Investigating the Impact of Cytochrome P450 Metabolism on Chemical-mediated Transcription Factor Transactivation

Agnes L. Karmaus[1], Amber Daniel[1], Victoria Hull[1], Emily Reinke[1], Alex Medvedev[2], David Allen[1], Nicole Kleinstreuer[3], and Warren M. Casey[3]

[1]Inotiv, Research Triangle Park, NC, USA; [2]Attagene, Inc., Morrisville, NC, USA; [3]NIH/NIEHS/DNTP/NICEATM, Research Triangle Park, NC, USA

Profiling chemical effects on transcription factor activity is an important new approach methodology (NAM) that can help characterize the mechanisms by which chemicals may perturb biological systems. The Attagene cis-FACTORIAL™ assay uses a reporter system to detect activity of 46 transcription factors to provide a quantitative assessment of chemical activity. A new version of this assay, CYP-FACTORIAL, includes addition of nine key cytochrome P450 (CYP450) enzymes to enable the evaluation of chemical effects on transcription factor activity with and without CYP-mediated phase 1 metabolism. This supports achieving a better understanding of whether CYP-mediated oxidation results in an altered bioactivity profile. The current study evaluates 24 chemicals across four test concentrations in the CYP-FACTORIAL assay. Preliminary results suggest that the transcription factors showing the greatest difference in response with CYP450 metabolism are activation of the estrogen receptor, aryl hydrocarbon receptor, metal regulatory transcription factor 1, heat shock factor 1 (HSF1), and oxidative stress response (nuclear factor erythroid 2–related factor 2 or Nrf2) pathways. The integration of metabolism into a multiplexed in vitro assay system helps provide additional insight needed to understand chemical-elicited bioactivity, thereby facilitating the development of human-relevant NAMs. This project was funded with federal funds from NIEHS, NIH under Contract No. HHSN273201500010C.