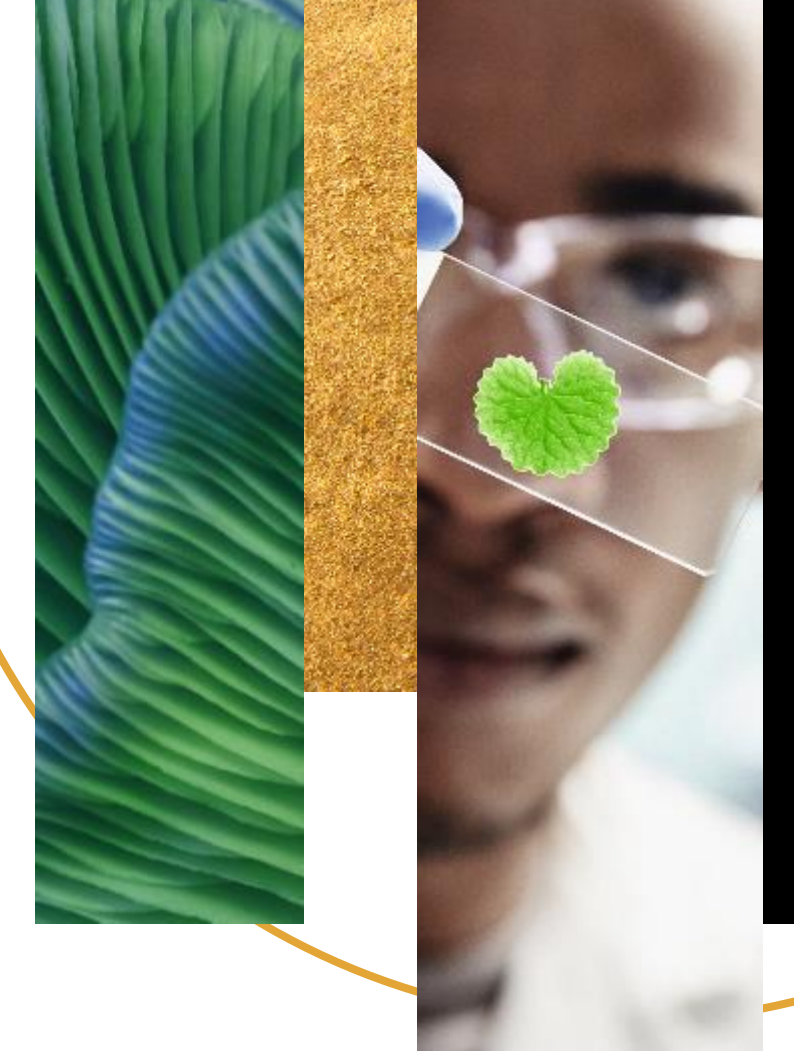


# DEVELOPMENT OF A DEFINED APPROACH FOR EYE HAZARD IDENTIFICATION OF NEAT SOLIDS ACCORDING TO THE THREE UN GHS CATEGORIES

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## 1 INTRODUCTION

The regulatory acceptance of Defined Approaches (DA) for assessing the hazard identification potential of chemicals remains an active topic for regulatory toxicology. Currently, two DA for non-surfactant liquids (DAL-1 and DAL-2) have been adopted by the Organisation for Economic Cooperation and Development (OECD TG 467, 2022) to discriminate between three United Nations Globally Harmonized System of Classification (UN GHS) categories i.e., Category 1 (Cat. 1) on "serious eye damage"; Category 2 (Cat. 2) on "eye irritation" and No Category (No Cat.) for chemicals "not requiring classification and labelling" for eye irritation or serious eye damage.

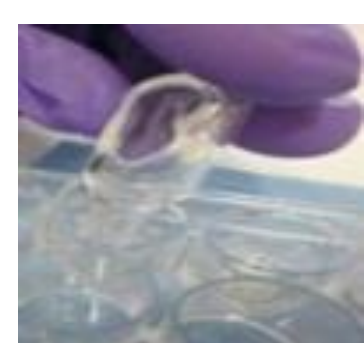
The purpose of the current project was to expand the applicability domain to solids with a new DA for solid chemicals. The DA for neat solids (DAS) is based on a combination of a Reconstructed human Cornea-like Epithelium (RhCE) test method, the SkinEthic™ Human Corneal Epithelium (HCE) Eye Irritation Test (EIT) (OECD TG 492), and the Bovine Corneal Opacity and Permeability (BCOP) test method using the laser light-based opacitometer (LLBO) (OECD TG 437).

## 2 MATERIALS AND METHODS

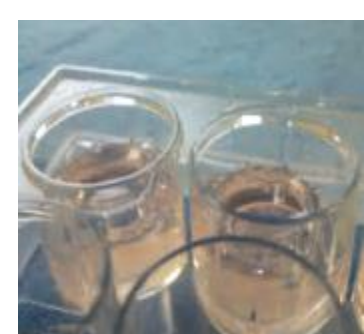
### PROTOCOLS AND PREDICTION MODELS

#### SKINETHIC™ HCE EITS (TG 492)

**Exposure:**  
4 hrs (neat solids)



Rinse + Post Soak: 30 min  
Post-incubation: 18 hrs



Viability assessment

SkinEthic™ HCE EITS	
Cell viability	Prediction
> 50%	No Cat.
≤ 50%	NPCM

NPCM: No stand-alone prediction can be made

#### BCOP LLBO (TG 437)

Mounting of corneas in holders  
Incubation: 1 hr  
LLBO opacity measurement (T0)

**Exposure:**  
4 hrs (20% w/v)



Washing

LLBO opacity measurement (T240)

Application of fluorescein (1.5 hrs)  
Determination of fluorescein (OD<sub>490</sub>)

BCOP LLBO	
Endpoints	Prediction
LIS ≤ 30	No Cat.
LIS > 30 & Opacity ≤ 145 & OD ≤ 2.5	NPCM
LIS > 30 & Opacity ≥ 145 and/or OD > 2.5	Cat. 1

LIS: *in vitro* irritancy score  
LIS = mean opacity (lux/7) + (15 x permeability)

### THE CHEMICALS DATASET

The reference set used to develop the DAS covers a broad range of uses and chemical classes and represented the most important drivers of *in vivo* Cat. 1 and Cat. 2 classifications. The two main subgroups for No Cat. (CO=0 and CO>0) were also included. The distribution grouped by driver of classification and by set is shown in the table below.

The performance of the DAS was assessed against the proposed minimum performance of 75% for Cat. 1, 50% for Cat. 2 and 70% for No Cat. as agreed by the OECD expert group for the DAL (OECD SD 354, 2022).

UN GHS	Driver	Training set	Test set	Total
Cat. 1	CO mean ≥ 3	7	2	9
	CO = 4	6	6	12
	CO pers D21	7	3	10
Total		20	11	31
Cat. 2	CO mean ≥ 3	5	2	7
	Conj mean ≥ 2	7	4	11
Total		12	6	18
No Cat.	CO > 0	6	5	11
	CO = 0	33	16	49
Total		39	21	60

## 4 CONCLUSIONS

- The Defined Approach for solids performs better than each individual test methods
- The prediction reached the minimum performance values of 75% Cat. 1, 50% Cat. 2, and 70% No Cat. established by the OECD expert group.
- The new DA for solids is proposed to be integrated in the OECD Test Guidelines 467 for DAs on Eye irritation as Part III.

## 3 RESULTS & DISCUSSION

### PERFORMANCE OF INDIVIDUAL METHODS

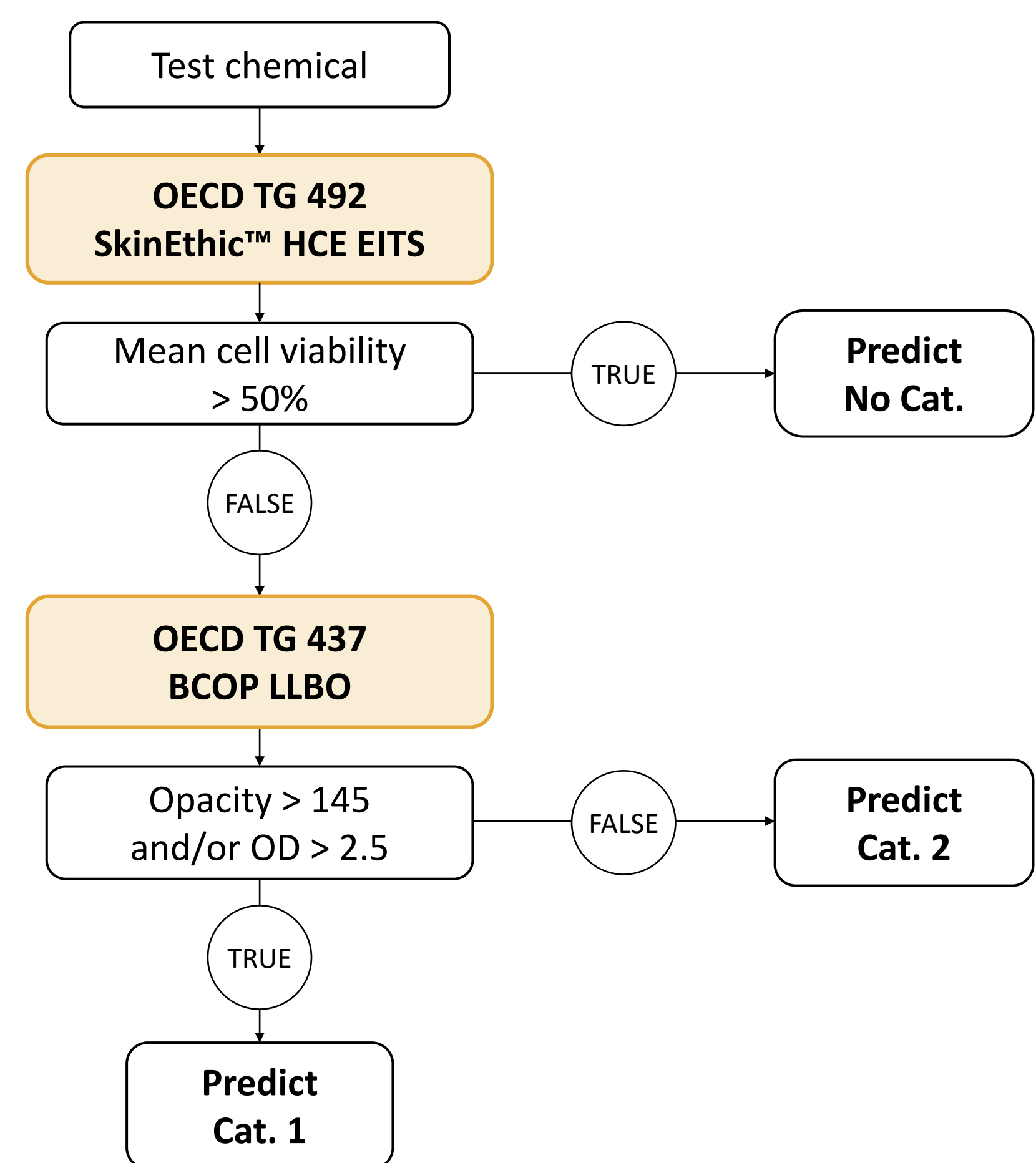
UN GHS	SkinEthic™ HCE EITS		BCOP LLBO		
	NPCM	No Cat.	Cat. 1	NPCM	No Cat.
Cat. 1 (N=31)	100%	0%	77.4%	19.4%	3.2%
Cat. 2 (N=18)	81.8%	18.2%	30.6%	38.9%	30.6%
No Cat. (N=60) <sup>a</sup>	30.0%	70.0%	5.6%	50.3%	44.2%

<sup>a</sup> 6 *in vivo* No Cat. solids have no LLBO data (True Negative with SkinEthic™ HCE EITS)

Defined approach for solids (DAS)

- SkinEthic™ HCE EITS: identify No Cat.
- BCOP LLBO: identify Cat. 1

### DEFINED APPROACH



### PERFORMANCE DEFINED APPROACH

UN GHS	DAS prediction		
	Cat. 1	Cat. 2	No Cat.
<b>Cat. 1 criteria</b> DAS N=31	≥ 75% 77.4%	≤ 25% 22.6%	≤ 5% 0.0%
<b>Cat. 2 criteria</b> DAS N=18	≤ 30% 29.5%	≥ 50% 52.3%	≤ 30% 18.2%
<b>No Cat. criteria</b> DAS N=60	≤ 5% 1.7%	≤ 30% 28.3%	≥ 70% 70.0%

OECD Performance criteria

The performance of the DAS to distinguish between the three UN GHS categories was compared with the minimum performance values for each category and all OECD defined criteria were met.

