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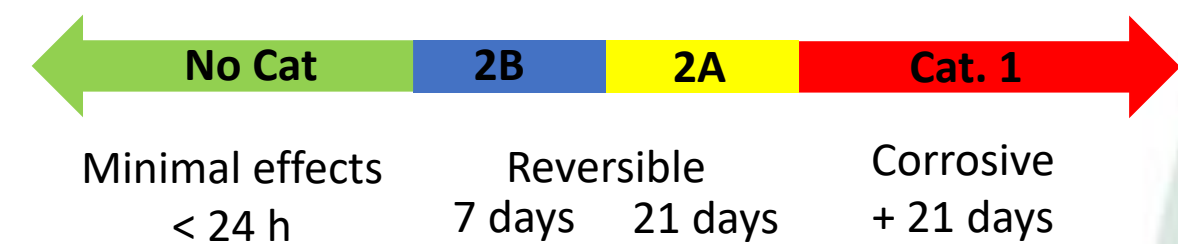
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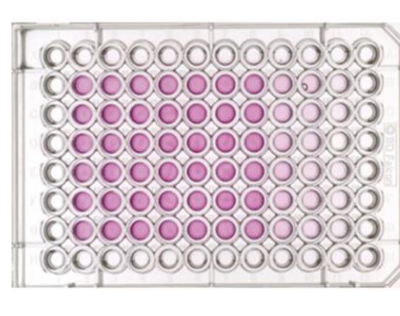
Introduction

For the registration of agrochemical formulations, acute eye toxicity assessment is required by regulatory agencies. The Draize rabbit eye test (OECD TG 405) has worldwide acceptance to assess eye irritation, even it has been increasingly questioned. The test distinguishes four categories considering reversible and non-reversible ocular lesions according to UN GHS Categories 1 (severe eye damage), 2A and 2B (reversible eye damage) and No Category (minimal effects).



Bovine Corneal Opacity and Permeability (BCOP) and Short Time Exposure (STE) are methods for identifying Cat. 1 and No Cat. products, according to OECD TG 437 and TG 491 respectively. Histopathological analysis of the corneas, after the BCOP test, is recommended to identify the depth of the damage as complementary information.

As the limitation of these alternative methods is to classify in the middle-range categories (2A and 2B), the aim of this work was to create a strategy to identify these categories.

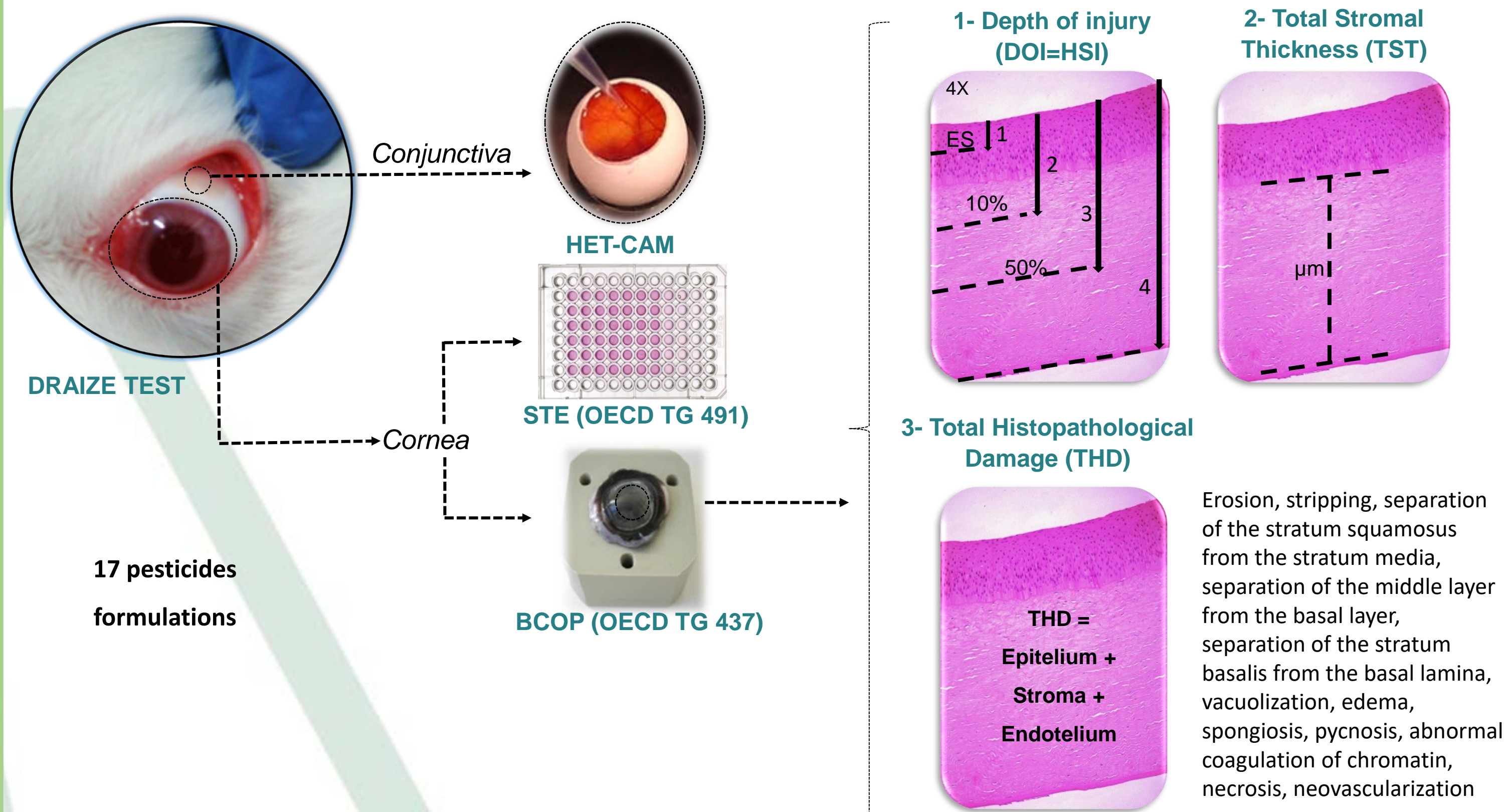


ALTERNATIVE METHODS
in vitro/ex vivo/ in silico/ in chemico



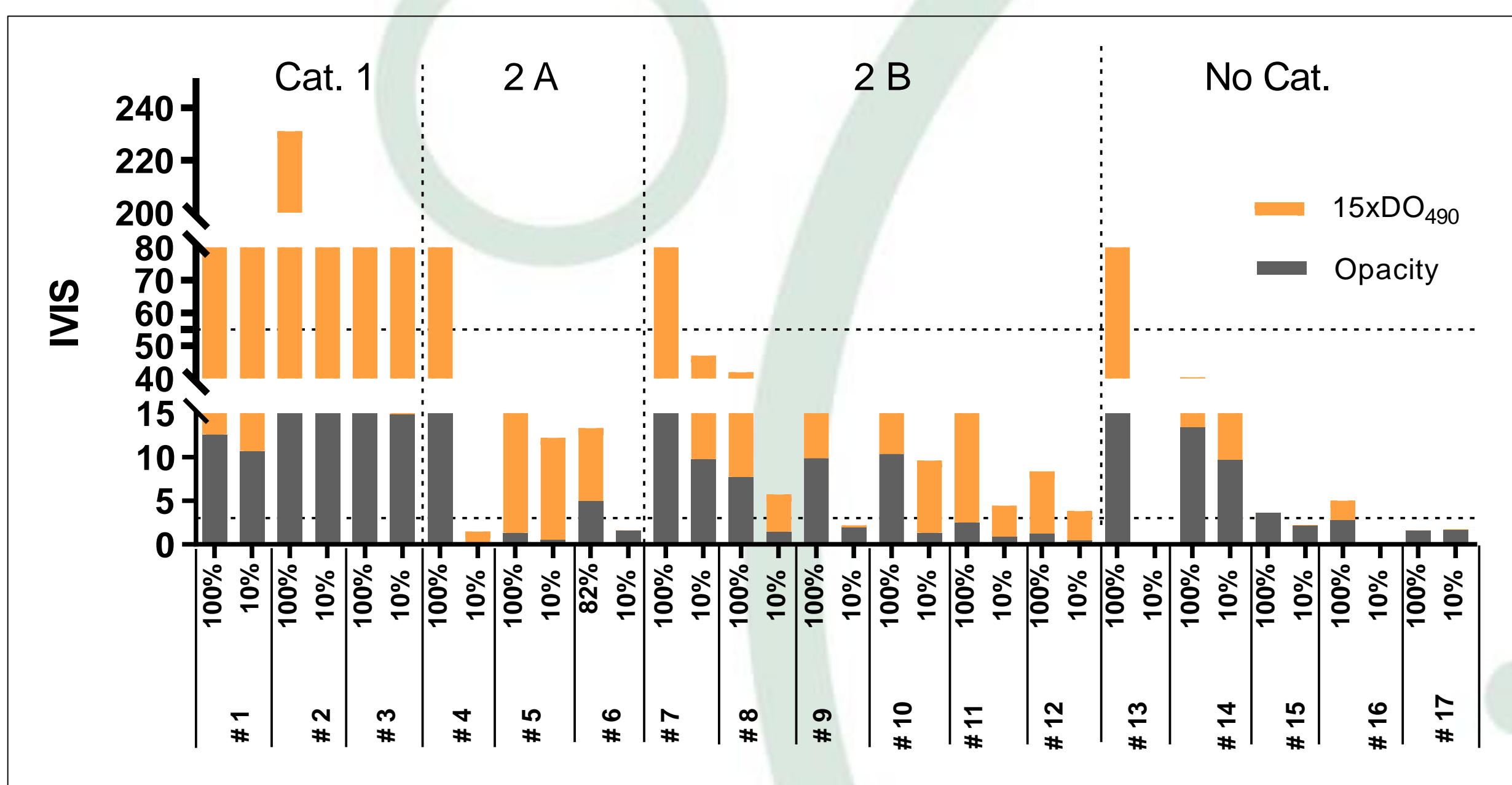
Materials and Methods

The STE and BCOP methods were used to test 17 pesticides manufactured by ATANOR SCA. These products had been previously classified in categories 1, 2A, 2B or No Cat. using the Draize eye irritation test.

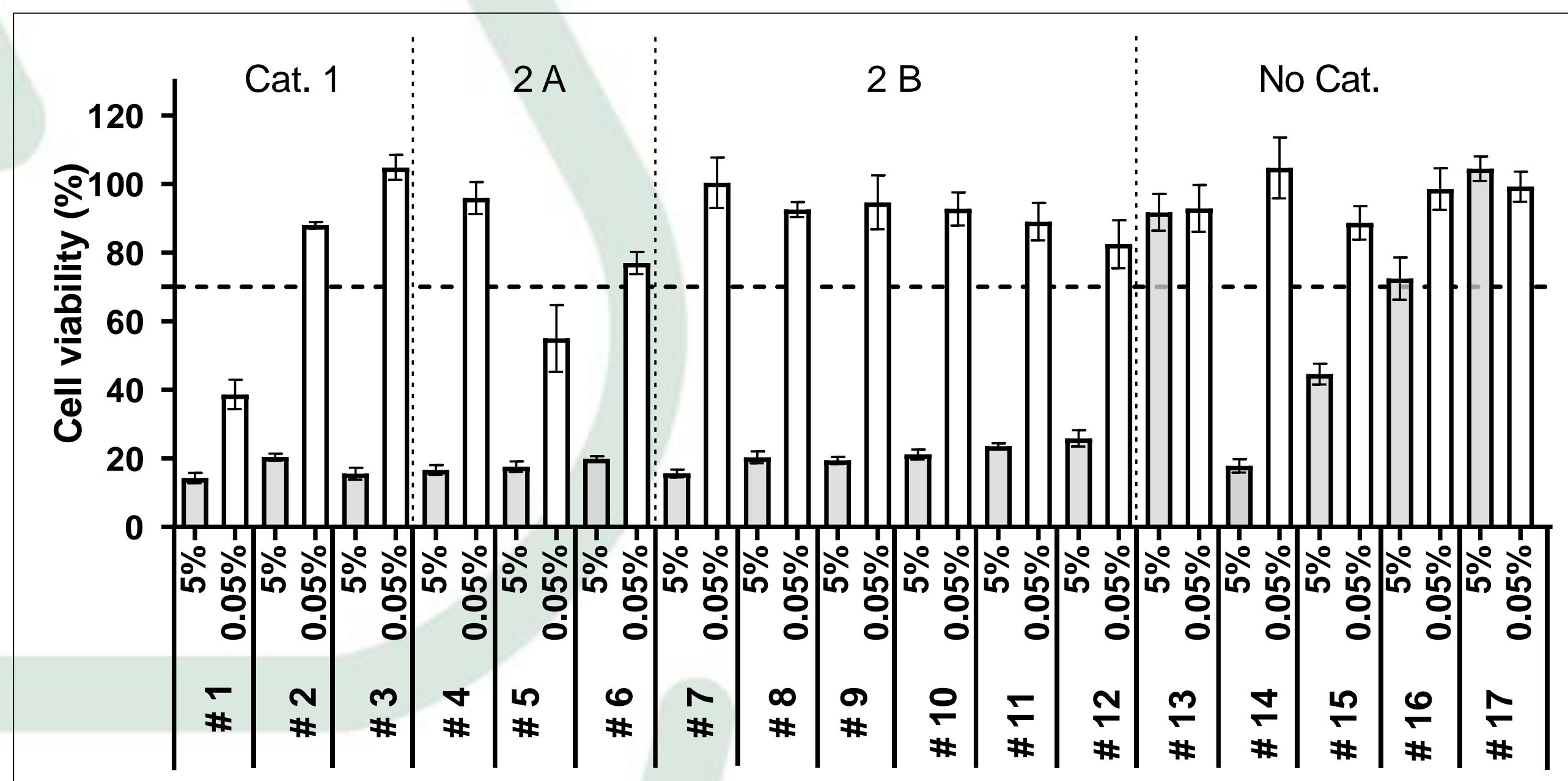


Results

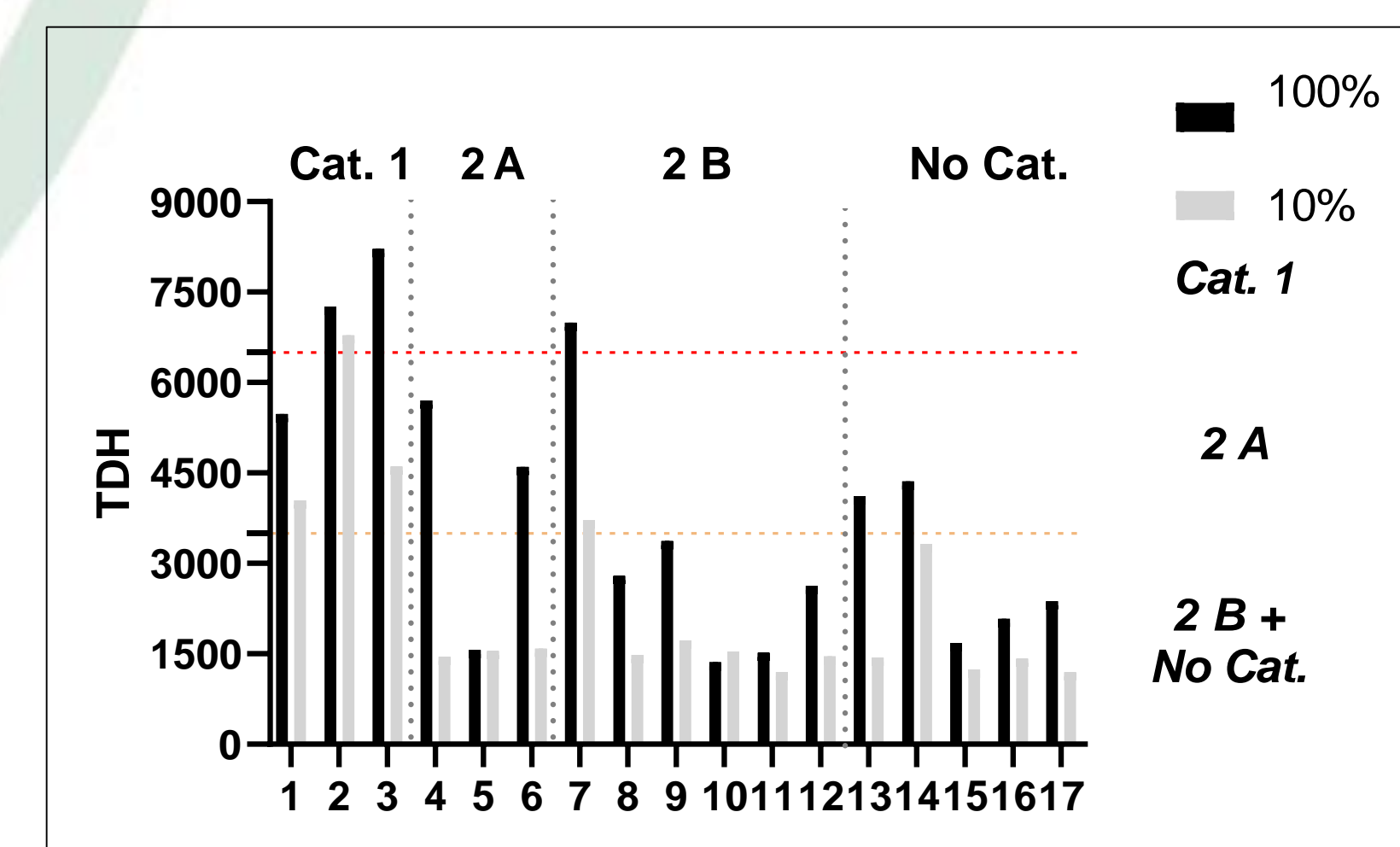
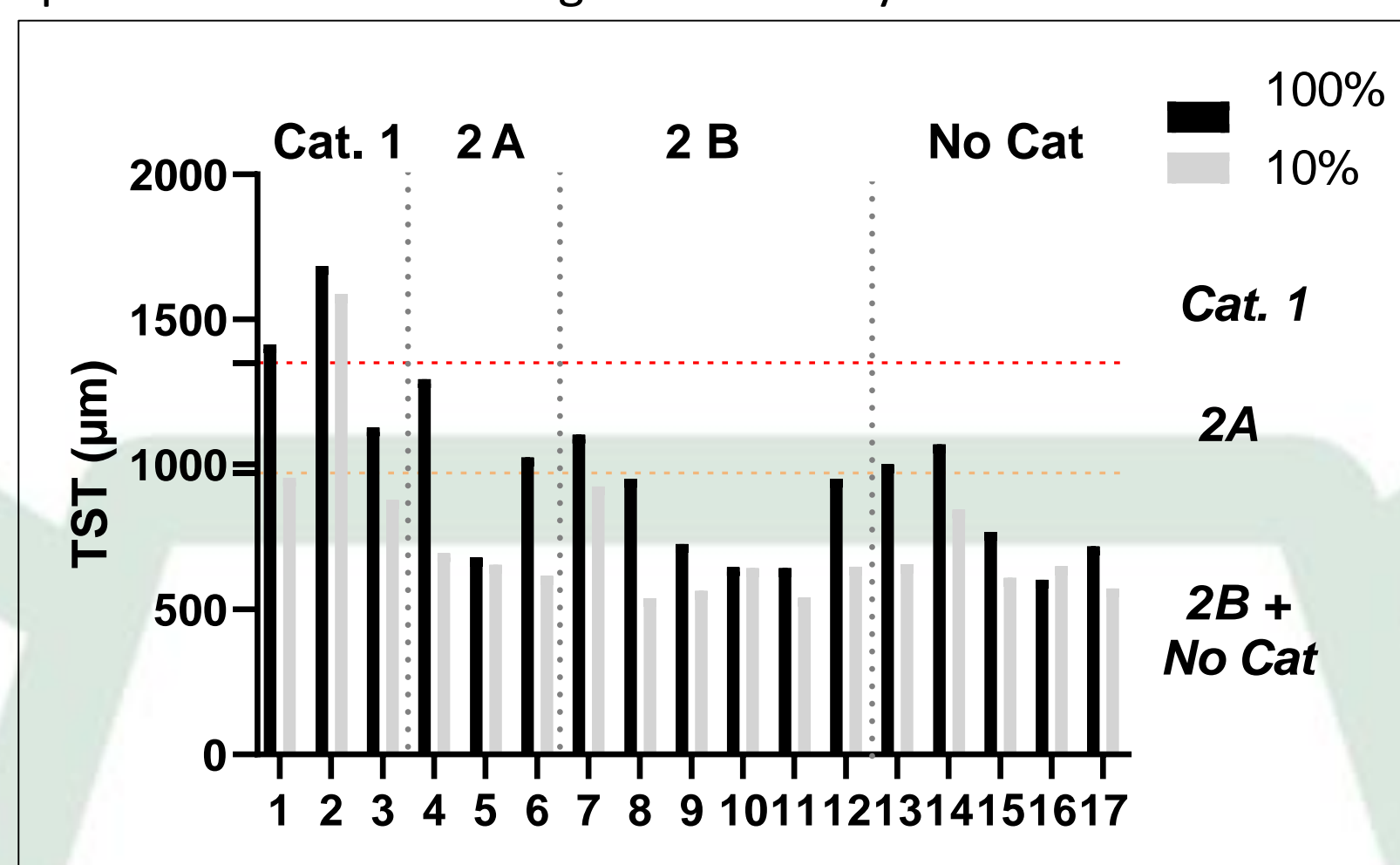
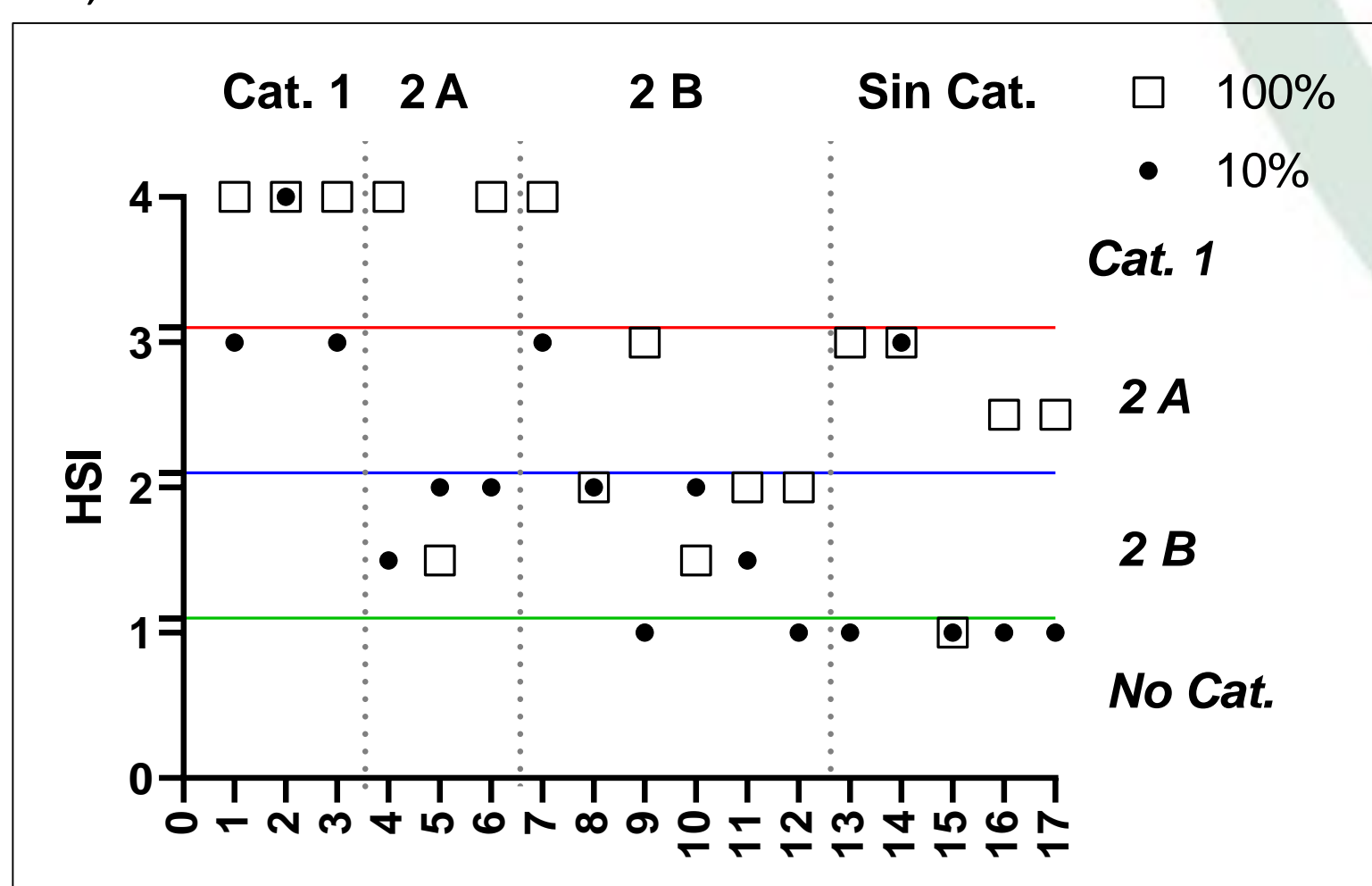
BCOP. IVIS values obtained in the evaluation of the 17 neat and at 10% pesticides. The horizontal dotted lines at the IVIS 3 and 55 values are the cutoffs according to OECD Guideline No. 437. The vertical dotted lines show the categories reported in vivo according to the GHS system.



STE. Cell viability % (mean ± SD) obtained in the evaluation of the 17 pesticides by STE, diluted at 5% (gray bar) and 0.05% (white bar). The horizontal dotted line indicates the cutoff (70%) for classifying substances. The vertical dotted lines show the categories reported in vivo according to the GHS system.



Results for the 17 pesticides evaluated neat and a 10% dilution. HSI= average histological score index using modified Redden. TST (µm) average of the total stromal thickness. TDH average represent total histopathological damage in epithelium, stroma and endothelium. The vertical dotted lines show the categories reported in vivo according to the GHS system.



Weight of evidence for a new product to be evaluated
Analysis of existing information (in vivo or in vitro), composition, physicochemical properties

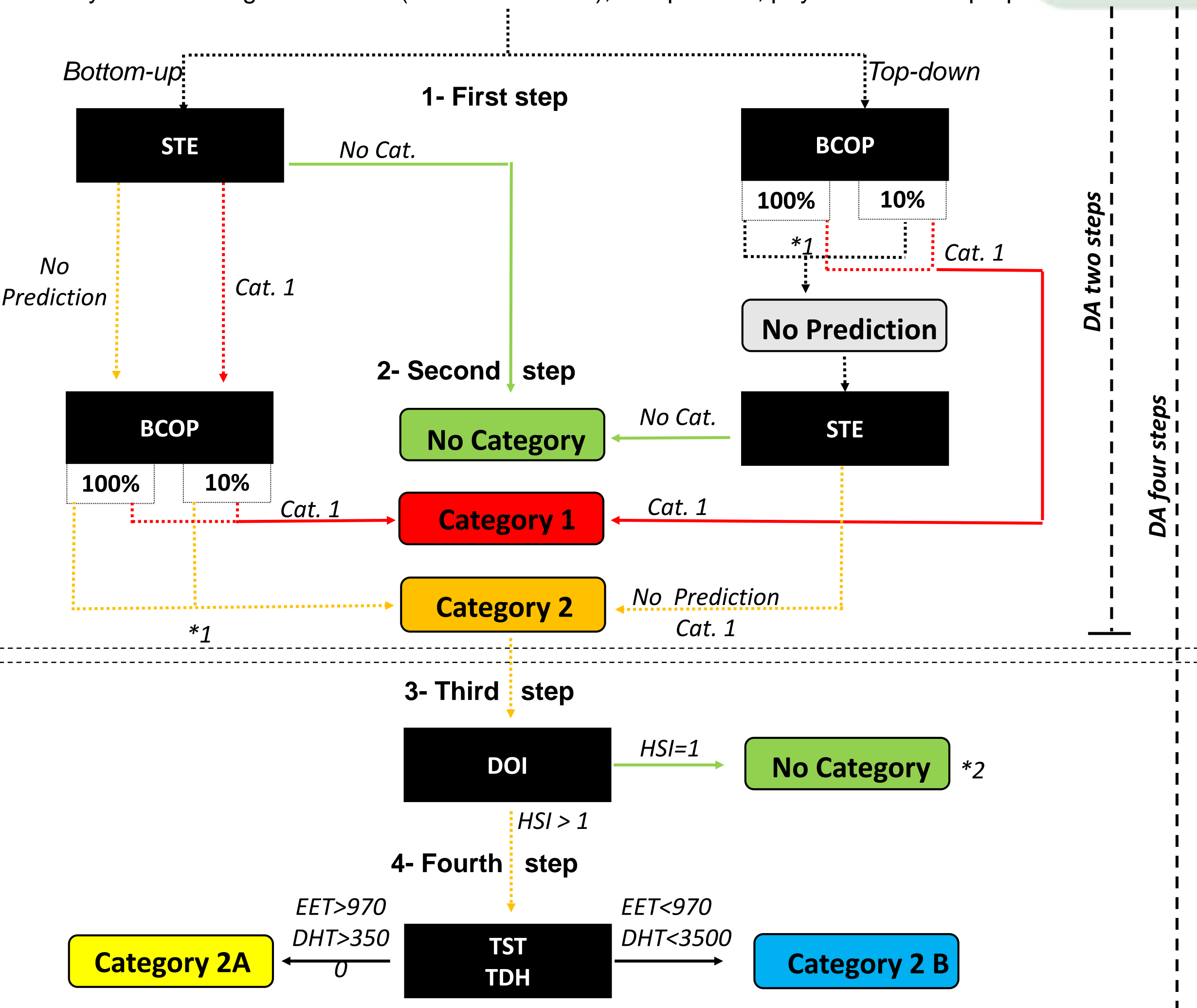


Table 1. Evolution of the classifications of agrochemicals obtained with the different alternative methodologies used within the AD, those resulting from the use of two- and four-step ADs, and their respective in vivo classifications

ID	Classification							in vivo
	STE	BCOP	DOI	TST	TDH	DA 2 steps	DA 4 steps	
# 1	Category 1	Category 1	Category 1	Category 1	2 A	Category 1	Category 1	Category 1
# 2	No Prediction	Category 1	Category 1	Category 1	Category 1	Category 1	Category 1	Category 1
# 3	No Prediction	Category 1	Category 1	2 A	Category 1	Category 1	Category 1	Category 1
# 4	No Prediction	Category 1	Category 1	2 A	2 A	Category 2	2 A	2 A
# 5	Category 1	No Prediction	2 B	2B+No Cat	2B+No Cat	Category 2	2 B	2 A
# 6	No Prediction	No Prediction	Category 1	2 A	2 A	Category 2	2 A	2 A
# 7	No Prediction	Category 1	Category 1	2 A	Category 1	Category 2	2 A	2 B
# 8	No Prediction	No Prediction	2 B	2B+No Cat	2B+No Cat	Category 2	2 B	2 B
# 9	No Prediction	No Prediction	2 A	2B+No Cat	2B+No Cat	Category 2	2 B	2 B
# 10	No Prediction	No Prediction	2 B	2B+No Cat	2B+No Cat	Category 2	2 B	2 B
# 11	No Prediction	No Prediction	2 B	2B+No Cat	2B+No Cat	Category 2	2 B	2 B
# 12	No Prediction	No Prediction	2 B	2B+No Cat	2B+No Cat	Category 2	2 B	2 B
# 13	No Category	Category 1	2 A	2 A	2 A	No Category	No Category	No Category
# 14	No Prediction	No Prediction	2 A	2 A	2 A	Category 2	2 A	No Category
# 15	No Prediction	No Prediction	No Category	2B+No Cat	2B+No Cat	Category 2	No Category *	No Category
# 16	No Category	No Prediction	2 A	2B+No Cat	2B+No Cat	No Category	No Category	No Category
# 17	No Category	No Category	2 A	2B+No Cat	2B+No Cat	No Category	No Category	No Category

Table 2. Distribution of agrochemicals according to the classification obtained using the DA of two and four steps with their in vivo results

DA 2 steps	Classification in vivo			Analysis in vitro vs in vivo (%)			
	Cat. 1	Cat. 2	No Cat.	FP	FN	P	E
Cat. 1	3	-	-	0	0	100	100
Cat. 2	-	9	2	18,2	0	88,2	75
No Cat.	-	-	3	0	40	88,2	100

DA 4 steps	Classification in vivo				Analysis in vitro vs in vivo (%)			
	Cat. 1	Cat. 2A	Cat. 2B	No Cat.	FP	FN	P	E
Cat. 1	3	-	-	-	0	0	100	100
2A	-	2	1	1	50	33,3	82,4	85,7
2B	-	1	5	-	16,7	16,7	88,2	90,9
No Cat.	-	-	-	4	0	20	94,1	100

Conclusion

By STE test, all formulations were tested at 5 and 0.05%. By the BCOP test, liquid products were tested neat and at 10% and solids at 10%. After BCOP, an histopathological analysis was performed and 3 different models were used to analyse: depth of injury, stromal thickness, and total histopathological damage. Following a 4-step decision tree, we obtained the same classification for 14 of the 17 agrochemicals. An analysis by category shows: Cat. 1 100% accuracy and specificity; Cat 2A 82.4% accuracy and 86.7% specificity; Cat 2B 88.2% precision and 90.9% specificity and No Cat. 94.1% precision and 100% specificity. Finally, we successfully established an in-house strategy for testing agrochemicals eye irritation.

Funding

