Dose Response Studies Supporting In Vitro-Based Safety Assessments for Chemicals Inducing Oxidative Stress

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Oxidative stress is involved in the development of a variety of diseases including allergic and inflammatory skin diseases. To advance the transition to in vitro toxicity testing described in the National Academies of Sciences report, “Toxicity Testing in the 21st Century (TT21C): A Vision and A Strategy”, a series of assays to provide proof-of-concept in vitro risk assessments have been developed for key readouts of Nrf2 oxidative stress toxicity pathway: oxidative homeostasis, Nrf2 transactivation, transcriptional regulation, protein and DNA oxidative damage (protein carbonylation, protein nitration, 8-hydroxy-2’-deoxyguanine), and cell fate (apoptosis, cytotoxicity). Dose-response data from these assays were collected from human immortal (HaCaT) and neonatal primary (HEKn) keratinocytes with curcumin and hydrogen peroxide exposure. Sorting biomarker response by no observed effect levels (NOELs) or benchmark dose (BMD) helped define the regions associated with sub-threshold, adaptive and adverse cellular responses. Primary keratinocytes were more susceptible to protein and DNA damage than the HaCaT cells. Transient protein damage (resolved within 24 hrs) occurred at much lower doses than pathway enrichment in the gene array studies. However, doses associated with transcriptomic pathway activation were similar to sustained protein and DNA damage and cytotoxicity. It appears that transcriptional activity response may not be the key protective mechanism against oxidative damage at low doses. We postulate that post-translational processes may be more important for resolution of protein damage at low doses. Further research is being conducted to evaluate the post-translational processes driving dose-response for cellular damage at low doses and to identify the tipping point for adversity in the oxidative stress pathway. This effort supports development of in vitro-based safety assessments for chemicals inducing oxidative stress.