In vitro Toxicology

Oral Irritation

Background Information

- The oral mucosa is routinely exposed to a variety of food and drink products, pharmaceuticals and chemicals including mouthwash and oral care products.

- The EpiOral™ 3D tissue consists of normal human-derived epithelial cells which form a highly differentiated model of human buccal (inner cheek) phenotypes. The model is a multi-layered tissue with organised basal cells and multiple non-cornified layers.

- Morphologically the EpiOral™ model closely parallels native buccal human tissue with an in vivo-like lipid profile.

- The EpiOral™ model also contains cytokeratins K13 and K14, and naturally occurring antimicrobial peptides known as human beta defensins (HBD).

- This model has been used with several common tests of cytotoxicity and irritation including 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), release of interleukin 1-alpha (IL-1α) as well as other cytokines.

- Cyprotex utilises the EpiOral™ model to provide an effective non-animal alternative approach for assessing oral irritation and toxicity.

Protocol

- Model Used
  MatTek EpiOral™ human 3D tissue model (other models e.g., EpiGingival™ available on request)

- Number of Replicates
  n = 2 or n = 4

- Exposure Times
  0.5, 1, 2 and 4 hr (depending on endpoint)

- Negative Control
  Sterile ultrapure water

- Positive Control
  1% solution of Triton X-100

- Reference Control
  Alcohol-containing mouthwash/rinse

- Endpoints
  MTT
  Interleukin-1α (IL-1α)
  Membrane Integrity (LDH) (optional)
  Oxidative Stress (GSH) (optional)
  Interleukin-8 (IL-8) (optional)
  Histopathology (optional)
‘The EpiOral™ model provides an effective non-animal alternative approach for assessing oral irritation and toxicity.’

**Figure 1**
Graph illustrating the mean % cell viability (n=2) over time when the EpiOral™ tissue model is exposed to the negative control, sterile water and the positive control, 1% Triton-X100

Sterile water had no effect on the cell viability of the tissue whereas the 1% Triton X-100 reduced the viability of the tissue to 8.4% over 4 hr with an ET_{50} of 1.3 hr.

**Figure 2**
Graph illustrating the mean IL-1α release (n=2) from the tissues over time when the EpiOral™ tissue model is exposed to the negative control, sterile water and the positive control, 1% Triton-X100.

In the wells treated with 1% Triton X-100, IL-1α levels in the basal media increased dramatically with time compared to the background levels in the wells treated with sterile water.

**References**