



## **Increased Sensitivity of the EpiDerm Skin Irritation Protocol Evaluated in the ECVAM Skin Irritation Validation Study**

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During 2001-2003, refined EPISKIN and EpiDerm *in vitro* skin irritation protocols were developed (Cotovio et al., 2005; Kandárová et al., 2005), which resulted good correlation between *in vivo* and *in vitro* data. Both methods were based on the idea of a common protocol, comprised of 15 min application and 42h post-exposure period, and measuring the reduction of tissue viability using MTT endpoint. In 2004, the two skin models proceeded into the ECVAM validation study with the aim of replacing acute skin irritation tests performed in rabbits. The study revealed that the EPISKIN assay (based on MTT endpoint) showed sufficient sensitivity and specificity to be endorsed as a replacement of the *in vivo* test. The EpiDerm model was recognized as a validated constituent within OECD testing strategy.

Faller and Bracher (2002), Schaefer-Korting et al. (2007) and others demonstrated that differences in the barrier properties of the reconstructed human epidermal models exist. We hypothesized that the "false negative" outcomes observed for EpiDerm using the common protocol were likely due to EpiDerm's enhanced barrier. Therefore, we modified the common protocol by extending the exposure time from 15 min to 60 min. Using this modified protocol, we re-tested 60 readily available chemicals from pre-validation and validation studies and obtained significant increased sensitivity, without loss of specificity, using EpiDerm. The modified assay provided a very balanced outcome, resulting in total accuracy of 80%. An inter-laboratory study will be performed with chemicals endorsed by ESAC to evaluate the reproducibility of the improved performance of EpiDerm method.

Taking into account evidence about the variability (Gilman et al., 1978; Weil and Scala, 1971; Worth and Cronin, 2001) and borderline predictive capacities of the Draize test in terms of human health effects (Calvin, 1992; Campbell and Bruce, 1981; Robinson et al., 2000; Basketter et al., 2004), it can be concluded that both EpiDerm skin irritation protocols provided sufficient levels of sensitivity and specificity. The presentation will summarize results obtained with the two EpiDerm protocols compared to *in vivo* rabbit data, existing and recently performed human patch study (Jírová et al., 2007).

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