Modeling Steroidogenesis Disruption Using High-Throughput *In Vitro* Screening Data

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Environmental chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis) causing adverse reproductive and developmental effects. Historically, a lack of assays resulted in few chemicals having been evaluated for effects on steroidogenesis. The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes, yet the only enzyme in the pathway for which a high-throughput screening (HTS) assay has been developed is aromatase (CYP19A1), responsible for the aromatization of androgens to estrogens. Recently, the ToxCast HTS program adapted the OECD validated H295R steroidogenesis assay using human adrenocortical carcinoma cells into a high-throughput model to quantitatively assess the concentration-dependent (0.003-100 µM) effects of chemicals on 10 steroid hormones including progestagens, androgens, estrogens and glucocorticoids. These results, in combination with two CYP19A1 inhibition assays, comprise a large dataset amenable to clustering approaches supporting the identification and characterization of putative mechanisms of action (pMOA) for steroidogenesis disruption. In total, 514 chemicals were tested in all CYP19A1 and steroidogenesis assays. 216 chemicals were identified as CYP19A1 inhibitors in at least one CYP19A1 assay. 208 of these chemicals also altered hormone levels in the H295R assay, suggesting 96% sensitivity in the ability of hormone quantification to identify CYP19A1 inhibitors. Interestingly, only 18 of these chemicals were identified in both CYP19A1 assays, 17 were also identified in the steroidogenesis assay. Of these 17 chemicals, all elicited effects on estrogen levels and 13 had effects on androgen levels, as expected with CYP19A1 inhibition. The steroidogenesis assay data were also used to model CYP17A1 inhibition. We suggest that increases in progestagens and glucocorticoids with simultaneous decreases in the levels of androgens and estrogens comprise a profile indicative of CYP17A1 inhibition, which correctly identified known CYP17A1 inhibitors such as conazole fungicides including prochloraz. Cumulatively, our examples demonstrate that computational profiling of *in vitro* data provides a model that characterizes pMOAs for steroidogenesis disruption to support the identification of endocrine disruptive chemicals. This abstract does not necessarily reflect US EPA policy.