Synopsis of Original Research Paper

## **Evaluation of genotoxicity using 3-dimensional human** epidermal model

## Hajime Kojima

Japanese Center for the Validation of Alternative Methods (JaCVAM), National Center for Biological Safety and Research National Institute of Health Sciences (NIHS)

- [Objective] To evaluate a risk assessment of genotoxicity when human skin is affected by chemicals, we tried to perform a Comet assay using a 3-dimensional human epidermal model (LabCyte EPI-MODEL, Japan).
- [Materials & Methods] Mitomycin C, methylmethanesulfonate (MMS) and 4-NQO (4-Nitroquinoline 1-Oxide) were utilized as test chemicals. Each test chemical solution was applied directly to the surface of the models and treated for 4 hours, then washed off and incubated for 20 hours after the treatment and maximal dosage was calculated according to the cytotoxicity. As to the Comet assay, each test chemical solution refer to the cytotoxicity was applied to them and treated for 4hr. Cells were detached by Liberase solution (Roche) or Trypsin solution (GIBCO), and an adequate cell suspension was obtained.
- [Results] More single cells could be efficiently retrieved using Trypsin solution, especially with treatment for 25min, than using Liberase solution. Comet signals mediated by Mitomycin C (150μM<) and 4-NQO were shown by our protocol. However, those by MMS were not clear response.
- [Discussion] We established a practical, rapid and easy Comet assay protocol using a 3-dimensional human epidermal model. To define the difference in genotoxical action between the epidermal model and *in vivo*, it is necessary to perform additional researches for minimal dosage of unknown chemicals that show genotoxicity to the human epidermis.