Identifying Androgen Disrupting Chemical Metabolites using Intracellular Xenobiotic mRNA Transfection Method

Evan Brown [1, 2], Daniel Hallinger [2], Steve Simmons [2]

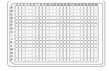
1. Oak Ridge Institute for Science Education (ORISE), Oak Ridge, TN; 2. Rapid Assay Development Branch (RADB), ORD, U.S. EPA, Durham, NC

Evan C. Brown | brown.evan@epa.gov | 980-395-2201

Background

- The existence of 10,000+ chemicals lacking thorough toxicity data necessitates the use of *in vitro* high-throughput screening (HTS) assays for hazard identification and chemical prioritization.
- A major limitation of existing high-throughput assays is their lack of endogenous cellular metabolism and subsequent inability to identify metabolically-induced changes in toxicity.
- As such, our laboratory developed an intracellular mRNA transfection method to confer Cytochrome P450 (CYP) metabolism to cell-based HTS assays (DeGroot *et al.* 2018).
- This method was developed to be applied to a wide-range of existing assay types; however, it had yet to be deployed on a cell-based HTS assay measuring an endocrine-related endpoint.
- Therefore, we sought to use our mRNA transfection method on a novel androgen receptor dimerization assay (AR2) to evaluate the effect of CYP metabolism on antagonists of the human androgen receptor.
- **Identification of bio- activated or deactivated metabolites can better characterize a compound's interaction with the AR pathway, leading to improved hazard estimates.**

Experimental Design



0 hr: Plate HepG2-AR2 cells (C)

18 hr: Transfect CYP mRNAs (C)

24 hr: Administer chemicals (E)

42 hr: Dispense substrate and read AR assay endpoints (C, CS)

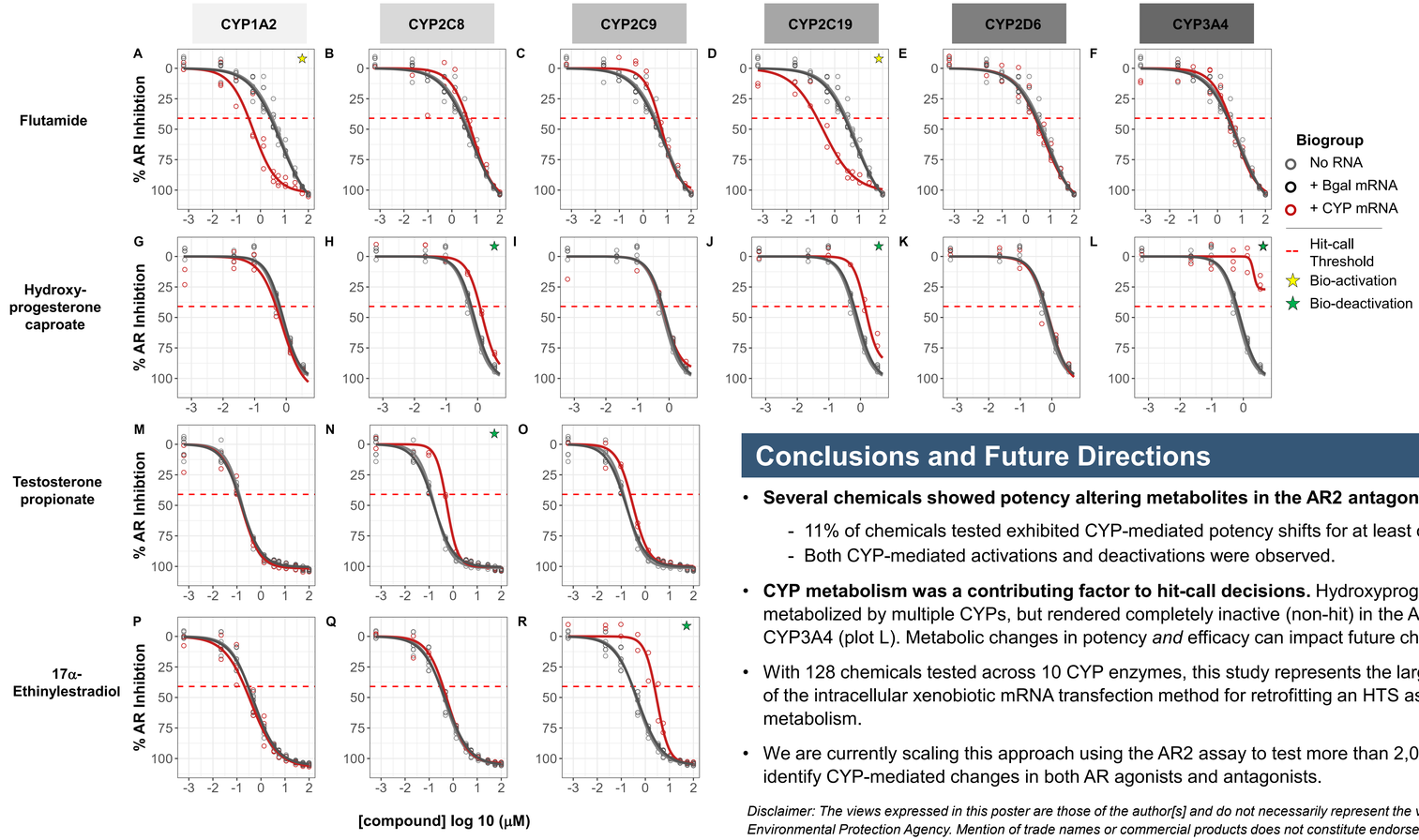
Laboratory Automation Key

- C: Certus FLEX Micro Dispenser
- E: Echo 555 Liquid Handler
- CS: CLARIOstar Microplate Reader

Experimental Details: 384-well plates were seeded with 8,000 cells/well. After 18hr, cells were transfected with 50ng CYP/control mRNA and allowed to acclimate for 6hr to promote translation. Following, chemicals/controls were administered. 10nM R1881 was used as AR maximal positive control for antagonist mode. After 18hr chemical exposure, furimazine was added to evaluate the AR-nanoluciferase endpoint and Resazurin (with 2hr incubation) was added to evaluate the post-hoc viability endpoint.

Results

A 128-chemical panel was tested among the 10 most prevalent human liver CYPs and AR antagonist bioactivity shifts were observed.



Micromolar concentrations are shown in log 10 scale along the x-axis. Response is shown as % AR Inhibition (of maximal positive control) in the downward direction. Dose-response hill curves were fit using the tcpl_lite R script. Grey and black curves indicate negative (non-CYP) controls and red curves correspond to the associated CYP column header. The red dashed line specifies the threshold used to define AR antagonist activity.

Abbreviations: Androgen Receptor (AR), Cytochrome P450 (CYP), micromolar (μM)

Conclusions and Future Directions

- **Several chemicals showed potency altering metabolites in the AR2 antagonist assay.**
  - 11% of chemicals tested exhibited CYP-mediated potency shifts for at least one CYP enzyme.
  - Both CYP-mediated activations and deactivations were observed.
- **CYP metabolism was a contributing factor to hit-call decisions.** Hydroxyprogesterone caproate was metabolized by multiple CYPs, but rendered completely inactive (non-hit) in the AR2 antagonist assay by CYP3A4 (plot L). Metabolic changes in potency *and* efficacy can impact future chemical prioritization.
- With 128 chemicals tested across 10 CYP enzymes, this study represents the largest deployment to date of the intracellular xenobiotic mRNA transfection method for retrofitting an HTS assay with CYP metabolism.
- We are currently scaling this approach using the AR2 assay to test more than 2,000 ToxCast chemicals to identify CYP-mediated changes in both AR agonists and antagonists.