H295R Human Adrenocortical Carcinoma Cells as a Screening Platform for Steroidogenesis

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Proper biosynthesis and metabolism of steroid hormones is essential for development and reproduction. Disruption of steroidogenesis by environmental toxicants results in altered hormone levels causing adverse reproductive and developmental effects. H295R human adrenocortical carcinoma cells were used to evaluate the effect of chemicals on steroidogenesis. Using a 96-well format, cells were pre-stimulated with 10μM forskolin for 48 hr to induce steroidogenesis followed by chemical exposure for 48 hr. Media were removed and 13 hormone analytes were quantified by HPLC-MS/MS including progestagens (i.e., pregnenolone [PREG], progesterone [PROG], and their hydroxylated metabolites), glucocorticoids, androgens, and estrogens. Initially, 311 unique ToxCast Phase I chemicals (primarily pesticides) were tested at a single maximal non-cytotoxic concentration. 220 chemicals were found to alter the levels of at least one hormone. Based on the single concentration analysis, 96 chemicals disrupting 4≤ hormones were selected for six-point concentration-response evaluation (0.003 – 100 μM). Concentration-dependent disruption of at least one hormone was observed with 68 of the 96 selected chemicals. By evaluating the effects of chemicals on 13 hormones this assay provides valuable mechanistic insight into the possible targets for chemical perturbance in the steroidogenic pathway. For example, <10 chemicals altered PREG or 17αOH-PREG while 27 and 35 chemicals had an effect on PROG and 17αOH-PROG levels, respectively. These results demonstrate that the chemicals evaluated likely do not target CYP17a hydroxylase activity. However, 33 chemicals altered testosterone levels and 38 chemicals concentration-dependently altered estradiol levels revealing significant disruption of subsequent dehydrogenation and aromatization steps. Cumulatively, these results suggest CYP17a lyase and hydroxysteroid dehydrogenase activity are the likely targets for the disruption of steroidogenesis by the subset of ToxCast Phase I chemicals evaluated. *This abstract does not necessarily reflect US EPA policy.*