Human lung epithelial type II cells (A549) were cultured on microporous membranes at the air/liquid interface and were exposed to various test gases (i.e. nitric oxide, sulphur dioxide, acetaldehyde, ammonia gas, tetrafluoroethane, argon and synthetic air).

Cytotoxicity was measured by counting the viable cell numbers (CASY® technology). Gas-mediated genotoxicity was elucidated by the COMET assay. Non toxic, inherent gases (synthetic air, tetrafluoroethane and argon) displayed no cyto- or genotoxicity. Toxic gases such as nitric oxide, sulphur dioxide, acetaldehyde, and ammonia gas showed dose dependent effects on the viability of the exposed cells. EC_{50} values obtained from the experiments were compared to LD_{50} literature based values from mice and rat acute inhalation experiments. Furthermore all data have been cross validated amongst the participating laboratories. Tail-moment values retrieved by the COMET assay showed a dose dependent correlation for nitric oxide, sulphur dioxide, and ammonia gas indicating dose dependent DNA damage.

This extended pre-validation study across laboratories presents a promising, novel, and non-invasive method for acute inhalation toxicity screening.

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P12-05
Alternative in vitro phototoxicity test using reconstructed skin model, KeraSkin®

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The reconstructed human skin model KeraSkin® has similar morphology, characteristics, and even biochemical marker expressions to native human skin. This model had been showed usefulness as an alternative testing model in skin irritation and corrosion test. Although there are strong urge to restrict the animal experiments for toxicity test, the alternative in vitro phototoxicity tests using KeraSkin® is not yet been established. Therefore, this study was conducted to validate the in vitro phototoxicity test method using KeraSkin®. Nine of phototoxic or non-phototoxic chemicals were topically treated onto KeraSkin®, and after 24 h incubation, the KeraSkin® were exposed to 6 J/cm^2 of UVA. Test chemicals were removed, and cell viability was quantified by MTT assay after incubation for another 24 h. Predictions of phototoxic potentials were highly reproduced, and the established prediction standard was effective showing consistency with previously reported in vivo test results. In conclusion, in vitro alternative phototoxicity test method using KeraSkin® was successfully established. Surely KeraSkin® can be used as a good alternative test method for assessment of phototoxicity of the chemicals.

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P12-06
Development of a new reconstituted human cornea model to assess the eye irritation

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Alternative methods to the Draize eye irritation test, such as the BCOP, HET-CAM, ICE, and IRE, are used to evaluate the ocular irritation potential of cosmetic, livelihood articles or industrial chemicals. In order to improve the sensitivity and specificity of alternative eye irritation test, we developed a novel three-dimensional human corneal model that uses a normal human corneal epithelial cells.

In this study, two laboratories have tested 20 reference chemicals using the same study protocol. The results were compared to previously published in vivo eye irritation as well as existing data obtained in the other three-dimensional corneal model test.

A good intra/inter-laboratory reproducibility and correlation with in vivo and other in vitro model results were obtained. Our new three-dimensional model, developed from normal human corneal epithelial cells, is reproducible and is accurately predicting model of eye irritation test.

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P12-07
Evaluating the micronucleus induction potential for the genotoxicity assay using the KeraSkin® human skin model

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The micronucleus test is part of a battery of genotoxicity screening programs. The in vitro micronucleus assay is mutagenic test system for the detection of chemicals which induce the formation of numerical or structural chromosomal damage. The reduction and replacement of in vivo toxicity testing require the development of in vitro models to predict the genotoxic or other tests. Reconstructed human skin models present various advantages structurally and functionally as compared to mouse skin for human risk assessment. KeraSkin®, reconstructed human tissue model, reflect metabolically complexities of in vivo and human specific responses and is used in safety or efficacy screening tests. In this study, five of genotoxins or non-genotoxins were applied KeraSkin® model and evaluated genotoxic potential using Giemsa staining. A good reproducibility and correlation with in vivo and other in vitro model results were obatained. Our KeraSkin® model has a higher predictive potential of micronucleus assay and utility as part of in vitro genotoxicity assay.

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