HepaRG Cells as a New Approach Methodology Follow Up to a Positive Response in Human TK6 Cell Micronucleus Assay: Naphthalene Case Study

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Abstract

We are evaluating the use of metabolically competent HepaRG cells combined with CometChip? for DNA damage and the micronucleus (MN) assay as a follow up for in vitro positive genotoxic response as alternatives to in vivo genotoxicity testing.. Naphthalene is genotoxic with rat liver S9 in human TK6 cells inducing a nonlinear dose-response for the induction of micronuclei in the presence of rat liver S9. To follow up this response, we used metabolically competent HepaRG cells as a New Approach Methodology (NAM) alternative to animals for genotoxicity assessment of naphthalene. In HepaRG cells, naphthalene genotoxicity was assessed using 12 concentrations of naphthalene with the top dose used for assessment of genotoxicity of 1.7 mM corresponding to 45% cell survival. In contrast to human TK6 cell with S9, Naphthalene was not genotoxic in either the HepaRG MN Assay or the Comet Assay using CometChip?. The lack of genotoxicity in both the MN and comet assays in HepaRG cells is likely due to Phase II enzymes removing phenols preventing further bioactivation to quinones and efficient detoxication of naphthalene quinones or epoxides by glutathione conjugation. In contrast to CYP450 mediated metabolism, these Phase II enzymes are inactive in rat liver S9 due to lack of appropriate cofactors causing a positive genotoxic response. This data indicates that rat liver S9-derived BMD10 over-predicts naphthalene genotoxicity BMD calculations when compared to hepatocytes. Metabolically competent hepatocyte models like HepaRG cells should be considered as human-relevant NAMs for use genotoxicity assessments to reduce reliance on rodents.

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