IN VITRO SKIN and EYE IRRITATION ASSESSMENT OF IS-001 AND IS-002 NOVEL IMAGING AGENTS

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ABSTRACT

Background and Purpose: IS-001 and IS-002 are imaging agents to visualize the ureter and Prostate Specific Membrane Antigen (PSMA) in prostate tumors (respectively) during robot-assisted surgical procedures. These compounds are now in clinical trials.

Methods: The skin and eye irritation of IS-001 and IS-002 were studied in reconstructed human epidermis and cornea-like epithelial human tissues using MatTEk EpiSkin and EPiOcular kits, respectively.

Results: Both agents have a strong green color. This presented a challenge and potential interference with measuring the final blue color read of the MTT cell viability assays. Several additional tests for potential color interference were performed in both skin and eye irritation assays. The cell viability following imaging agent incubation with the killed and alive skin and eye tissues was measured and compared. All acceptance criteria with all necessary positive and negative controls were met for both skin and eye assays confirming the validity of these in vitro human tissue assays. The final cell viability in the EPiOcular assay after subtraction of the interferences of the killed tissue and colored control were 87.1% and 82.0% for IS-001 and IS-002, respectively. According to the OECD TG432 Guidelines, the viability above 60% confirms that the tested agent is not an irritant and does not require classification and labeling for eye irritation or serious eye damage. Similarly, after adjustment of the potential color interference of the agents incubated with the skin tissue, the final viability was 81.9% and 91.0% for IS-001 and IS-002, respectively. According to the OECD TG439 Guidelines, compounds with viability above 50% are not considered irritants and do not require irritation labeling.

Conclusions: Based on the obtained in vitro data, both IS-001 and IS-002 are not considered irritants for either skin or eye, and no irritation categories are assigned to these compounds.

ACKNOWLEDGMENT

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OBJECTIVES

The main goal of this study was to assess possible skin and ocular irritation potential of IS-001 and IS-002, manufactured as the novel imaging agents, using OECD approved *in vitro* assays.

MATERIALS AND METHODS

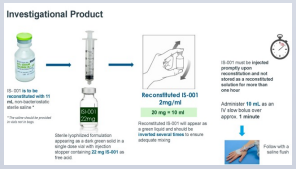
IS-001 and IS-002 were reconstituted in the sterile Normal saline at the concentration of 2 mg/ml (as is used in clinic).

SKIN IRRITATION ASSAY . In Vitro EpiDerm™ Skin Irritation Test (EPI-200-SIT) was used with MatTek Corporation's Reconstructed Human Epidermal Model EpiDerm™ kit. The tissue consists of normal human-derived epidermal keratinocytes, which have been cultured to form a multilayered highly differentiated model of the human epidermis. It consists of organized basal, spinous, and granular layers, and a multilayered stratum corneum containing intercellular lamellar lipid layers arranged in patterns analogous to those found in vivo. A generic description of general and functional conditions that reconstructed human skin models complied with the new OECD Test Guidelines 431 *In vitro* Skin Corrosion: Human Skin Model and **439 *In vitro* Skin Irritation: Reconstructed Human Epidermis test Method**.

The EpiDerm™ tissues (surface 0.6 cm^2) are cultured on specially prepared cell culture inserts and were shipped to CBT as kits, containing 24 tissues on shipping agarose together with the necessary amount of culture media, DPBS, 6-well plates, and 24-well plates. In addition, the MTT kit (containing MTT concentrate, diluent, and extractant) was provided by MatTek. A **5% SDS** (in H_2O) solution was used as positive control (PC) and tested concurrently with the test chemicals.

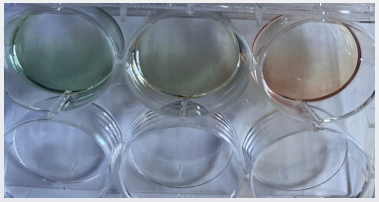
OCULAR ASSAY . The EpiOcular™ human cell construct (*OCL-200-EIT kit with the 24-well plate tissue inserts*, MatTek Corporation, MatTek IVLSL) was used in the Ocular Irritation assay. The use of EpiOcular™ cultures offers features appropriate to model ocular irritation: (1) the model is composed of **stratified human keratinocytes in a three-dimensional structure**, and (2) test materials can be applied topically to the model so that water insoluble materials may be tested. The toxicity of the test article (and thus the ocular irritation potential) was evaluated by the relative viability of the treated tissues relative to the negative control-treated tissues. Methyl Acetate was used as a Positive Control (PC).

Viability was determined by the MTT assay (*MTT test kit, MTT-100, MatTek).* Data were presented in the form of relative survival (relative MTT conversion).



RESULTS

Figure 1. MTT interference (reduction)



IS-001 + MTT IS-002 + MTT MTT control

Figure 2. MTT extracted from the Epithelium tissues

NC (water) IS-001 IS-002

Table 1. Skin Tissues Cell Viability

	Mean	SD	Mean of	SD	CV %	Acceptance
Agent/Conditions	of OD	of OD	viability [%]	of viability		criteria
Negative Control	2.748	0.344	100.0	12.51	12.51	od >0.8<2.8
Positive Control	0.091	0.001	3.3	0.02	0.62	Viability<20
IS-001	2.127	0.355	82.8	12.92	15.62	
IS-002	2.515	0.111	91.5	4.04	4.42	
IS-001 (killed cells)	0.144	0.007	5.2	0.26	4.96	
IS-002 (killed cells)	0.137	0.004	5.0	0.16	3.18	
NC (killed cells)	0.122	0.004	4.4	0.16	3.51	
IS-001 (No MTT)	0.003	NA	0.1	NA	NA	
IS-002 (No MTT)	0.005	NA	0.2	NA	NA	
NC (No MTT)	0.003	NA	0.1	NA	NA	

Acceptance criteria <=18%

Final viability for IS-001 after subtraction of killed cell control 81.9% and for IS-002 91.0%

Figure 3. Relative Skin Tissue Viability

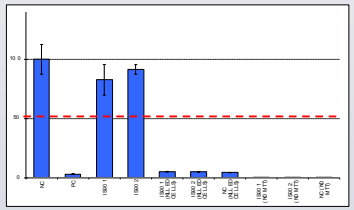


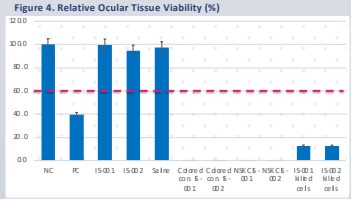
Table 2. Ocular Tissue Cell Viability

Chemical/ Tissue	Mean	Dif.	Mean of	Dif. of	Dif./2	Classification	
	of OD	of OD	viabilities [%]	viabilities			
Negative Control (NC)	1.796	0.157	100.0	8.73	4.36	NI	qualified
Positive Control (PC)	0.710	0.248	39.5	13.83	6.92	I	qualified
IS-001	1.792	0.126	99.8	7.04	3.52	NI	qualified
IS-002	1.702	0.111	94.8	6.15	3.08	NI	qualified
Saline	1.752	0.125	97.5	6.94	3.47	NI	qualified
Colored Con IS-001	0.002	0.000	0.1	0.03	0.01	NA	qualified
Colored Con IS-002	0.003	0.000	0.2	0.02	0.01	NA	qualified
NSKC IS-001	0.006	0.007	0.3	0.40	0.20	NA	qualified
NSKC IS-002	0.003	0.001	0.2	0.03	0.02	NA	qualified
IS-001 killed cells	0.231	0.047	12.8	2.63	1.31	NA	qualified
IS-002 killed cells	0.229	0.209	12.7	11.61	5.81	NA	qualified

Final viability for IS-001 after subtraction of the interferences of the killed tissue and colored control were 87.1% and 82.0%

NI - Not Irritant I-Irritant NA – Not Applicable

Figure 4. Relative Ocular Tissue Viability (%)



NSKC- Not Specific Killed Control (No MTT)

DISCUSSION

The assessment of skin and eye irritation has typically involved the use of laboratory animals. More recently, the reliable *in vitro* methods **were validated and adopted by OECD** which allow to classify chemicals according to the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS).

OECD Test Guidelines (TG) TG **439 In Vitro Skin Irritation:** Reconstructed Human Epidermis Test Methods addresses the human health endpoint skin irritation. It is based on the in vitro test system of reconstructed human epidermis (RhE), which closely mimics the biochemical and physiological properties of the upper parts of the human skin, i.e., the epidermis. The RhE test system uses human derived non-transformed keratinocytes as cell source to reconstruct an epidermal model with representative histology and cytoarchitecture.

One limitation of this assay method is a possible interference of the test substance with the MTT endpoint. A colored test substance or one that directly reduces MTT (and thereby mimics dehydrogenase activity of the cellular mitochondria) may interfere with the MTT endpoint. However, these test substances are a problem only if at the time of the MTT test (i.e., 42 hours after test substance exposure) sufficient amounts of the test substance are still present on (or in) the tissues. In case of this unlikely event, the (true) metabolic MTT reduction and the contribution by a colored test material or (false) direct MTT reduction by the test material can be quantified

According to the EU and GHS classification (R38/ Category 2 or no label), an irritant is predicted if the mean relative tissue viability of three individual tissues exposed to the test substance is reduced below 50% of the mean viability of the negative controls.

OECD Test Guideline (TG) No. 492

Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage confirms validated EpiOcular RhCE as one of 4 in vitro platforms validated for irritation assessment of chemicals that do not meet the requirements for classification as UN GHS Category 1 or 2, i.e., they are referred to as UN GHS **No Category.**

A limitation of this Test Guideline is that it does not allow discrimination between eye irritation/reversible effects on the eye (Category 2) and serious eye damage/irreversible effects on the eye (Category 1), nor between eye irritants (optional Category 2A) and mild eye irritants (optional Category 2B), as defined by UN GHS.

OECD/OCDR TG No. 492 adopted the following classification in 2019: If the test article-treated tissue viability is > 60.0% relative to negative control-treated tissue viability, the test article is not requiring classification and labeling (No Category) and no further testing requires.

CONCLUSIONS

Based on the obtained in vitro data, both IS-001 and IS-002 are not considered irritants for either skin or eye, and no irritation categories are assigned to these compounds (No category for either skin or eye irritation). The in vitro methods with reconstructed human tissues were tested with the positive, negative controls and 2 tested materials and showed that all acceptance criteria were met. This confirmed the suitability and validity of these *in vitro* assays to test eye and skin irritation potential without the use of animals.