Activity profiles of 676 ToxCast Phase II compounds in 231 biochemical high-throughput screening assays

Sipes NS, Reif DR, Houck K, Martin MT, Kothiya P, Richard A, Judson R, Knudsen TB, Dix DJ, and Kavlock R

National Center for Computational Toxicology, ORD, US EPA, RTP, NC, USA

Understanding potential health risks posed by environmental chemicals is a significant challenge elevated by large numbers of diverse chemicals with generally uncharacterized exposures, mechanisms and toxicities. The present study is a performance evaluation and critical analysis of 231 high-throughput cell-free assay results for 676 chemicals (including a number of failed pharmaceuticals, alternative plasticizers and food additives) in Phase II of EPA's ToxCast™ project, and comparison to previous results for 309 ToxCast Phase I compounds. Biochemical high-throughput screening profiled G-protein-coupled and nuclear receptors, kinases, phosphatases, CYPs, histone deacetylases, ion channels and transporters. A primary screen tested all Phase II chemicals at 25 µM concentration (or 10 µM for CYP assays) and a secondary screen re-tested over 14,000 chemical-assay pairs in 8-point concentration series from 0.023 to 50 µM (or 0.009–20 µM for CYPs). Mapping relationships on half-maximal activity concentration (AC50) revealed 5484 active chemical-assay pairs for 510 unique chemicals and 216 unique assays. On average a chemical affected 2.5 assays and an assay was affected by 7.5 chemicals at AC50≤10µM, versus 3.4 and 3.7, respectively for Phase I, but the percent affected remained constant (1%). Among the most promiscuous chemicals were tributyltin methacrylate, crystal violet, and tributyltin chloride; the most promiscuous assays were CYP2C19, CYP2C9, and the dopamine transporter. Known (e.g. caffeine perturbation of adenosine receptors and carbosulfan inhibiting acetylcholinesterase activity) and unknown (e.g. cyclopamine binding ion channels and methotrexate binding to somatostatin receptors) chemical activities were observed. A combination of these *in vitro* results along with *in vivo* toxicity data are being used to generate hypotheses about potential molecular initiating events associated with adverse outcomes for this diverse chemical set.