

***In vitro* Evaluation of Steroidogenesis: H295R Assay**

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EPA's Computational Toxicology Communities of Practice

January 28, 2016

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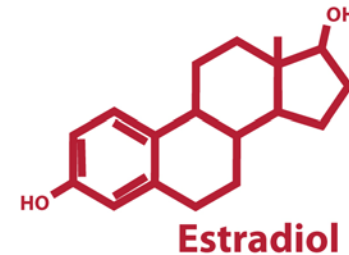
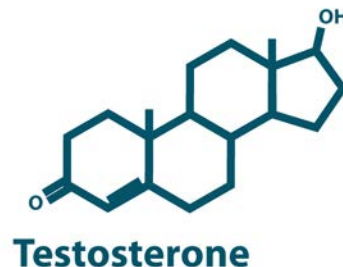
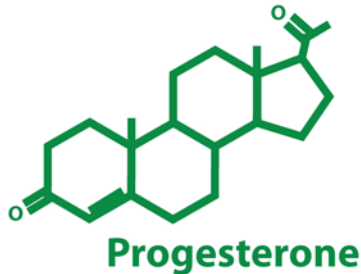
Endocrine Disrupting Chemical

“an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects...” (World Health Organization)

- EDCs have largely been tested by evaluation of effects on nuclear receptors (ER, AR, etc.)
- Altering hormone levels via disruption of biosynthesis or metabolism can also contribute to endocrine disruption
 - This is difficult to assay *in vitro*
 - OECD validation of **H295R steroidogenesis assay** in 2011.

Sex Hormone Biosynthesis & Metabolism

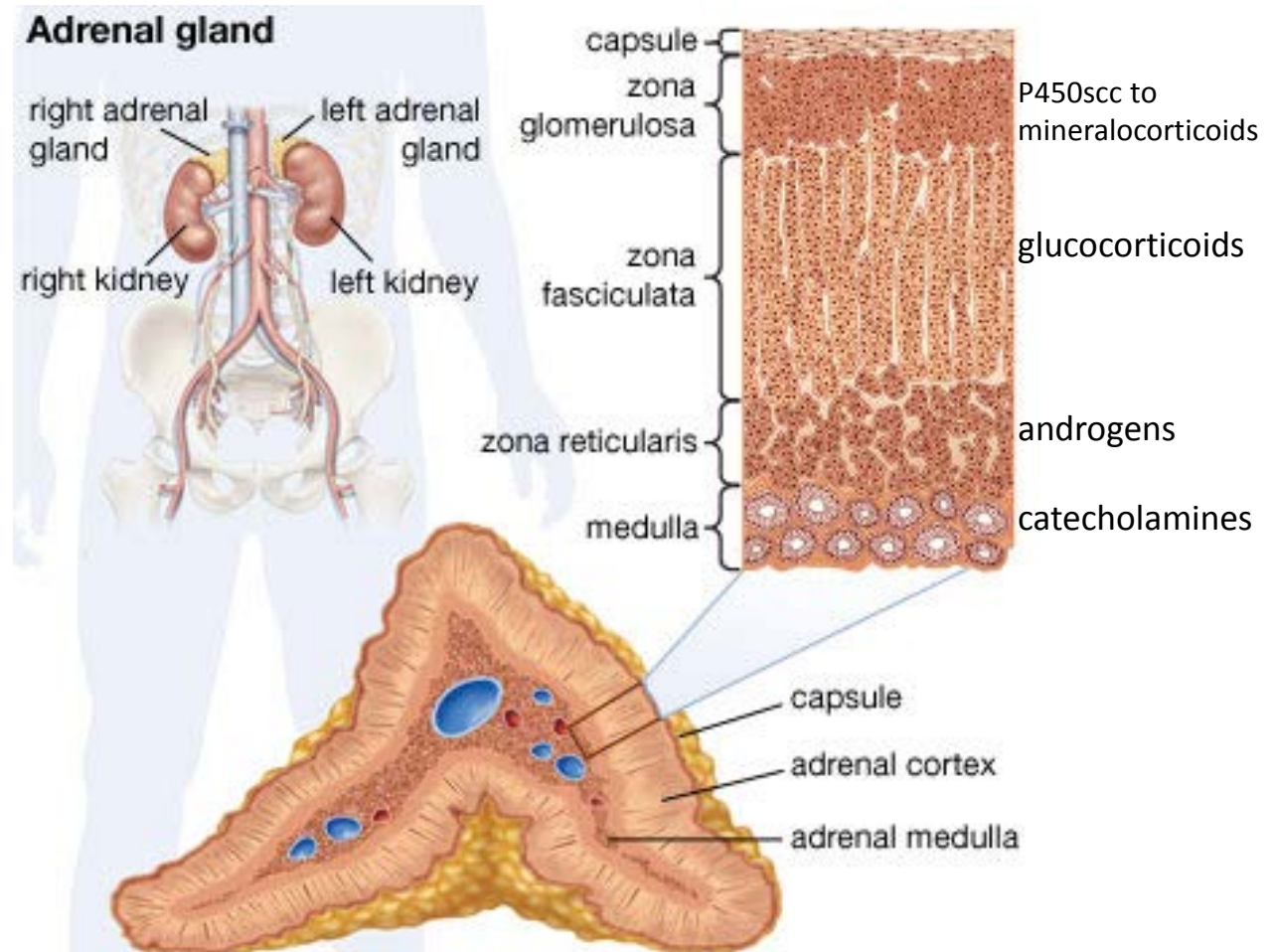
- Proper steroidogenesis is essential:
 - *In utero* for fetal development
 - In adults for reproductive function



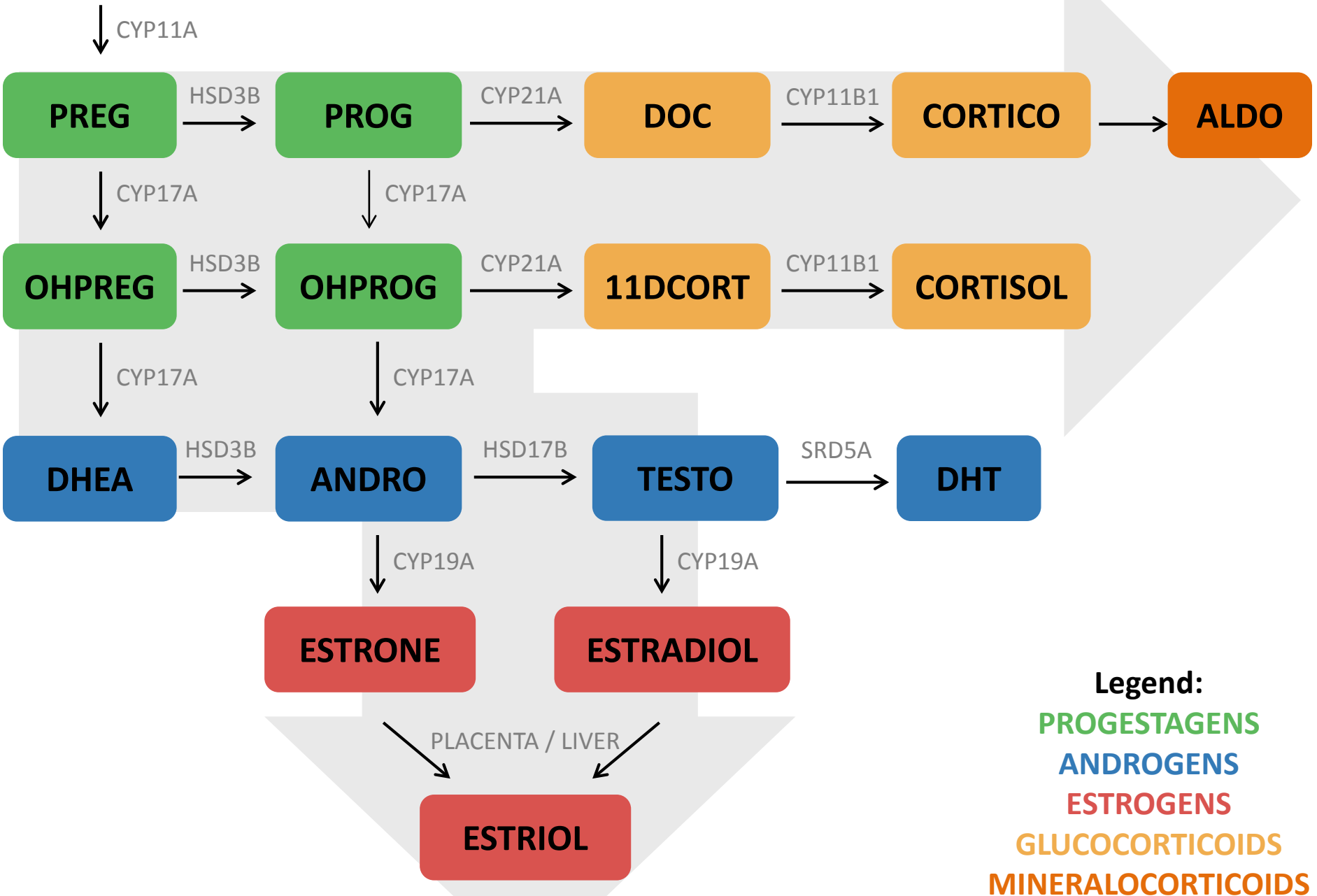
- >90% of steroidogenesis occurs in the gonads
- Adrenal gland (glucocorticoids)

Adrenal Steroidogenesis: H295R Cells

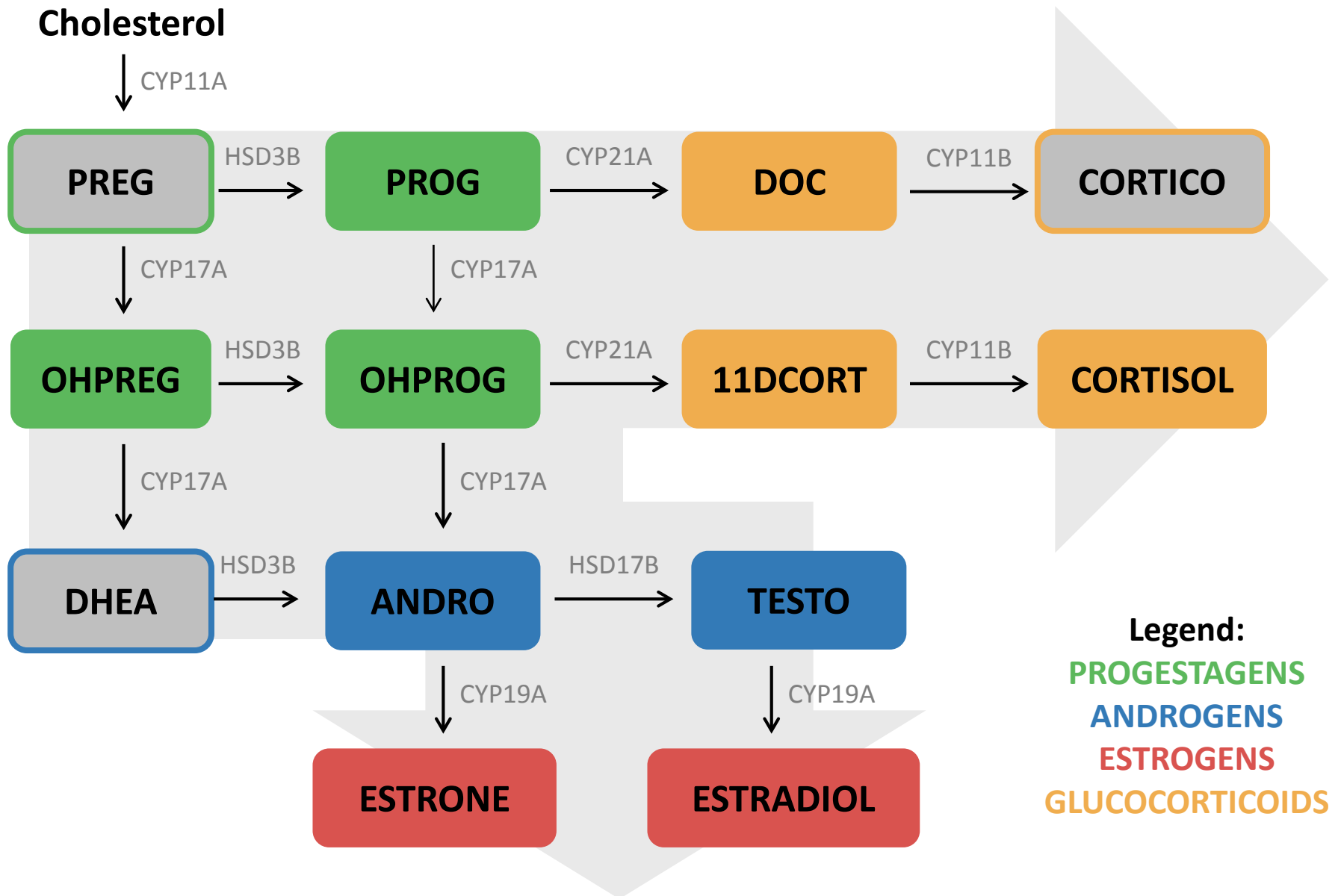
- Model characterized by John Giesy & Markus Hecker (MSU)
- Human origin: African American female donor
- Adrenocortical Carcinoma
- Characteristics of undifferentiated fetal adrenal cells



Cholesterol



Steroidogenic Pathway Assayed



H295R OECD Test Guideline

OECD Guideline Assay

- No pre-treatment or cell stimulation
- 48 hr chemical treatment in concentration-response
- Quantification of **estradiol** and **testosterone**

Comparison to OECD Guideline

OECD Guideline H295R Assay

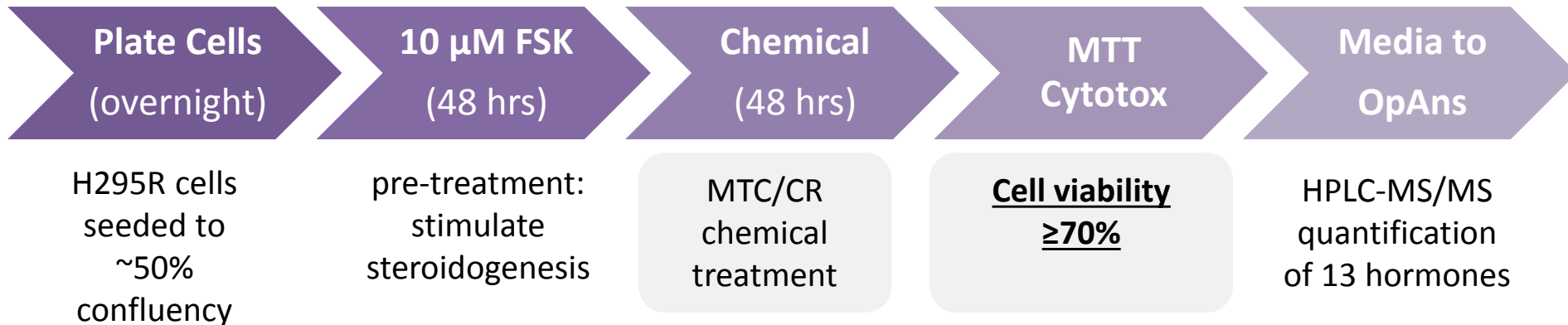
- No pre-treatment
- 48 hr chemical treatment in concentration-response
- Quantification of estradiol and testosterone

ToxCast H295R Assay

- 48 hr Forskolin stimulus
- 48 hr chemical treatment for MTC and conc-response
- Quantification of 13 hormones across pathway
- 3 stage approach

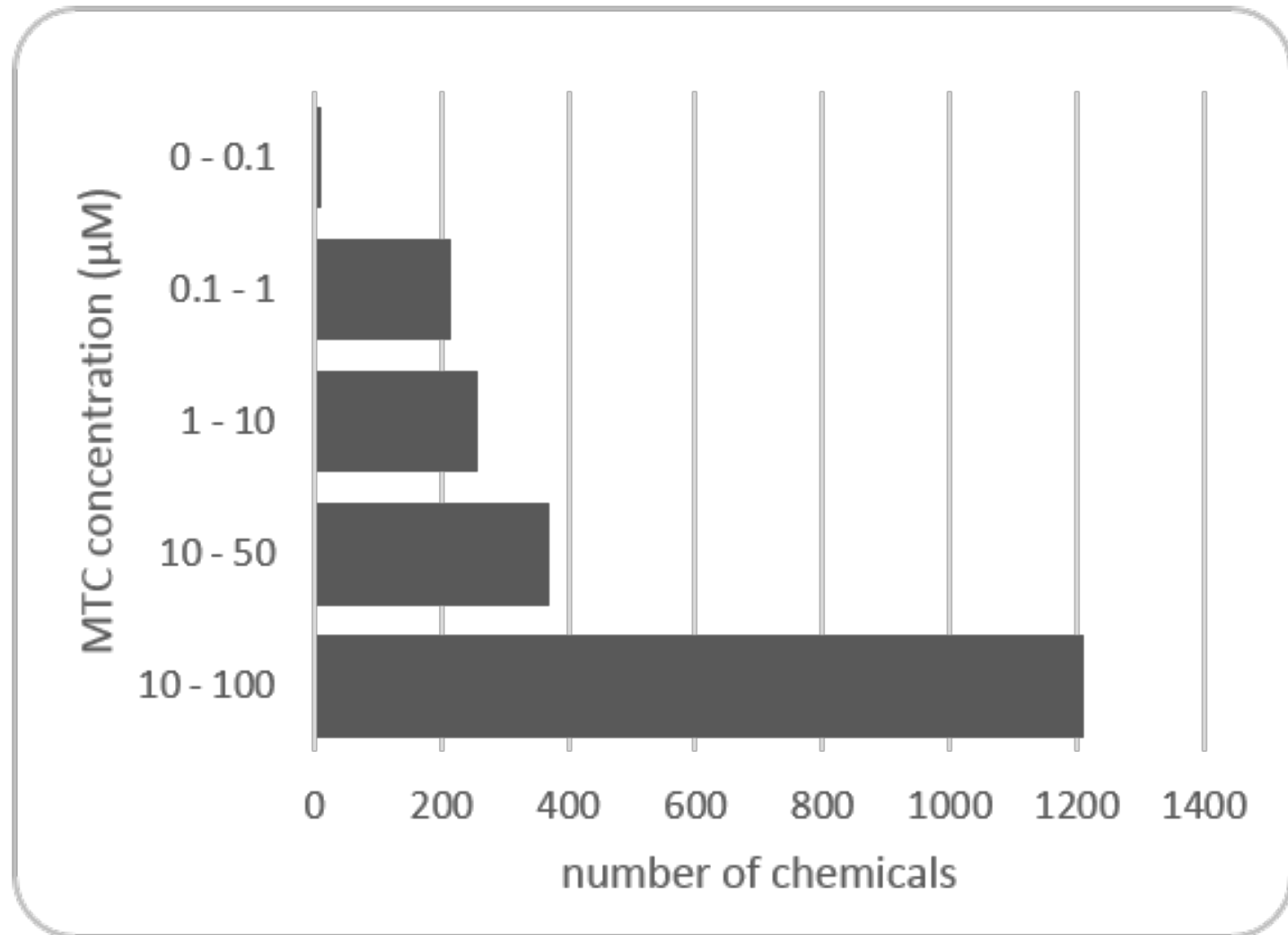
Study Design

- ToxCast three stage approach:
 1. Determine maximum tolerated concentration (MTC)
 2. Hormone quantification for MTC treatment alone
 3. Concentration-response for selected chemicals



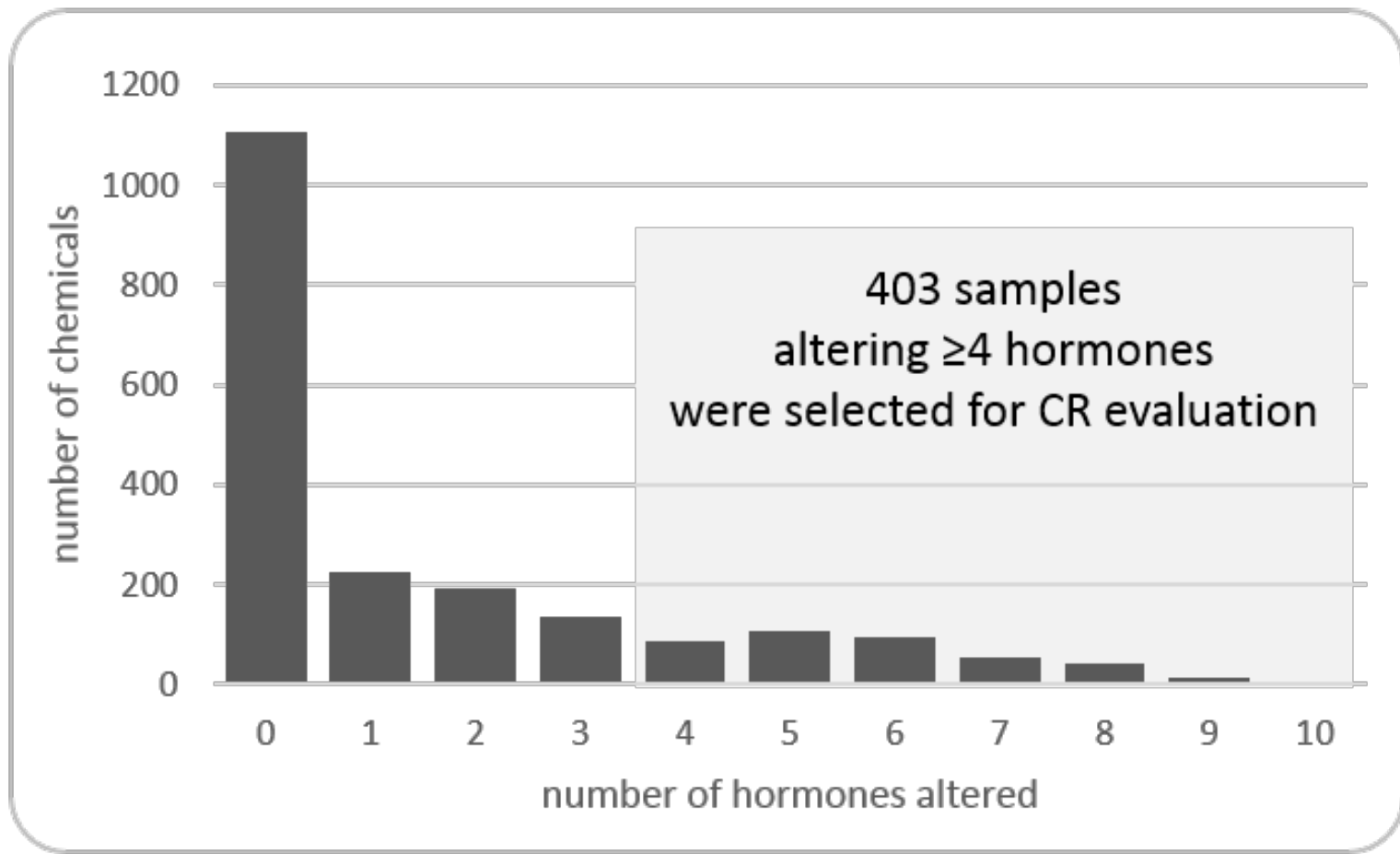
Stage 1: MTC determination

2060 samples



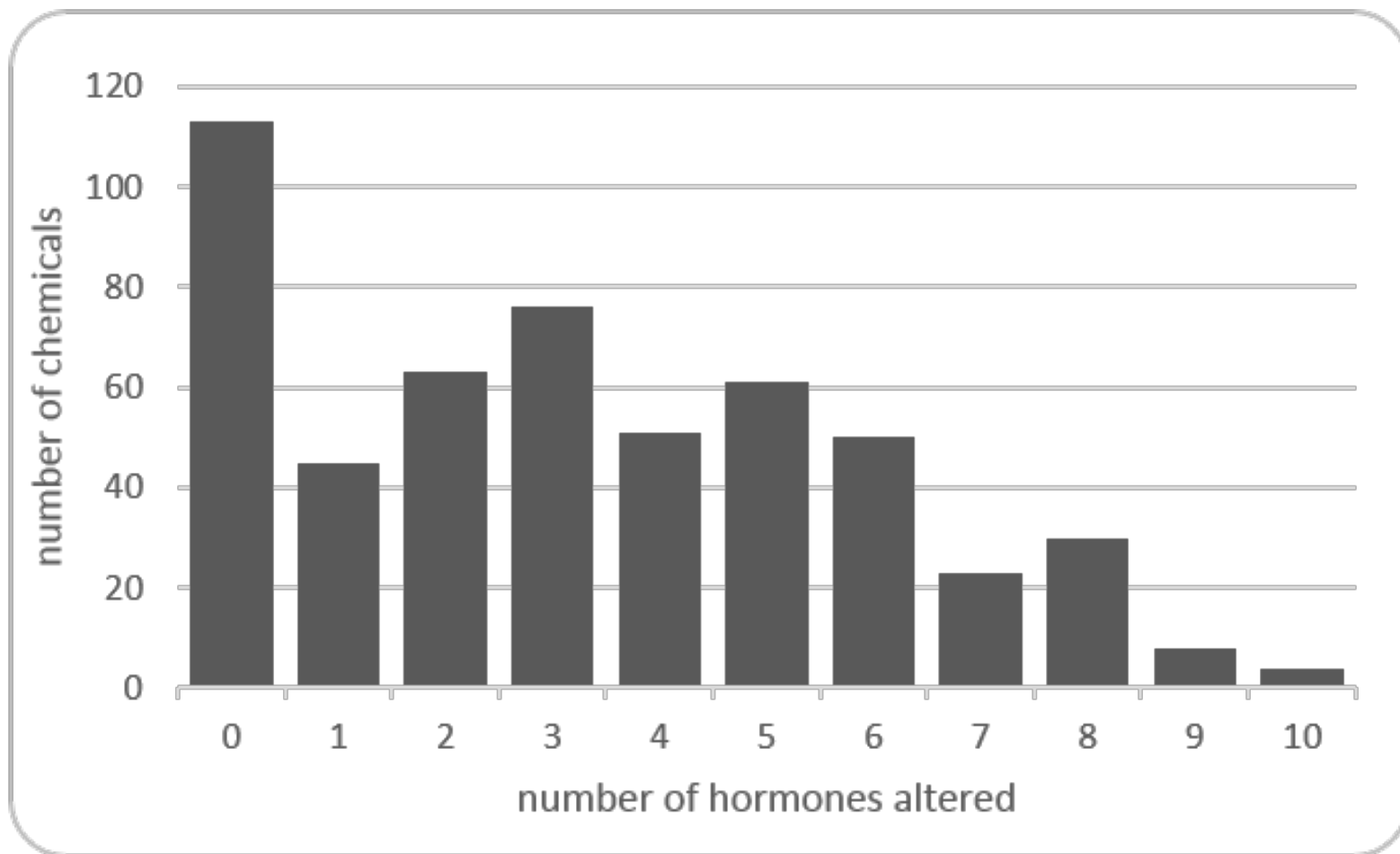
Stage 2: MTC hormone screen

2060 samples



Stage 3: CR hormone evaluation

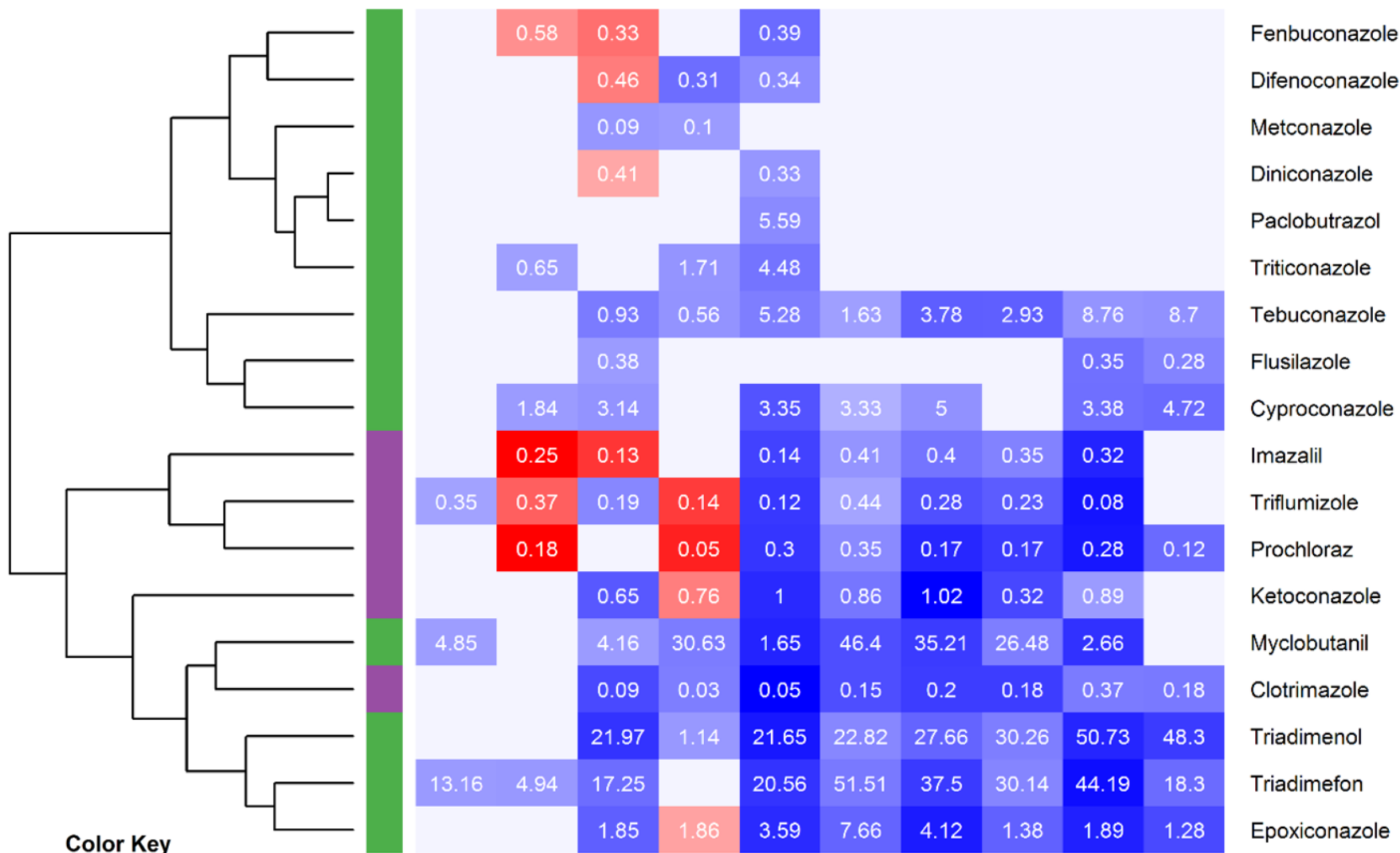
524 samples



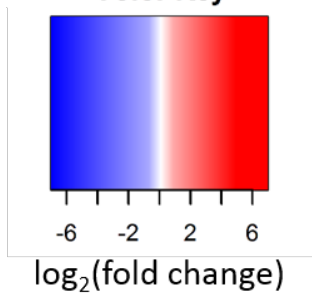


Prochloraz-mediated effects on steroidogenesis

Prochloraz is a fungicide known to inhibit CYP17A1 (circled in red).

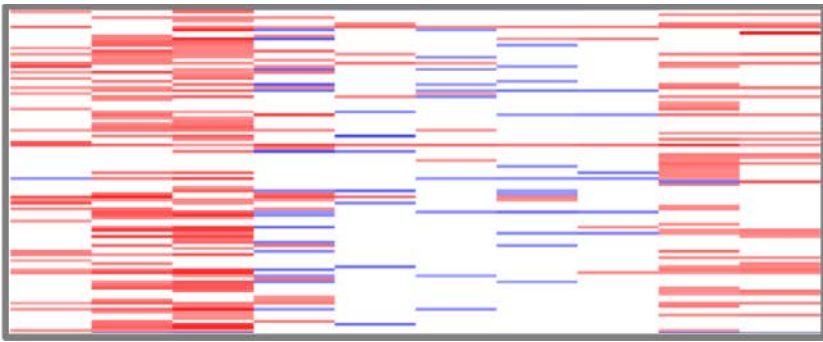


Color Key

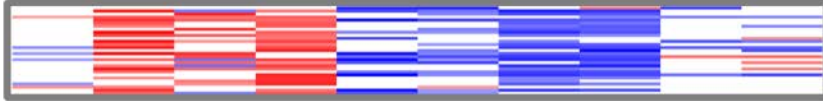


Profiling chemical effects on steroidogenesis

A



B



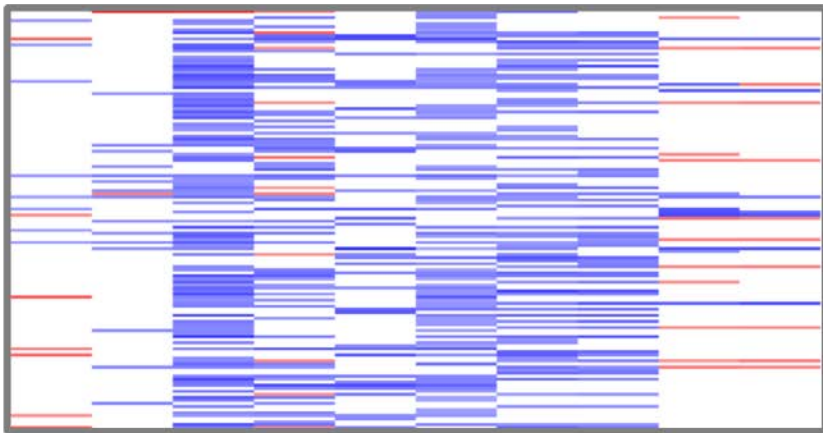
C



D



E



OH-PREG

PROG

OH-PROG

DOC

CORTISOL

11DCORT

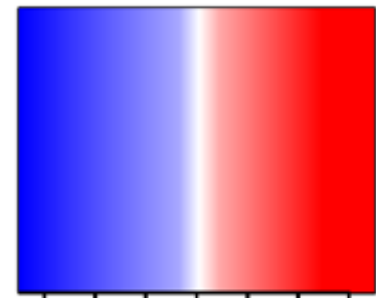
ANDRO

TESTO

ESTRONE

ESTRADIOL

Color Key



-6

-2

2

6

log₂(fold change)

Summary

- The modified H295R assay used for ToxCast high-throughput screening successfully evaluated 2060 chemical samples
 - 524 chemicals were evaluated in conc-response
 - Profiling across 10 hormone endpoints across the pathways provides valuable insight into potential mechanisms of action

Future Directions

- Integrating steroidogenesis results with other complementary assays including aromatase inhibition assays

Acknowledgements

Collaborators

Colleen Toole (CeeTox)

Ken Lewis (OpAns)

Dayne Filer

Matt Martin

Colleagues

ToxCast Scientists

EDSP Workgroup