Characterizing the Impacts of Assay Design on Cytotoxic Concentration Range

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The Tox21 and ToxCast high-throughput screening (HTS) programs have provided an abundance of *in vitro* assay data from multiple cell lines and technologies. Among these data, it has been observed that heightened assay activity at higher concentrations is likely driven by cytotoxicity and/or generalized cell stress, rather than target-mediated activity, motivating the characterization of cytotoxic concentration ranges using the concept of a cytotoxicity "burst". To further refine HTS bioactivity data curation and contextualize chemical activity in the Integrated Chemical Environment's (ICE; ice.ntp.niehs.nih.gov/) curated HTS pipeline, we evaluated how assay design components, such as cell line or detection technology, impact the estimated cytotoxic concentration range. Data from the U.S. Environmental Protection Agency's *invitroDBv3.4* database were used to derive chemical lowest effective concentrations (LECs) in all assays. LECs were analyzed with unsupervised machine learning to identify associations between cytotoxicity, non-cytotoxic assay activity, and the relevant technological and biological features of assays as well as chemical structural features and physicochemical properties, that could help define chemical-specific cytotoxicity points. For each chemical/assay technology combination, the cytotoxicity point concentration was predicted and compared to cytotoxic concentration ranges derived from the cytotoxicity "burst" approach, which pools all cytotoxicity assay data. This work serves to help better understand the relationship between assay design, chemical features, and cytotoxicity endpoints, and will bolster confidence in bioactivity interpretation from in vitro assay data in support of chemical prioritization. This project was funded with federal funds from NIEHS, NIH under Contract No. HHSN273201500010C.