Linking ToxCast Signatures with Functional Consequences: Proof-of-Concept Study using Known Inhibitors of Vascular Development

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The USEPA's ToxCast program is developing a novel approach to chemical toxicity testing using high-throughput screening (HTS) assays to rapidly test thousands of chemicals against hundreds of *in vitro* molecular targets. This approach is based on the premise that *in vitro* HTS bioactivity signatures can be linked to biological pathways that, when sufficiently perturbed, could lead to adverse outcomes. One putative adverse outcome pathway (AOP) linked to a ToxCast HTS bioactivity signature is disruption of embryonic vascular development leading to developmental toxicity. The present study examined two known effectors of vascular development: the receptor tyrosine kinase inhibitor 5HPP-33 (a thalidomide analogue), and the MetAP-2/noncanonical (NC) Wnt signaling inhibitor, TNP-470. ToxCast vascular disruption bioactivity profiles for these compounds were compared with their effects in three functional assays: rat whole embryo culture (WEC), the zebrafish embryotoxicity test (ZET), and the rat aortic explant (AE) assay. Mid-somite stage rat and cleavage stage zebrafish embryos were cultured in control medium or medium containing 5HPP-33 or TNP-470 for 48 or 120 h, respectively, followed by evaluation for developmental defects. AE samples were cultured for three days in control medium, four days in treated media, and then evaluated for microvessel outgrowth. 5HPP-33 was embryolethal at ≥ 15 or 1 μM in WEC and ZET, respectively, and inhibited microvessel outgrowth in cultured AEs at ≥ 0.46 μM. Consistent with its different antiangiogenic mode of action, TNP-470 caused cranial and caudal abnormalities at ≥ 0.025 or ≤ 0.1 μM in the WEC and ZET assays, respectively. In the AE assay, TNP-470 treatment stunted vessel outgrowth at ≤ 0.0025 μM. Although both compounds caused effects across the multiple assays, further work is needed to determine if the developmental effects are specifically due to perturbation of their presumed molecular initiating events (VEGFR-inhibition, MetAP-2/NC-Wnt disruption) and to establish correlates to the observed ToxCast bioactivity profiles. This study illustrates a potential tiered approach in which functional in vitro assays can be used to further evaluate suspected positives based on ToxCast HTS assay signatures.

This abstract does not necessarily reflect USEPA policy.