Insights from Profiling Transcription Factor Transactivation with CYP450 Metabolism Integration

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Profiling chemical effects on transcription factor activity can help characterize the mechanisms by which chemicals perturb biological systems. Such profiles can contribute to a predictive approach to characterizing chemical effects that avoids animal testing. The Attagene cis-FACTORIAL™ assay uses a reporter system to quantify the activity of 46 transcription factors to provide a quantitative assessment of chemical effects. A new version of this assay, CYP-FACTORIAL ™, adds nine key cytochrome P450 (CYP450) enzymes to evaluate effects on transcription factor activity with and without CYP-mediated Phase 1 metabolism. This supports evaluation of whether CYP-mediated oxidation results in an altered bioactivity profile. This study examined activity of 24 chemicals across four test concentrations in the cis-FACTORIAL™ and CYP-FACTORIAL ™ assays. Results suggest that alterations in CYP450 metabolism have the greatest effects on transcription factors activating the estrogen receptor (ER), aryl hydrocarbon receptor (AhR), and oxidative stress response (NRF2) pathways. Comparisons of profiles of test vs. reference chemicals identified a highly conserved polycyclic aromatic hydrocarbon toxicity signature involving activation of AhR, NRF2, and ER. Interestingly, a profile in which ER and AhR are activated but NRF2 is not activated correlated to non-toxic compounds, suggesting the possibility of using differences between signatures to predict toxic outcomes. Integrating the profiling approach with metabolism in a multiplexed in vitro assay system allows this assay platform to provide insight into chemically induced bioactivity and thus facilitates the development of mechanistically based, human-relevant predictive testing approaches. This project was funded by NIEHS under Contract No. HHSN273201500010C.