

Effects of ToxCast Phase I Chemicals on Steroidogenesis in H295R Human Adrenocortical Carcinoma cells

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Steroid hormones are essential for proper 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. Cells were pre-stimulated with 10 μ M forskolin for 48 hr to induce steroidogenesis followed by chemical treatment for 48 hr. Media were removed and 13 hormone analytes were quantified by HPLC-MS/MS including progestagens (pregnenolone [PREG], progesterone [PROG], and their hydroxylated metabolites), glucocorticoids (corticosterone, cortisol, and their deoxy-precursors), androgens (dehydroepiandrosterone, androstenedione, and testosterone), and estrogens (estrone and estradiol). Initially, 311 unique ToxCast Phase I chemicals (primarily pesticides) were tested at a single non-cytotoxic concentration. 220 chemicals were found to alter the levels of at least one hormone analyte. Based on the single concentration analysis, 96 chemicals disrupting 4 \leq hormones were selected for six-point concentration-response evaluation (0.003 – 100 μ M). Concentration-dependent disruption of at least one hormone was observed with 68 of the selected chemicals. By evaluating the effects of chemicals on 13 hormones this assay provides valuable mechanistic insight into the possible targets for chemical perturbation in the steroidogenic pathway. For example, <10 chemicals altered PREG or 17 α OH-PREG while 27 and 35 chemicals had an effect on PROG and 17 α OH-PROG levels, respectively. These results demonstrate that the chemicals evaluated likely do not target CYP17 α 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 CYP17 α lyase and hydroxysteroid dehydrogenase activity are the most 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.*