Subcategorization of Eye Irritants Using the EpiOcular Time-to-Toxicity Test

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Abstract

Background: As per OECD TG 405 "Acute Eye Irritation/ Corrosion", albino rabbits were traditionally used to assess the eye damage/eye irritation of test materials. However, in 2015, OECD TG 492 "Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage" was accepted and validated for the use of in vitro ocular tissue models. Initially, this TG allowed for distinguishing between substances and mixtures not requiring classification and those that must be labeled for eye irritation or serious eye damage. Differentiation between materials causing serious eye damage and less-severe eye irritation was not included in the TG. Recently, OECD TG 492B was accepted which allows for distinguishing between chemicals that: a) do not require labeling for serious eye damage or eye irritancy (No Category or No Cat), b) cause serious eye damage (Category 1 or Cat 1), and c) are eye irritants (Category 2 or Cat 2) according to the UN GHS ocular hazard categories.

Methods: Results from 2 studies, the CON4EI project (2017) and the ALT4EI project (2022), were combined and re-analyzed. Prediction models for liquids and solids were developed as part of the CON4EI project which involved a set of 80 chemicals (38 liquids and 42 solids). An additional 64 chemicals were tested within the ALT4EI project. When combined, a robust final set of 144 reference chemicals – 78 liquids and 66 solids, was used to confirm the new testing strategy.

Results: The performance criteria, established by the OECD expert group overseeing OECD TG 492B, were met for all 144 chemicals. This data set was used to develop the EpiOcular™ time-to-toxicity test method for eye hazard identification of liquid and solid chemicals according to the three UN GHS. Based on the new testing strategy for liquids, 78.7% of Cat 1 (N=27), 63.5% of Cat 2 (N=26) and 82.0% of No Cat (N=25) were correctly identified. Using the new testing strategy for solids, 75.0% of Cat 1 (N=28), 59.4% of Cat 2 (N=16) and 80.3% of No Cat (N=22) were correctly predicted. Overall, the new test method correctly predicted 76.8% of Cat 1 (N=55), 61.9% of Cat 2 (N=42), and 81.2% of No Cat (N=47) test articles.

Conclusion: The EpiOcular™ time-to-toxicity test method is a novel approach for subcategorizing both liquid and solid compounds. The prediction models that were developed for liquids and solids are capable of distinguishing substances and mixtures into the 3 UN GHS ocular hazard categories: No Cat, Cat 2, and Cat 1. The new test method will further reduce the need to perform animal tests for determination of the eye irritation potential of materials.

Methods

EpiOcular Tissue Model: EpiOcular tissues were cultured at the air-liquid interface which attains in vivo-like differentiation and allows for topical application of test articles (**Figure 1**). Normal human cells derived from tissue explants are used to produce the EpiOcular tissues. The EpiOcular tissues are produced under Good Manufacturing Practices (GMP) and ISO 9001:2015.
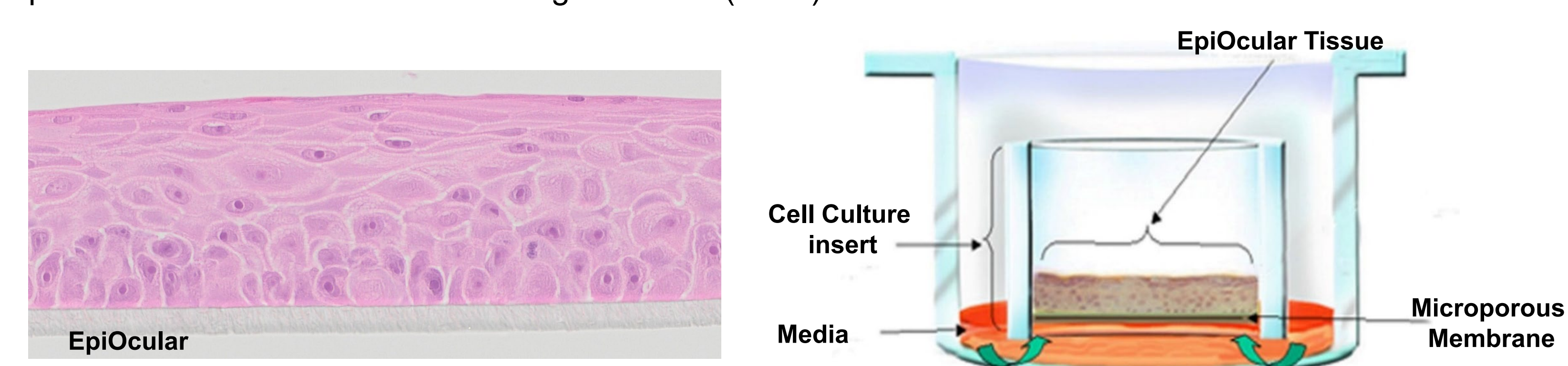

Figure 1: EpiOcular (OCL-200) Model A) Representative formalin-fixed, paraffin embedded, H&E stained tissue cross-section. The tissue structure closely parallels human corneal epithelium. B) Schematic showing tissue model cultured at air liquid interface (tissue fed from basolateral side only) allowing for topical application of test materials.

Chemicals: 144 chemicals (78 liquids and 66 solids) were tested within this project in at least two independent runs. Within a single experiment, a Negative Control (NC, ultrapure water) and a Positive Control (PC, 0.3 % Triton X-100) were concurrently tested on N=2 tissues replicates.

	All	Liquids			Solids		
	Total	CON4EI	ALT4EI	Total	CON4EI	ALT4EI	Total
Total	144	38	40	78	42	24	66
Cat. 1	55	17	10	27	21	7	28
Driver							
CO mean ≥ 3	19	7	4	11	7	1	8
CO pers D21	20	4	6	10	8	2*	10
CO=4	16	6	-	6	6	4	10
Cat. 2	43	13	13	26	14	2	16
Driver							
CO mean ≥ 1	25	8	11	19	5	1	6
Conj mean ≥ 2	17	5	2	7	9	1	10
No Cat.	47	8	17	25	7	15	22
Subgroup							
CO > 0	12	-	6	6	-	6	6
CO = 0	35	8	11	19	7	9	16

* one chemical (2.5-dimethyl-2,5-hexanediol, CASRN 110-03-2) was classified Cat. 1 based on conjunctival persistence (Conj pers) on D21 only

Table 1: *Distribution by UN GHS and driver of classification for the enlarged set of chemicals (78 liquids and 66 solids).* CO: corneal opacity; CO pers. D21: CO persistence on day 21; Conj: conjunctival redness (CR) and/or conjunctival chemosis (CC).
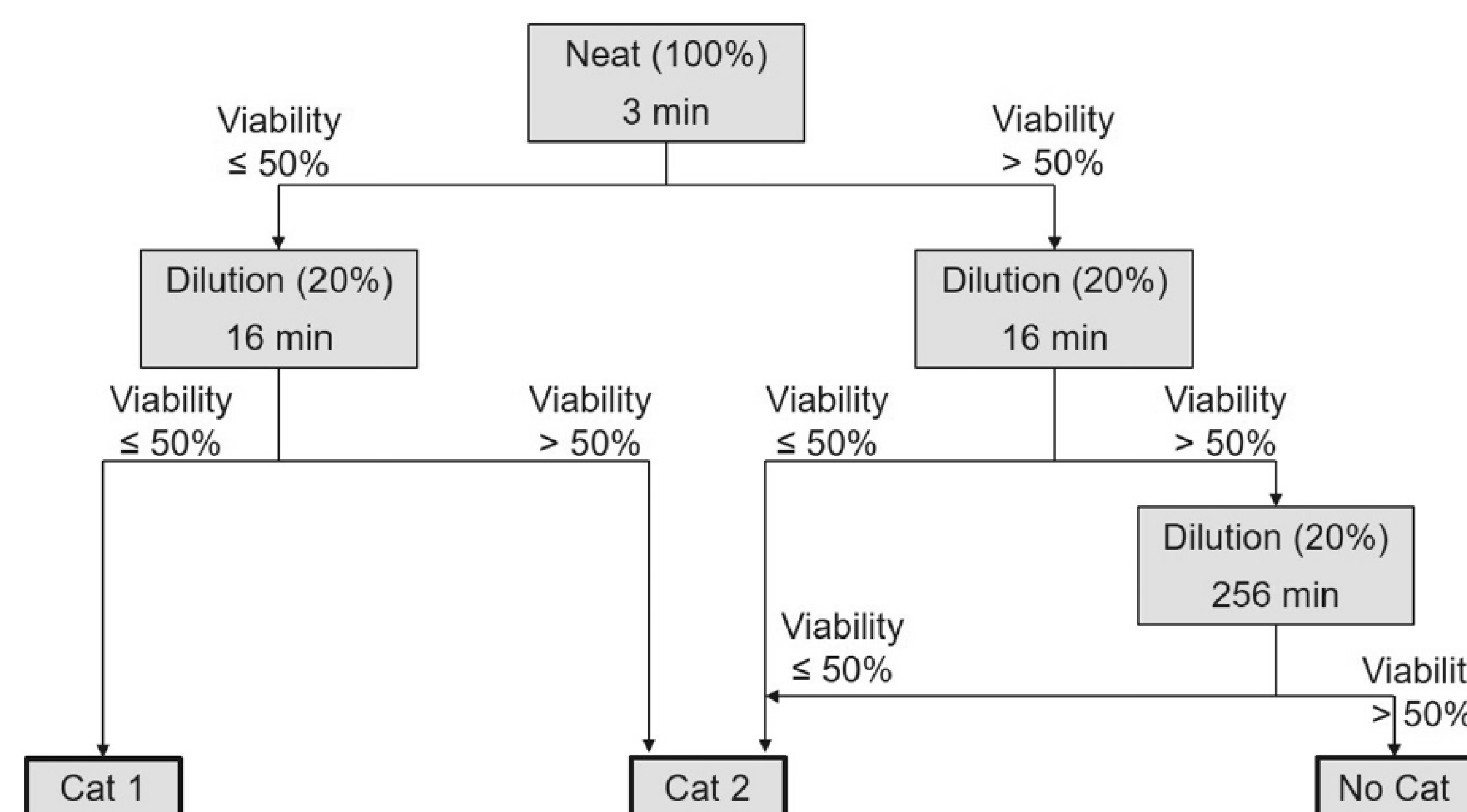

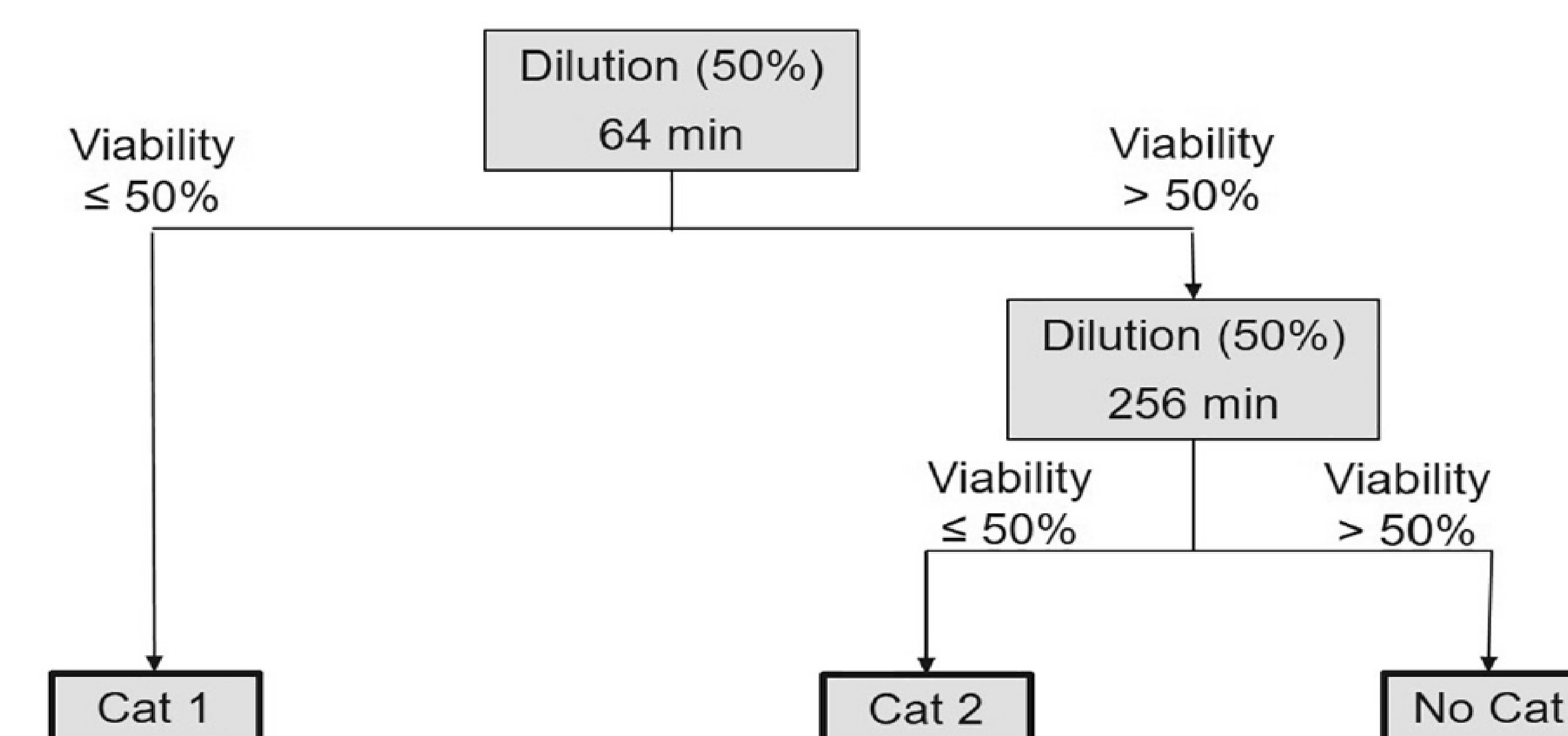**Figure 2: EpiOcular Time-to-toxicity assay - the CON4EI prediction model for liquids.**

Figure 3: EpiOcular Time-to-toxicity assay - the CON4EI prediction model for solids.

	N	Overall			N	Liquids			N	Solids		
UN GHS	144	Cat. 1	Cat. 2	No Cat.	78	Cat. 1	Cat. 2	No Cat.	66	Cat. 1	Cat. 2	No Cat.
Cat. 1	55	**76.8%**	22.3%	0.9%	27	**78.7%**	19.4%	1.9%	28	**75.0%**	25.0%	0.0%
CO mean ≥ 3	19	81.6%	18.4%	0.0%	11	90.9%	9.1%	0.0%	8	68.8%	31.3%	0.0%
CO pers D21	20	66.3%	31.2%	2.5%	10	62.5%	32.5%	5.0%	10	70.0%	30.0%	0.0%
CO=4	16	84.4%	15.6%	0.0%	6	83.3%	16.7%	0.0%	10	85.0%	15.0%	0.0%
Cat. 2	42	27.4%	**61.9%**	10.7%	26	28.8%	**63.5%**	7.7%	16	25.0%	**59.4%**	15.6%
CO mean ≥ 1	25	32.0%	62.0%	6.0%	19	34.2%	57.9%	7.9%	6	25.0%	75.0%	0.0%
Conj mean ≥ 2	17	20.6%	61.8%	17.6%	7	14.3%	78.6%	7.1%	10	25.0%	50.0%	25.0%
No Cat.	47	3.2%	15.6%	**81.2%**	25	0.0%	18.0%	**82.0%**	22	6.8%	12.9%	**80.3%**
CO > 0	12	4.2%	29.2%	66.7%	6	0.0%	33.3%	66.7%	6	8.3%	25.0%	66.7%
CO = 0	35	2.9%	11.0%	86.2%	19	0.0%	13.2%	86.8%	16	6.3%	8.3%	85.4%

Table 3: Performance of the original prediction model (CON4EI project) for the liquids (Figure 1) and solids (Figure 2) – overall set

	Test method		
UN GHS	Cat. 1	Cat. 2	No Cat.
Cat. 1	≥ 75%	≤ 25%	≤ 5%
Cat. 2	≤ 30%	≥ 50%	≤ 30%
No Cat.	≤ 5%	≤ 30%	≥ 70%

Table 2: Performance metrics for the assessment of the predictivity of a test method / testing strategy / defined approach

Conclusions

- Based on the results of the CON4EI project, a prediction model for eye hazard assessment according to the three UN GHS categories was developed (**Figure 2** and **Figure 3**). This prediction model was developed based on a set of 80 chemicals (38 liquids and 42 solids).

- The set of reference chemicals was enlarged to 144 chemicals (78 liquids and 66 solids) (**Table 1**).

- The performance of four additional prediction models was assessed, but the original prediction model (CON4EI) resulted in the best performance.

- The performance criteria established by the OECD expert group on eye/skin irritation/corrosion and phototoxicity were met (**Table 2**), 76.8% of Cat. 1 (N=55), 61.9% of Cat. 2 (N=42), and 81.2% of No Cat. (N=47) were correctly predicted (**Table 3**).

- The EpiOcular™ time-to-toxicity test method is a novel approach for subcategorizing both liquid and solid compounds. The prediction models that were developed have proven to be capable of classifying chemicals (substances and mixtures) **into the 3 UN GHS ocular hazard categories: No Cat, Cat 2, and Cat 1.**

References

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Letasiova S, Kandarova H, Adriaens E, Verstraelen S, van Rompay A R, Hudecova L, Markus J, Kaluzhny Y, Klausner M. EpiOcular™ time-to-toxicity test method for eye hazard subcategorization. Manuscript in preparation.