Application of Targeted Functional Assays to Assess a Putative Vascular Disruption Developmental Toxicity Pathway Informed By ToxCast High-Throughput Screening Data

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Chemical perturbation of vascular development is a putative toxicity pathway which may result in developmental toxicity. EPA's high-throughput screening (HTS) ToxCast program contains assays which measure cellular signals and biological processes critical for blood vessel development. By testing the Phase-1ToxCast chemicals in these assays, and comparing the results to prenatal DT study summary information derived from ToxRefDB, a vascular disruption signature was identified. This signature was correctly observed when the antiangiogenic thalidomide analogue, 5HPP-33, was tested in a ToxCast assay subset. There is utility in using targeted *in vitro* functional assays to explore the potential consequences of chemicals that test positive in the ToxCast program, both for chemicals without DT data (5HPP-33) or as an intermediate tier for DT data comparisons. Therefore, 5HPP-33 was tested in rat whole embryo culture (WEC) and *in vitro* rat aortic explant (AE) cultures. Mid-somite stage rat embryos were cultured in media containing 0, 1.6, 5, 15, 30 or 46 uM 5HPP-33 for 48 h followed by evaluation for developmental defects. To further confirm the direct effects of 5HPP-33 on angiogenesis, rat AE were cultured in media containing 0, 0.46, 4.6, 46, 93, or 247 uM 5HPP-33 for four days and the resulting inhibition of microvessel outgrowth was evaluated. In WEC, 5HPP-33 caused developmental defects and embryolethality at ≥ 15 uM. Consistent with an antiangiogenic mode of action in embryos, 5HPP-33 inhibited microvessel outgrowth in cultured AEs at ≥ 0.46 uM and completely abolished vessel outgrowth at 46 uM with a cell morphology similar to the outcome of cell-agent based *in silico* models informed by ToxCast data. Data from these targeted functional assays correlated with the *in vitro* HTS assay data for this vascular disrupting compound.

This abstract does not necessarily reflect US EPA policy.