MatTek CORPORATION

Protocol

EpiAirway Toxicity Testing Protocol

For use with EpiAirway™ Model (AIR-100)

I. EpiAirway™ Culture Preparation

a) 3 AIR-100 kits will be required for testing 3 chemicals of unknown airway toxicity potential (1 kit for preliminary dose range-finding experiment, and 2 kits for definitive toxicity experiment, including positive and negative controls).

b) Unpack and equilibrate EpiAirway tissues overnight according to standard procedures outlined in the EpiAirway use protocol (MatTek document # MK-24-007-0027).

c) Following equilibration, gently rinse the apical surface of EpiAirway tissues twice by adding 400 µl of phosphate buffered saline (PBS) to the culture insert and carefully aspirating to remove all liquid and mucus from the tissue surface. This process should remove all apical mucus.

d) Place rinsed EpiAirway cultures into 6-well plates containing 1.0 ml of fresh AIR-100-ASY medium.

II. Test Chemical Preparation

a) Prepare test chemicals solutions in either water or corn oil depending upon chemical solubility. A positive displacement pipettor is required for dispensing corn oil solutions.

b) Experiments will be conducted to determine the test chemical concentration required to inhibit the MTT metabolizing ability of the EpiAirway cultures to 75% of untreated control tissue metabolism (IC75).

c) For preliminary IC75 dose range–finding experiments, test chemical solutions of 10, 50, 250 and 500 mg/ml will be applied to the apical surface of the EpiAirway cultures (100 µl, n = 2 tissues per dose).

d) Doses for definitive IC75 determinations are based on results of range-finding experiments.

   i. For test chemicals with dose range-finding IC75 ≤ 10 mg/ml, definitive doses will be 0.1, 0.5, 2.5 and 12.5 mg/ml.

   ii. For test chemicals with dose range-finding IC75 > 10 ≤ 50 mg/ml, definitive doses will be 5, 15, 30 and 60 mg/ml.

   iii. For test chemicals with dose range-finding IC75 > 50 ≤ 250 mg/ml, doses will be 40, 120, 200 and 280 mg/ml.

   iv. For test chemicals with dose range finding IC75 > 250 ≤ 500 mg/ml, doses will be 200, 300, 420 and 550 mg/ml.

   v. For test articles with dose range finding IC75 > 500 mg/ml, doses will be 450, 650, 850 mg/ml and neat (100 µl, n = 3 tissues per dose).

   vi. Water will be used as negative control (MTT OD should be ≥ 1.455). Formaldehyde (14.7) mg/ml will be the positive control (MTT OD should be ≤ 75% of negative control).

III. Exposure conditions of dosed EpiAirway tissues.

a) After application of the test chemicals solutions, the EpiAirway tissue culture inserts will be sealed by covering the top opening of the inserts with an insert cap (provided by MatTek).

b) Dosed EpiAirway tissues will be placed in a 37 °C 5% CO2 incubator for 3 hrs.

IV. Toxicity Assessment of Dosed Cultures

At the conclusion of the 3 hr test chemical incubation, test chemicals will be removed from the apical surface of the EpiAirway tissues by gently rinsing 3 times with 400 µl of PBS and carefully aspirating.

Tissue viability will be determined by the MTT viability assay following the procedure outlined in the EpiAirway use protocol (MatTek document # MK-24-007-0027).

EpiAirway tissue culture medium may also be collected for assay of released cytokines. Alternate procedure: Following test chemical removal, EpiAirway tissues are incubated for an additional 18-24 hrs with fresh culture medium prior to Transepithelial Electrical Resistance (TER) measurement, MTT assay and cytokine analysis from the culture medium.

Test results should be compared to positive (known respiratory irritant) and negative (known respiratory non-irritant) control materials that are similar in nature to the test materials being evaluated. Negative control chemicals may include heptyl butyrate and methyl stearate. In addition to formaldehyde, positive control chemicals may include butyl methacrylate and heptanal as described by Neilson et al. (Neilson L, Mankus C, Thorne D, Jackson G, DeBay J, Meredith C. Development of an in vitro cytotoxicity model for aerosol exposure using 3D reconstructed human airway tissue; application for assessment of e-cigarette aerosol. Toxicol In Vitro. 2015 Oct;29(7):1952-62. doi: 10.1016/j.tiv.2015.05.018. Epub 2015 Jul 12.).