionate		1.25	IV	IV		IV		IV	IV
Isopropyl myristate		1.17	IV	IV		IV		IV	IV
Isopropyl palmitate		1.17	IV	IV		IV		IV	IV
4-(methylthio)benzaldehyde	#	0.92	IV	II	III	IV		III	IV
Benzyl salicylate	#	0.92	IV	IV		IV		IV	IV
Isopropanol	#	0.83	IV	IV		IV		IV	IV
Sodium bisulphite		0.75	IV	IV		IV		IV	IV
Lauric acid		0.58	IV	II	III	IV		III	IV
Allyl phenoxyacetate		0.38	IV	IV		IV		IV	IV
Dipropylene glycol		0.33	IV	IV		IV		IV	IV
Erucamide		0.25	IV	IV		IV		IV	IV
Benzyl benzoate		0.25	IV	IV		IV		IV	IV
4,4-Methylene bis-(2,6-ditert-butyl) phenol		0.17	IV	IV		IV		IV	IV
Sodium bicarbonate		0.08	IV	IV		IV		IV	IV
4-Amino-1,2,4-triazole diethyl phtalate		0.08	IV	IV		IV		IV	IV
1-bromo-4-chlorobutane	#	0	IV	II	III	IV		III	IV
3,3'-Dithiodipropionic acid		0	IV	IV		IV		IV	IV

CHEMICALS SET FOR RETROSPECTIVE DATA ANALYSIS

- **39 unique chemicals (41 data points) with paired *in vivo-in vitro* data (averaged EpiDerm™-based results available for both EX15 and EX60)[8-11]:**
- **3 (Category I); 13 (Category III) and 25 (Category IV)**
- ***Chemicals with two sets of *in vivo* data (EPA Category predicted as III and IV, respectively, by the animal studies: Linalol and Benzylalcohol)**

CHEMICALS SUB-SET FOR R&D WORK

- **13 chemicals tested using the proposed US EPA PM**
- **2 (Category II); 5 (Category III) and 6 (Category IV) and:**
  - **# correctly predicted (10)**
  - **+ under-predicted (1)**
  - **^ over-predicted (2)**

EPA Category determined using the *in vivo* Assay

EPA Category determined using the *in vitro* OECD TG439 Prediction Model

Single exposure: 60 or 15 minutes (dataset analyzed separately)
Single post-treatment period: 42 hours
Single cut-off value for tissue viability endpoint: 50%

- **The UN GHS PM could not discriminate between US EPA Hazard Categories II and III.**

By using the data from single exposure of 60 minutes:

- **Some US EPA Category IV materials were over-predicted as II/III.**

By reducing the exposure to 15 minutes:

- **Some of the Category III chemicals were under-predicted as Category IV.**
- **All Category IV materials were correctly predicted.**

EPA Category determined using the proposed *in vitro* US EPA Prediction Model

Combined data from two exposures: 60 and 15 minutes
Single post-treatment period: 42 hours
***Revised** single cut-off value for tissue viability endpoint: 20%*

- **US EPA Category II chemicals: separated with 100% concordance**
- **US EPA Category III chemicals:**
  - **Correctly predicted: 4**
  - **Under-predicted: 3**
  - **Over-predicted: 6**
- **US EPA Category IV chemicals: some over-predicted as Category III.**

By adding the 24 hours post-treatment period to the 15 minutes single exposure:

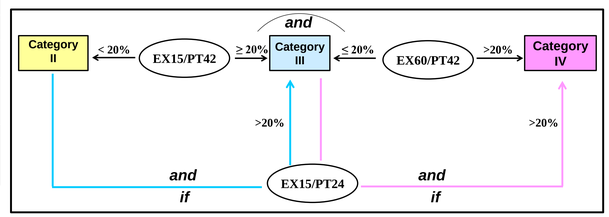
- **US EPA Category III materials: the over-prediction rate decreased:**
  - **Correctly predicted: 6**
  - **Under-predicted: 3**
  - **Over-predicted: 4**
- **US EPA Category IV chemicals: only Linalol and Benzylalcohol were over-predicted as Category III (predicted *in vivo* to be Category III and IV)**

CONCLUSIONS

- **Additional experiments and data analysis are needed to address the US EPA Category III materials still misclassified.**
- **Access to *in vivo* data is needed particularly those associated with formulations.**

US EPA Category determined *in vivo*	US EPA Category determined *in vitro*		
	II	III	IV
Sensitivity	100%	46.2%	92%
Category under predicted	0%	23.0%	NA
Category over predicted	NA	30.8%	8.0%

Proposed in vitro Prediction Model for assignment of US EPA hazard categories for skin irritation



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