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Xenobiotic Metabolizing Enzyme and Transporter Gene Expression in Primary Cultures of Human Hepatocytes Modulated by ToxCast™ Chemicals

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ToxCast™ chemicals were assessed for induction or suppression of xenobiotic metabolizing enzyme and transporter gene expression using primary human hepatocytes. The mRNA levels of 14 target and 2 control genes were measured: ABCB1, ABCB11, ABCG2, SLCO1B1, CYP1A1, CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4, UGT1A1, GSTA2, SULT2A1, HMGCS2, and control genes ACTB, GAPDH. These genes represent 5 nuclear receptor signaling pathways: AHR, CAR, PXR, PPARa and FXR. Gene expression was quantitatively measured by nuclease protection assays at 5 concentrations, 3 time points (6, 24, 48 hr), in 4 replicate wells. Hepatocytes from 2 male donors were isolated and cultured with 6 reference chemicals and 320 ToxCast phase I chemicals. CYP1A1/2 enzymatic activity and cell morphology were assessed in each well. Concentration-response curves were generated for 13,813 chemical, time and gene combinations. EC50 (effective concentration 50%) and Emax (effective maximum) values from the curves determined nuclear receptor pathways modulated by ToxCast chemical exposures. Chemical potency and efficacy were determined relative to reference chemicals and correlated with *in vivo* chronic hepatotoxicity endpoints from EPA's ToxRefDB database. Using ≥40% efficacy and <40 μM EC50, a preliminary analysis of the 5 nuclear receptor pathways at 48 hr was conducted based on expression of 5 representative genes for each pathway. Of the 320 ToxCast chemicals, 6 chemicals perturbed AhR (CYP1A1/2), 59 chemicals perturbed CAR (CYP2B6), 32 chemicals perturbed PXR (CYP3A4), 20 chemicals perturbed PPARa (HMGCS2), and 27 chemicals perturbed FXR (ABCB11). Comparisons to chronic toxicity data were made using sensitivity, specificity, and relative risk analyses. Induction of CYP2B6 was associated with rat liver apoptosis/necrosis, liver hypertrophy, proliferative thyroid lesions, and thyroid tumors, with relative risk values of 2.00, 2.26, 2.25, and 3.83, respectively.