Stereoids, which have an important role in a wide range of physiological processes, are synthesized primarily in the gonads and adrenal glands through a series of enzyme-mediated reactions. The activity of steroidogenic enzymes can be inhibited by endocrine disruptors (EDs). This study demonstrates the feasibility of using the computational model of adrenal steroidogenesis to predict in vitro adrenocortical steroid concentrations using H295R cells. This capability could be useful to help define mechanisms of action for poorly characterized chemicals and mixtures in support of the H295R steroidogenesis screening system.

**CELL PROLIFERATION EXPERIMENTS**
- Incubate cells in medium for 72 hr
- Incubate cells in new medium + stimuli + carrier for 72 hr
- Collect samples at 0, 8, 24, 48, 72 hr
- Measure cell and medium concentrations of E1 and E2 using ELISA and remaining 12 steroids using LC/MS/MS

**STEREOIDOGENESIS EXPERIMENTS**
- Baseline and two metyrapone concentrations (1, 10 μM)
- Collect samples at 0, 8, 24, 48, 72 hr
- Measure cell and medium concentrations of E1 and E2 using ELISA and remaining 12 steroids using LC/MS/MS

**MODEL PARAMETERS**
- First-order metabolic reaction rates
- Endocrine disruptors: partition coefficients for transport, competitive enzyme inhibition
- Assumed quasi-equilibrium for steroid transport
- Feedback control system of hypothalamus-pituitary-adrenal (HPA) axis regulates synthesis and secretion of adrenocortical steroids (cortisol (CORT), aldosterone (ALDO), testosterone (T), estradiol (E2)) by release of corticotropin releasing hormone (CRH) from hypothalamus, and adrenocorticotropic hormone (ACTH) from pituitary.

**DISCUSSION**
- Stereoids can predict dynamic steroid concentrations in H295R cells for baseline and two metyrapone doses
- Mechanistic model can improve understanding of dose-response behavior for environmental chemicals that alter activity of steroidogenic enzymes
- This capability could help define mechanisms of action for poorly characterized chemicals and mixtures in support of the H295R steroidogenesis screening system

**Graphical representation of parameters with highest sensitivities (green circles) for cortisol.**

**Competitive Enzyme Inhibition Equation**

$$
\frac{dC}{dt} = \frac{V_{max,cell} + V_{max,med}}{V_{max,cell} + V_{max,med}} \cdot P_{cell} - C_{cell}
$$

$V_{max,cell}$: volume of viable cells
$V_{max,med}$: volume of medium
$P_{cell}$: production rate of steroid $x$ in cells
$U_{cell}$: utilization rate of steroid $x$ in cells

**Dynamic Molecular Balances**
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**STEREOIDOGENESIS MODEL**
- Mathematical model based on in vitro experimental design
- Two compartments: medium and H295R cells
- Two pathways: transport and metabolism
- First-order cholesterol transport rate
- Assumed quasi-equilibrium for steroid transport
- Assumed quasi-equilibrium for steroid transport between medium and cells
- Assumed quasi-equilibrium for steroid transport
- Endocrine disruptors: partition coefficient for transport, competitive enzyme inhibition

**Equilibrium Equations**

$$
C_{cell} = \frac{q_x C_{cell}}{C_{x,med}}
$$

$C_{cell}$: concentration of steroid $x$ in cells
$C_{x,med}$: concentration of steroid $x$ in medium
$q_x$: equilibrium constant for steroid $x$

**THEME**
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