CertiChem, Inc.

George D. Bittner, PhD, CEO
11212 Metric Blvd, Suite 500
Austin, Texas 78758
gbittner@CertiChem.com

- To regulate EA in selected chemicals, the EPA in 2009 published a battery of Tier 1 EDSP assays that included: two in vitro (estrogen receptor (ER) binding, h-ERα-HeLa-9903 TA) EA assays and one in vivo (uterotrophic) assay to detect EA.

- The entire battery is costly ($50,000 - $150,000), time consuming (months) and requires nearly 600 animals per chemical.

- A less costly, less time consuming, commercially available battery of assays is needed as an alternate to the current Tier 1 EDSP assays for estrogenic activity (EA)

- The EPA in 2015 published a battery of 16-18 ToxCast ER Bioactivity Model Assays that interrogate key steps in the ER Adverse Outcome Pathway (AOP) as an in vitro alternate to the three Tier1 EDSP assays).

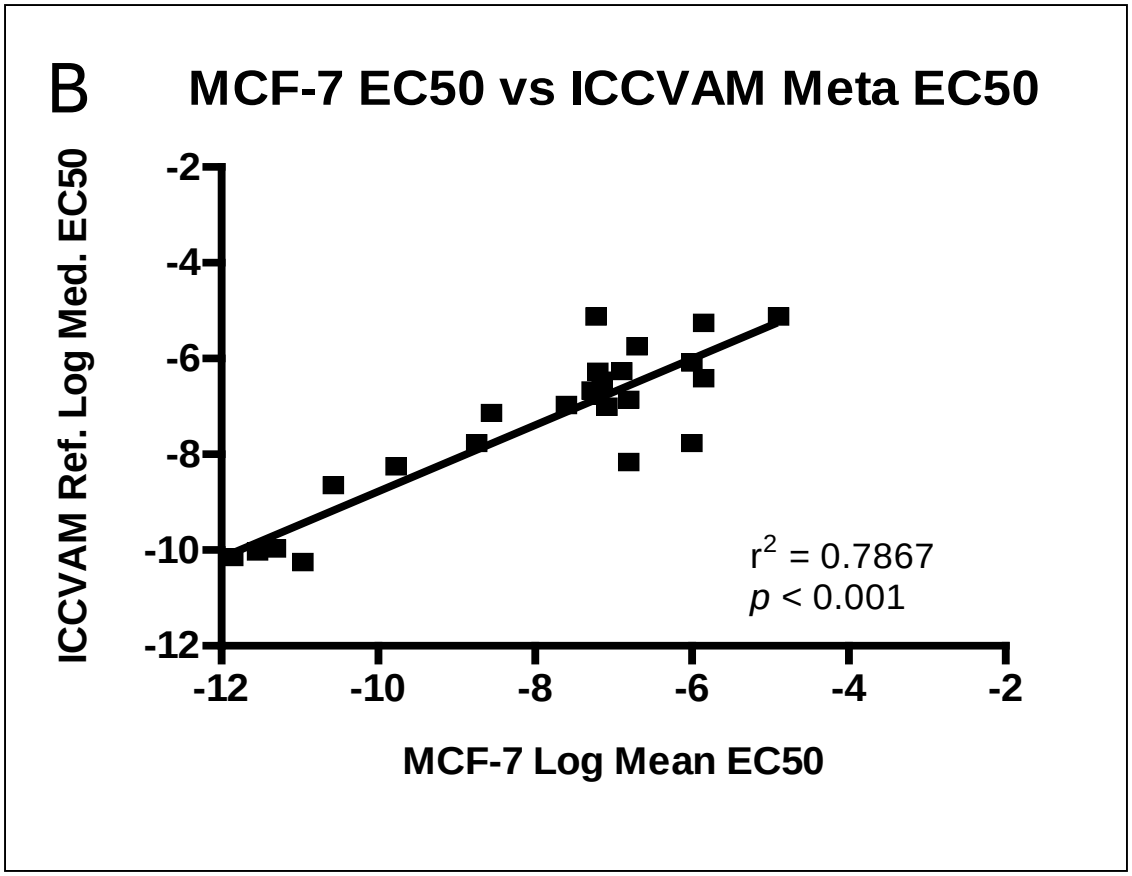
- Many of the assays in this set of assays are not freely commercially available and would be costly to run if they were available.

- CertiChem is developing an ER Battery of 4-5 robotic, in vitro assays to probe key ER AOP events (e.g., ER polymerization, ER translocation, ER binding, reporter gene transcriptional activation and cellular proliferation) to comprehensively assess ER bioactivity and meet EPA performance standards under development, as an alternative to the “ToxCast 16-AOP Battery”

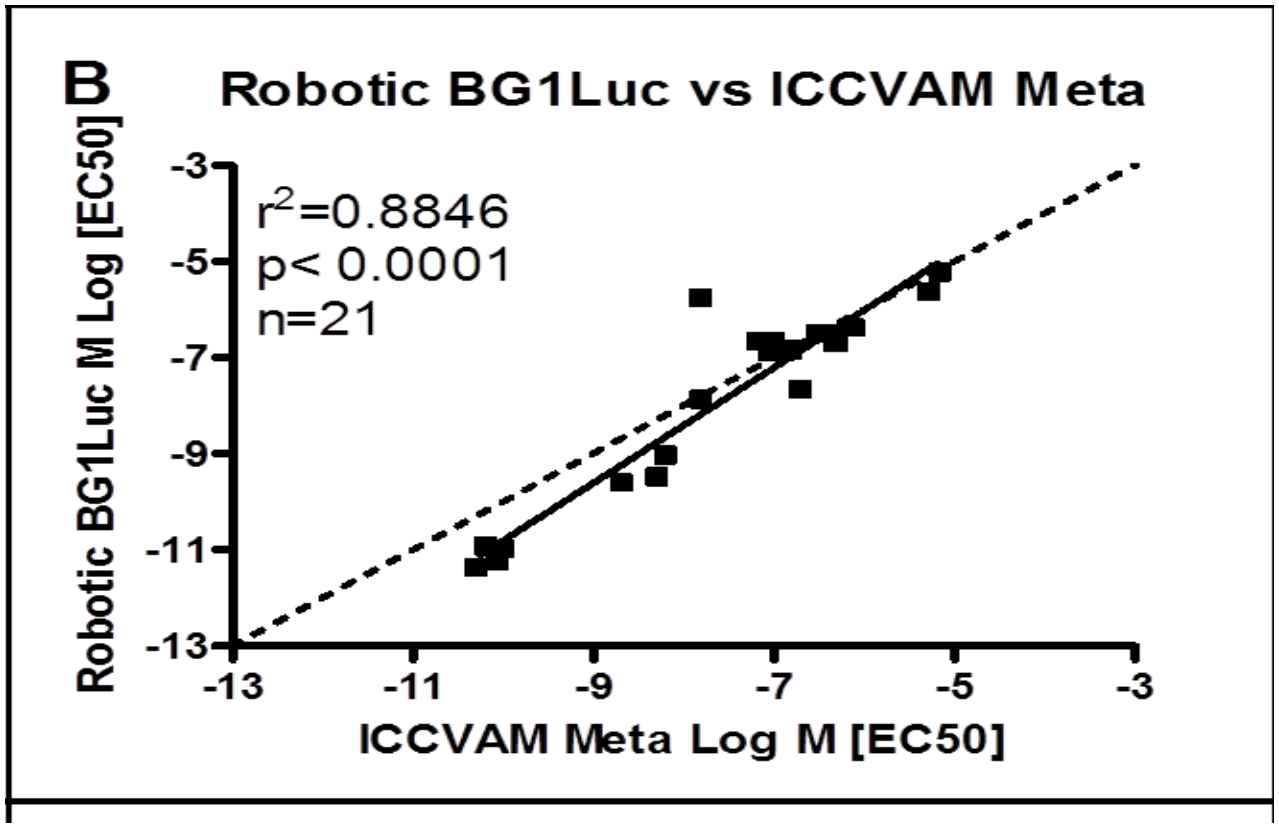
- For example, CertiChem's results for EPA test chemicals using robotic versions of MCF-7 and BG 1-Luc assays, with confirmation assays, have excellent concordance with ICCVAM meta-analysis data.

- MCF-7 assays alone had no false negatives or false positives for over 50 test chemicals

Accuracy, Concordance of MCF-7 assay



Accuracy, Concordance of BG1-Luc assay



- Conclusion: An ER Battery of 4-5 judiciously chosen, robotic, in vitro assays to probe key ER AOP events including an ER polymerization, ER translocation, ER binding, reporter gene transcriptional activation (BG-1 Luc) and cellular proliferation (MCF-7) assay to comprehensively assess ER bioactivity is needed at this time and would meet EPA performance standards. A binding assay would help eliminate false positives or false negatives due to toxicity (as do Confirmation Assays)