

**Development Of A Novel Micronucleus Assay Using The Human 3-D Skin Model, EpiDerm™**R. Curren<sup>1</sup>; G. Mun<sup>1</sup>; D. Gibson<sup>2</sup>; M. Aardema<sup>2</sup>

1. Institute for In Vitro Sciences, Inc., Gaithersburg, MD, USA.
2. The Procter & Gamble Co., Cincinnati, OH, USA.

The rodent in vivo micronucleus assay is an important part of a tiered testing strategy in genetic toxicology. However, this assay, in general, only provides information about materials available systemically, not at the point of contact, e.g. skin. Although in vivo rodent skin micronucleus assays are being developed, the results will still require extrapolation to the human. Furthermore, to fully comply with recent European legislation such as the 7th Amendment to the Cosmetics Directive, non-animal test methods will be needed to assess new chemicals and ingredients. Therefore we have begun development of a micronucleus assay using a commercially available 3-D engineered skin model of human origin, EpiDerm (MatTek Corp, Ashland, MA). We first evaluated whether a population of binucleated cells sufficient for a micronucleus assay could be obtained by exposing the tissue to 1-3 ug/ml cytochalasin B (Cyt B). The frequency of binucleated cells increased both with time (to at least 120 h) and with increasing concentration of Cyt B. Three ug/ml Cyt B allowed us to reliably obtain 40-50% binucleated cells at 48h. Mitomycin C (MMC) was then used (in the presence of 3 ug/ml Cyt B) to investigate toxicity and micronuclei formation in EpiDerm. Exposing the tissue directly through the growth medium for 48h gave a dose response for toxicity between 0.03 and 0.6 ug/ml. Maximum micronuclei induction (~5%) occurred at 0.06-0.6 ug/ml MMC. Experiments conducted with and without Cyt B indicated higher frequencies in the presence of Cyt B as expected. A topical application protocol was then developed using two 10 ul (per 0.64 cm<sup>2</sup> tissue) applications of MMC in ethanol 24 and 48h prior to harvest. Maximum micronucleus response (~8%) and toxicity occurred with applications of 6-60 ug/ml MMC. The background frequency of micronuclei was very low (~0.1%). These studies show that micronuclei can be reproducibly induced in a 3-D skin model and are the first steps in developing a routine "in vivo-like" assay for chromosomal damage in human tissue.

**ID# 2209****Location: Ballrooms A & B****Time of Presentation: Mar 10 8:30 AM - 11:30 AM****Category: Genotoxicity/DNA Repair, (Alternatives to Mammalian Models), (Skin)**