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

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## Abstract & Objectives

A delicate balance of steroid hormones is 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. 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 (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 maximum tolerated concentration (MTC). 220 chemicals were found to alter the levels of at least one hormone analyte. 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 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.*

*OBJECTIVES*

- *Develop high-throughput protocol for testing disruption of steroidogenesis capable of measuring many steroid analytes in the pathway*
- *Develop a cost-effective screening pipeline for testing effects of chemicals on H295R cells*
- *Characterize the profile of effects on steroidogenesis across a diverse library of chemicals*

## Study Design & Workflow


***Figure 1.*** *(**A**) **Study design**: H295R human adrenocortical carcinoma cells were pre-stimulated with 10 M Forskolin for 24 h prior to chemical treatment for 48 h. (**B**) **Workflow**: The maximum tolerated concentration for 311 ToxCast phase I chemicals was identified and evaluated for effects on steroidogenesis. Of the 220 chemicals altering at least one hormone at the MTC tested, 96 were selected for concentration-response evaluation. 68 chemicals concentration-dependently altered at least one hormone.*

## Steroidogenesis Pathway

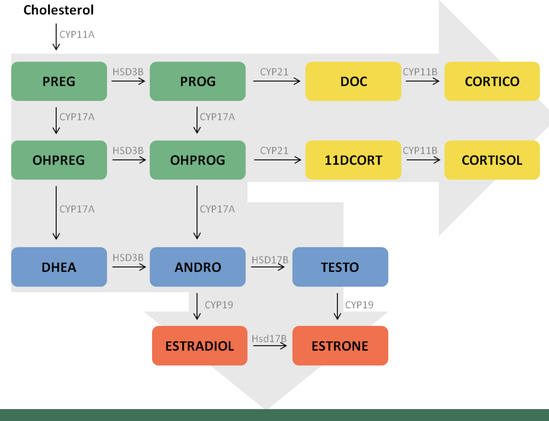


***Figure 3. Steroidogenesis Pathway:*** *Stimulation of steroidogenesis in immortalized H295R human adrenocortical carcinoma cells by 10 μM forskolin results in detectable levels of steroid hormone production. The steroid hormones evaluated in this study can be categorized into four classes: progestagens (represented in green), glucocorticoid/mineralocorticoid (yellow), androgens (blue) and estrogens (red). Gene symbols for the enzymes carrying out each step in steroidogenesis are indicated in grey. Briefly, cholesterol is committed to steroid hormone by CYP11A. Progestagens can be metabolized by CYP21 to form glucocorticoids and mineralocorticoids or by CYP17A to form androgens and subsequently estrogens via CYP19.*

## Chemical Effects on Steroidogenesis

***Table 1. Summary of Concentration-Response Evaluation of Hormone Concentrations for 96 ToxCast Phase I Chemicals***

| Hormone | Acronym | Quantification Range (ng/ml) | Average Concentration (ng/ml) | Chemicals with Conc.-Dependent Effects |
|---|---|---|---|---|
| pregnenolone | **PREG** | 2-400 | 3.3 | **9** (↑3, ↓6) |
| 17α-OH pregnenolone | **OH-PREG** | 5-1000 | 8.4 | **3** (↑1, ↓3) |
| progesterone | **PROG** | 0.2-40 | 0.4 | **27** (↑8, ↓19) |
| 17α-OH progesterone | **OH-PROG** | 0.2-40 | 15.2 | **35** (↑21, ↓14) |
| deoxycorticosterone | **DOC** | 0.5-100 | 3.9 | **29** (↑18, ↓11) |
| corticosterone | **CORTICO** | 0.5-100 | 0.6 | **19** (↑0, ↓19) |
| 11-deoxycortisol | **11DCORT** | 5-1000 | 246.7 | **20** (↑19, ↓1) |
| cortisol | **CORTISOL** | 0.5-100 | 17.4 | **21** (↑20, ↓1) |
| dehydroepiandrosterone | **DHEA** | 3-600 | 3.9 | **3** (↑2, ↓1) |
| androstenedione | **ANDRO** | 1-200 | 111.9 | **24** (↑23, ↓1) |
| testosterone | **TESTO** | 0.1-20 | 2.9 | **33** (↑32, ↓1) |
| estrone | **ESTRONE** | 0.03-6 | 2.1 | **38** (↑17, ↓21) |
| estradiol | **ESTRADIOL** | 0.03-6 | 0.2 | **36** (↑13, ↓23) |

## Stimulation of Steroidogenesis

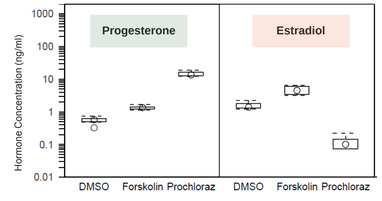


***Figure 4. Stimulation of H295R Cells:*** *Stimulation of steroidogenesis with 10 μM Forskolin induced hormone production. Conversely, treatment with 3 μM Prochloraz, inhibiting Cyp17 activity, increased progesterone levels while inhibiting estradiol. All treatments were conducted with a 24 hr pre-stimulus using 10 μM forskolin followed by 48 hr treatment in 0.1% DMSO.*

## Profiling Chemical Effects on Steroidogenesis

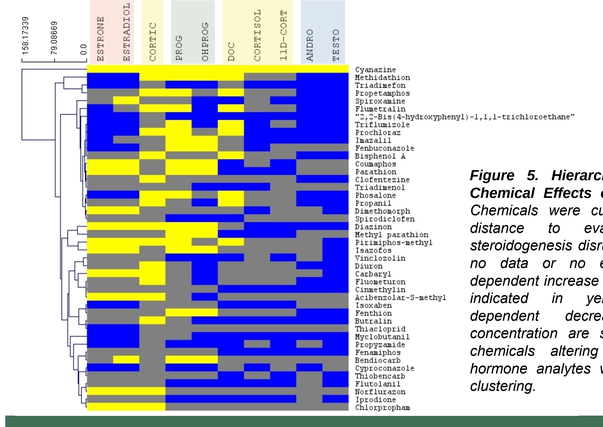


***Figure 5. Hierarchical Clustering of Chemical Effects on Steroidogenesis:*** *Chemicals were custered by euclidean distance to evaluate profiles of steroidogenesis disruption. Grey indicates no data or no effect, concentration-dependent increase in hormone levels are indicated in yellow, concentration-dependent decrease in hormone concentration are shown in blue. Only chemicals altering the levels of ≥3 hormone analytes were included in this clustering.*

## Summary

- H295R cells are a suitable model for high-throughput screening for chemical effects on steroidogenesis
- 24 hr stimulus with forskolin prior to chemical treatment allows for the detection of both increases and decreases in hormone levels
- 68 of the 96 chemicals selected for concentration-response evaluation elicited concentration-dependent effects on at least one hormone analyte
- Distinct profiles of steroidogenesis disruption can be observed among chemicals, demonstrating the utility of this model to not only identify chemicals that perturb steroidogenesis, but also the ability to evaluate possible mechanisms underlying altered steroidogenesis

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