

OECD TG 492B - SKINETHIC™ HCE TIME-TO-TOXICITY: OCULAR HAZARD ASSESSMENT OF CHALLENGING CHEMICALS WITH A HPLC-BASED FORMAZAN DETECTION METHOD

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1 INTRODUCTION

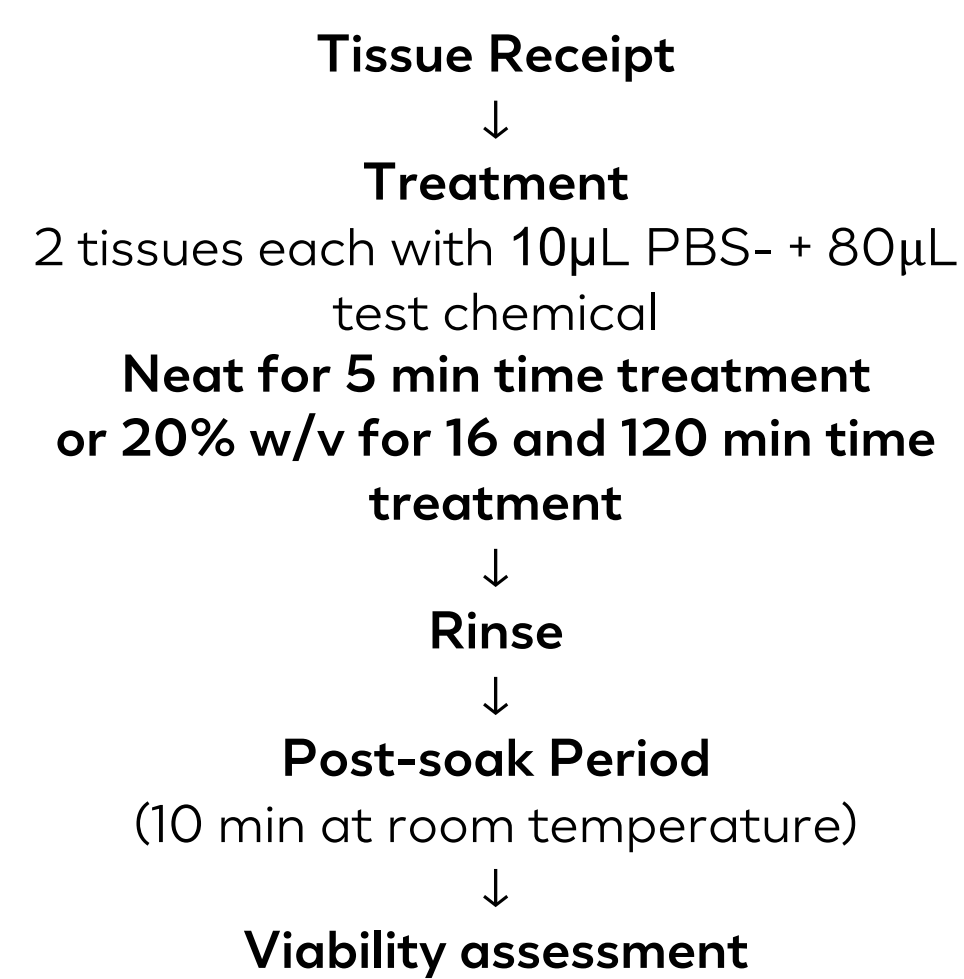
Eye Hazard Identification of a chemical could be assessed with the SkinEthic Human Corneal Epithelium (HCE) Time-to-Toxicity (TTT) method; the only stand alone NAM formally OECD adopted allowing the identification on its own of chemicals (substances and mixtures) not requiring classification (No Cat), requiring classification for eye irritation classification (Cat.2) and requiring classification for serious eye damage classification (Cat.1) according to with the UN GHS ocular hazard categories (OECD, TG 492B).

The potential of a chemical is typically determined by measuring cell viability by the colorimetric MTT reduction assay. A known limitation of the MTT reduction test is possible interference with the absorbance measurement by photometry for chemicals that are highly coloured. To illustrate the relevance of High-Performance Liquid Chromatography (HPLC)-spectrophotometry approach described in the OECD TG 492B, an evaluation of 6 "standard" chemicals and 9 direct MTT-reducers ± colour interfering chemicals was performed. Amongst those, 4 chemicals incompatible with use of standard photometry were included.

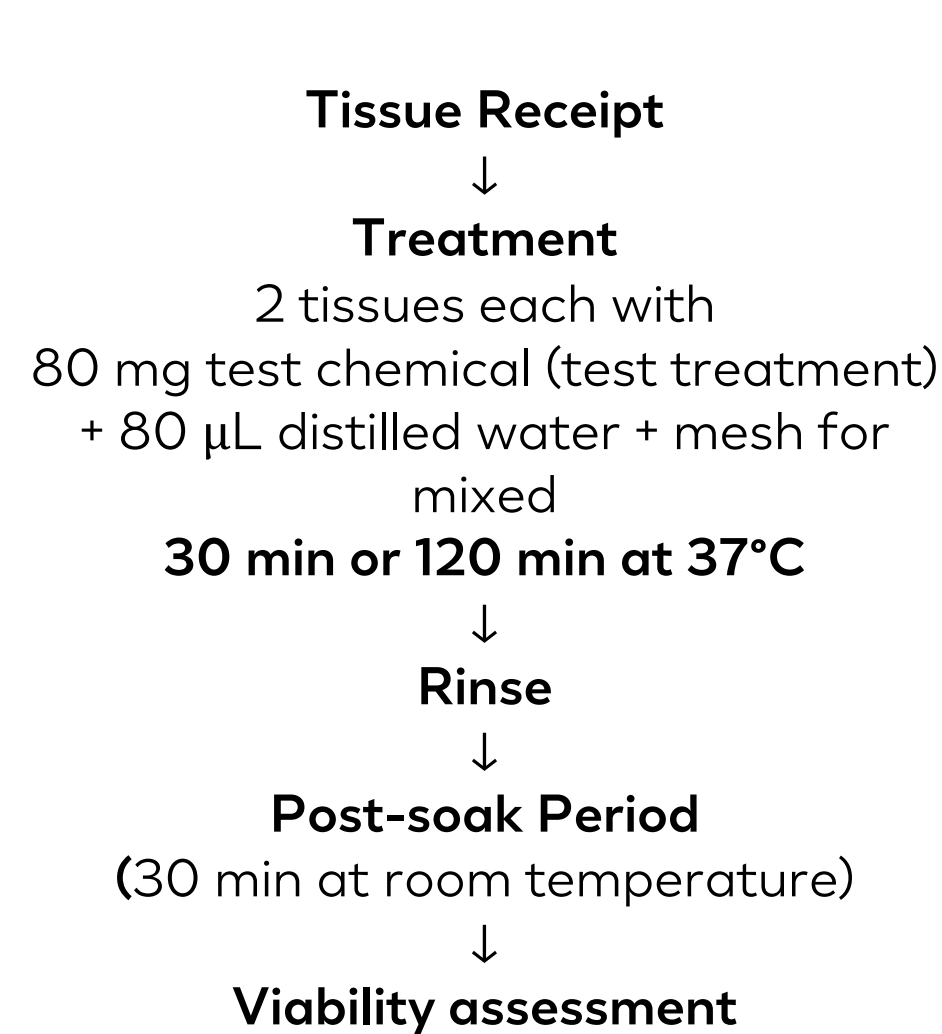
2 MATERIALS AND METHODS

PROTOCOLS

SkinEthic™ HCE TTT for liquids (TTL)



SkinEthic™ HCE TTT for solids (TTS)



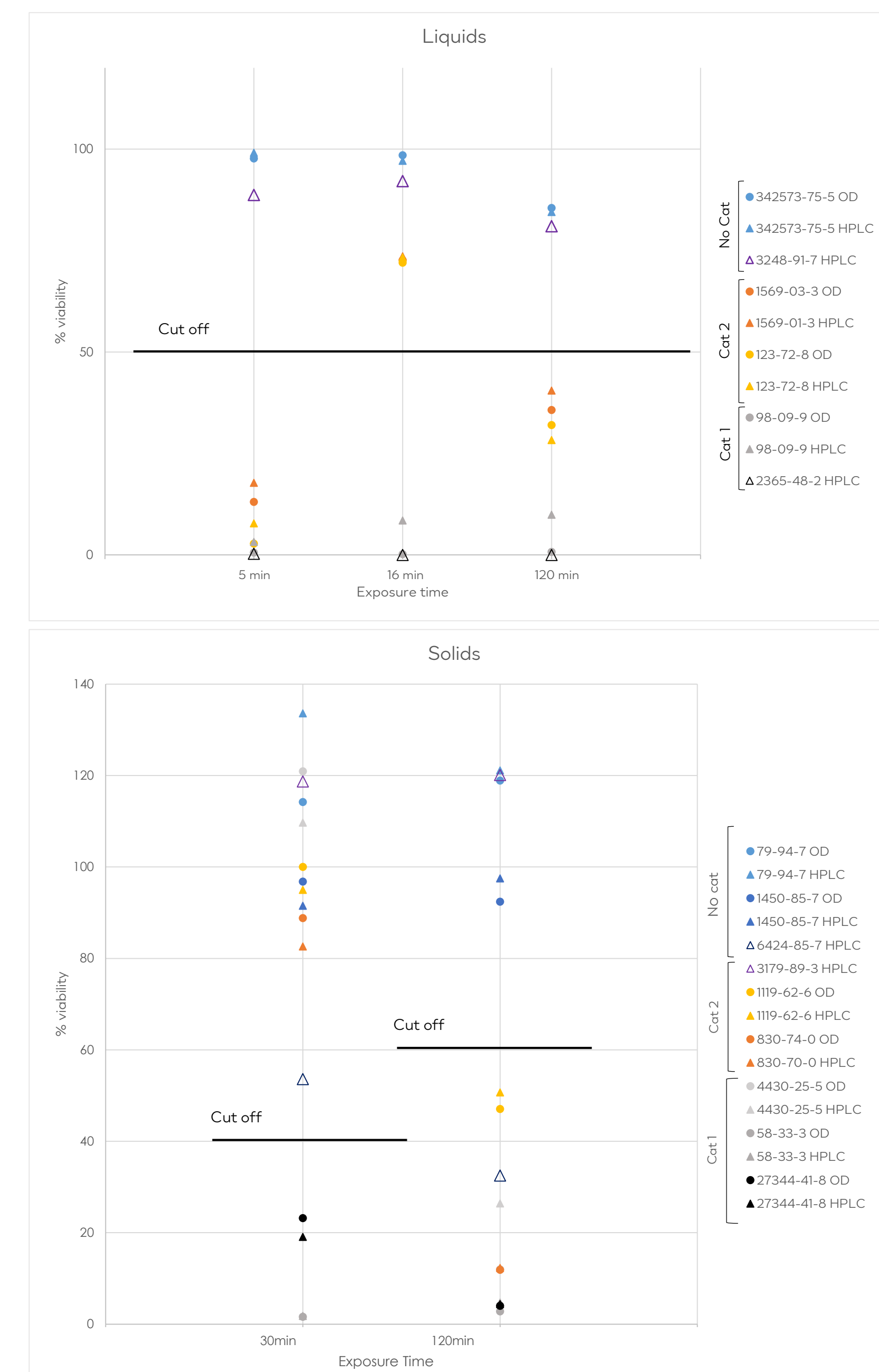
DETECTION SYSTEM



PREDICTION MODEL

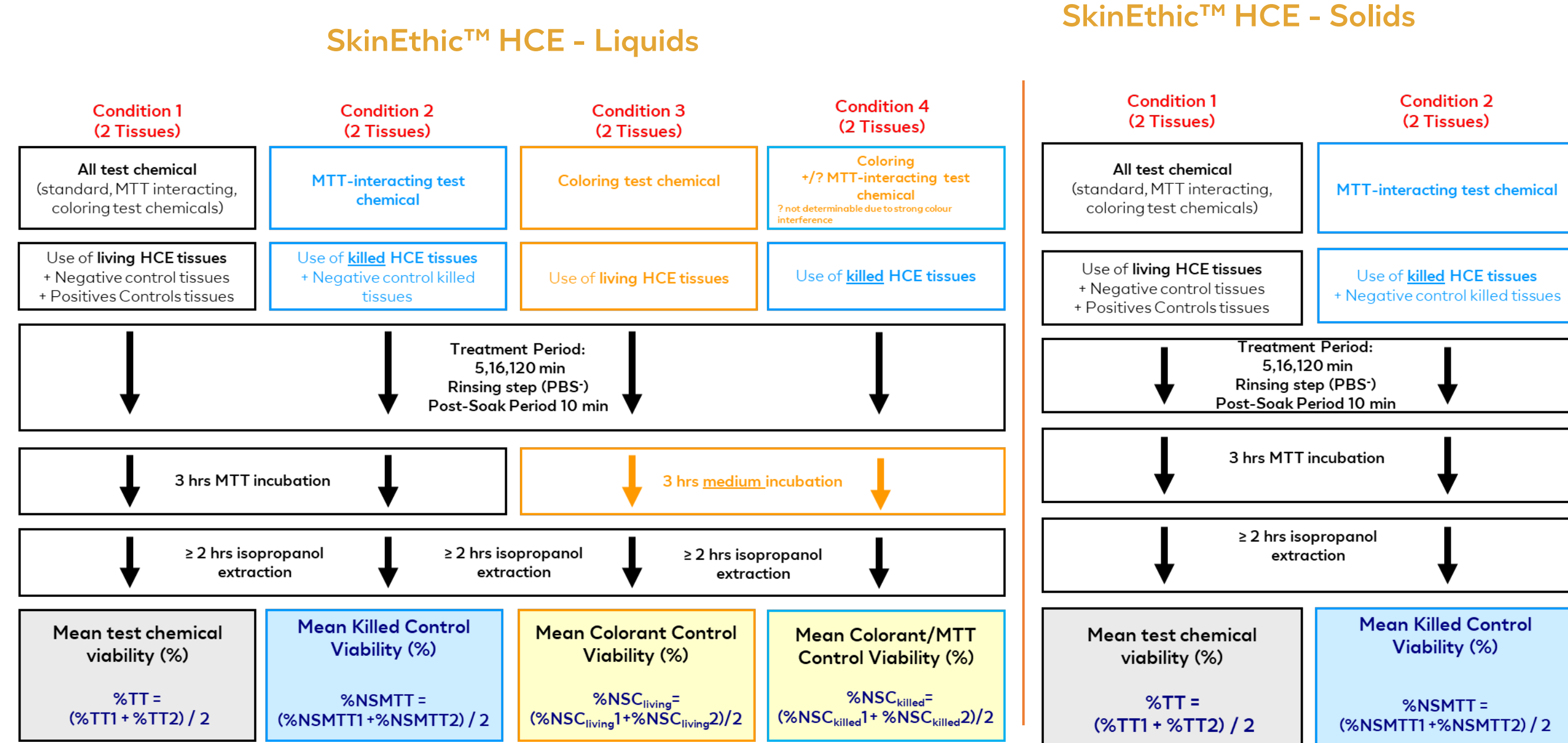
	No Category	Category 2	Category 1
TTL	Viability > 50% within all-time treatments	Any other combination of values other than those defined for No Cat. or Cat.1	Viability ≤ 50% within all-time treatments
TTS	Viability > 40% after 30 minutes and > 60% after 120 minutes		Viability ≤ 40% after 30 minutes and ≤ 60% after 120 minutes

3 RESULTS & DISCUSSION



CAS RN	Physical State	UN GHS	MTT reducer (Y/N)	Colour interference (Y/N)	In vitro Eye Irritation classification		OD-HPLC viability concordance (Y/N)
					OD	HPLC	
98-09-9	L	Cat 1	N	N	Cat 1	Cat 1	Y
27344-41-8	S	Cat 1	N	N	Cat 1	Cat 1	Y
2365-48-2	L	Cat 1	Y	N	Not compatible	Cat 1	/
58-33-3	S	Cat 1	Y	N	Cat1	Cat 1	Y
4430-25-5	S	Cat 1	Y	Y	Cat2	Cat2	Y
1569-01-3	L	Cat 2	N	N	Cat 2	Cat 2	Y
1119-62-6	S	Cat 2	N	N	Cat2	Cat 2	Y
123-72-8	L	Cat 2	Y	N	Cat 2	Cat 2	Y
830-74-0	S	Cat 2	Y	Y	Cat2	Cat 2	Y
342573-75-5	L	No Cat	N	N	No Cat	No Cat	Y
79-94-7	S	No Cat	N	N	No Cat	No Cat	Y
3248-91-7 (1%)	L	No Cat	Y	Y	Not compatible	No Cat	/
1450-85-7	S	No Cat	Y	N	No Cat	No Cat	Y
6424-85-7	S	No Cat	Y	Y	Not compatible	Cat2	/
3179-89-3	S	No Cat	N	Y	Not compatible	No Cat	/

ADAPTED CONTROLS DEPENDING ON TEST CHEMICAL PROPERTIES



The chemicals set was selected to achieved a representation of i) liquid and solid chemicals anticipated to produced interference, ii) amongst the three UN GHS categories. Analysis of the reduced MTT extracts was performed with both detection cell viability measurement systems.

- High-Performance Liquid Chromatography (HPLC) detection system allows separation of the Formazan dye from the chemical before its quantification.
- Similar cell viability outputs for the "standard" chemicals using both photometry and HPLC detection systems.
- Ocular hazard concordance of both detection systems, independently of the protocols and exposure treatment.
- For colour-interfering chemicals, use of HPLC/UPLC approach reduces the number of required controls and corrections and potentially decreases the uncertainties.
- HPLC measurement can be translated into a classification for strongly colored test substances when OD endpoint detection is not possible.

4 CONCLUSIONS

- Both detection systems have been validated for ocular hazard classification of standard chemicals.
- The applicability domain of the SkinEthic™ HCE Time-to-Toxicity method has been demonstrated for challenging chemicals using the HPLC-spectrophotometry system.
- Discrimination of the three UN GHS ocular hazard categories has been adopted in OECD TG 492B using HPLC-spectrophotometry as an alternative detection system.

REFERENCES

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 OECD (2022). *Test No. 492B: Reconstructed Human Cornea-like Epithelium (RHCE) Test Method for Eye Hazard Identification*, <https://doi.org/10.1787/0d603916-en>

