Developing in vitro bioassays for screening of chemical contaminants in California's recycled water

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WHY ENHANCE USE OF BIOLOGICAL SCREENING

- **Too many compounds**
- **Use ultimate endpoints of interest to screen for targets**
  - Adverse Outcomes to Biota
  - Bioavailability
  - Considers mixtures and unknowns
- **Potentially cheaper than measuring many chemicals**
  - High throughput;
  - Commercially available

CECs TO MONITOR FOR GROUNDWATER RECHARGE

Constituent	Chemical Class	Health-based MRL (ng/L)	Perf. indicator MRL (ng/L)
17-β estradiol	Natural hormone	•0.09 (1.0*)	•1.0
Caffeine	Food product	•35 (50*)	•50
Triclosan	Pers care prod	•50	•50
NDMA	Rxn by-product	•0.1 (2.0*)	•2.0
Sucralose	Food additive	•N/A	•100
Iopromide	Pharmaceutical	•N/A	•50
DEET	Pers care prod	•N/A	•50
Gemfibrozil	Pharmaceutical	•N/A	•50

* currently achievable by commercial services labs

STUDY OBJECTIVES & GOALS

- **Identify most promising bioassays**
  - Which endpoints are most relevant?
- **Optimize & evaluate selected bioassays**
  - Can they be standardized for robust, cost-effective monitoring?
- **Provide interpretive guidance & framework for results**
  - Do bioassays get us closer to effects or simply screen for exposure?
- **Transfer technology to stakeholders**
  - Deliver protocols for robust measurement and tips on best use of information

BIOASSAY SELECTION

- **Determine most relevant endpoints**
  - mode-of-action classifications (e.g. hormones as estrogens)
  - specificity for CEC “indicators” (e.g. gemfibrozil)
- **Perform literature survey**
  - peer-reviewed publications
  - Australian NWC & Global Water Coalition reports
  - vendor material
- **Interview vendors**
  - Are cell lines (or kits) commercially available? Proprietary or restricted use?
  - What is their performance for known agonists?
  - What level of customer/product support can be provided?

PRIORITY ENDPOINTS

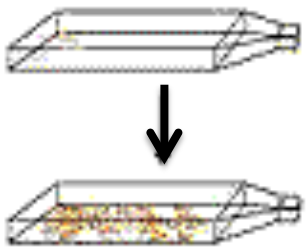
ENDPOINT	INDICATOR CEC	REF TOXICANT	CANDIDATE BIOASSAY(S)
estrogenicity	17β-estradiol	17β-estradiol	estrogen receptor (ER)
androgenicity	dihydrotestosterone	testosterone & derivatives	androgen receptor (AR)
progesterone activity	levonorgestrel	trenbolone levonorgestrel	progesterone receptor (PR)
thyroid hormone activity	triclosan	T3	Thyroid receptor (TR)
genotoxicity	NDMA	benzo[a]pyrene	Ames II, UMU, p53 reporter
aryl hydrocarbon reactivity	caffeine	TCDD; PCB126; benzo[a]pyrene	Aryl hydrocarbon receptor (AhR)
CEC-specific response	gemfibrozil	gemfibrozil	PPAR gamma

includes 3 of 4 "high priority" endpoints recently evaluated by Australian NWC (Chapman et al. 2011)

ADAPTATION OF IN VITRO ASSAY PROTOCOL

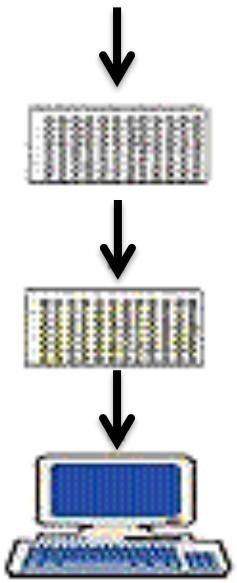
1. Stably transfected?
2. Division arrested

Cell culture



Seed cells and Transfection

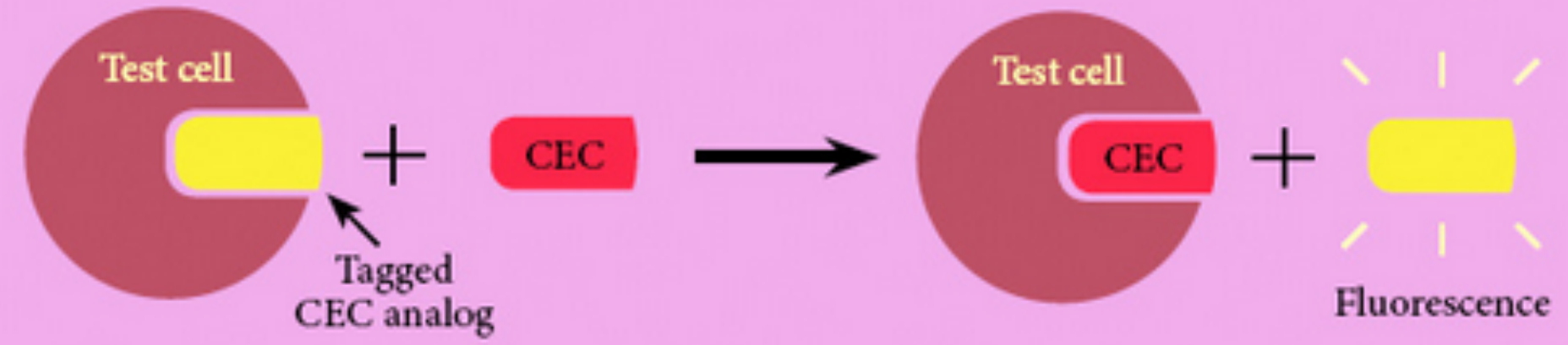
Apply organic extract of *sample*

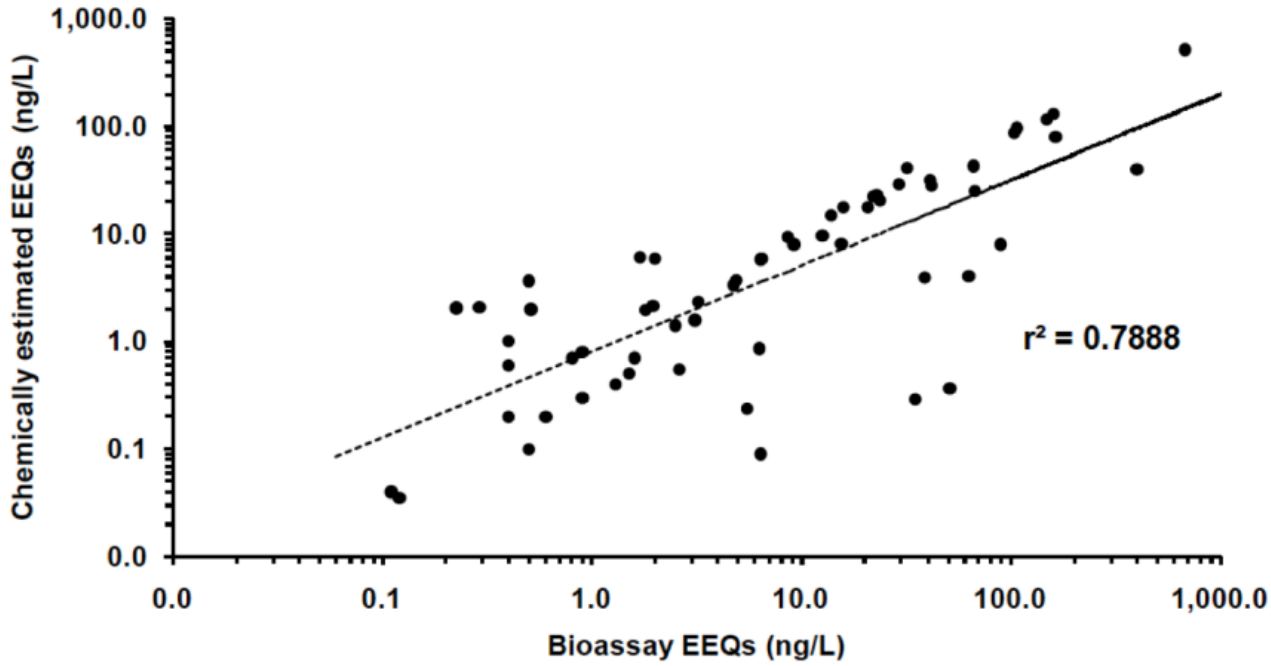


Add assay reagents and Incubation

Fluorescence or Luminescence reading

HOW IN VITRO BIOASSAYS WORK





Bulloch and Schlenk 2010

BIOASSAY COMPARISON

- **Relevance**
  - specificity for MOA or CEC
  - human or highly modified cell line
  - link to known tox pathways and/or apical endpoints
  - translatable results (e.g. TEQs?)
- **Robustness**
  - specificity
  - sensitivity
  - precision & reproducibility
  - interferences/blanks
  - stable vs. transient transfection
  - historical usage

BIOASSAY COMPARISON

- **Simplicity**
  - widespread availability of cell lines/test kits
  - protocol complexity (pre-growth; reagents; environmental controls)
  - required training & expertise
  - interpretation & translation of results
- **Time & Cost**
  - bioassay set-up & response time
  - capital & recurring costs
  - bioassay integration (multiplexing) & automation
  - data interpretation, management & reporting
- **Vendor support**
  - co-investment & leveraging
  - ready resources & expertise

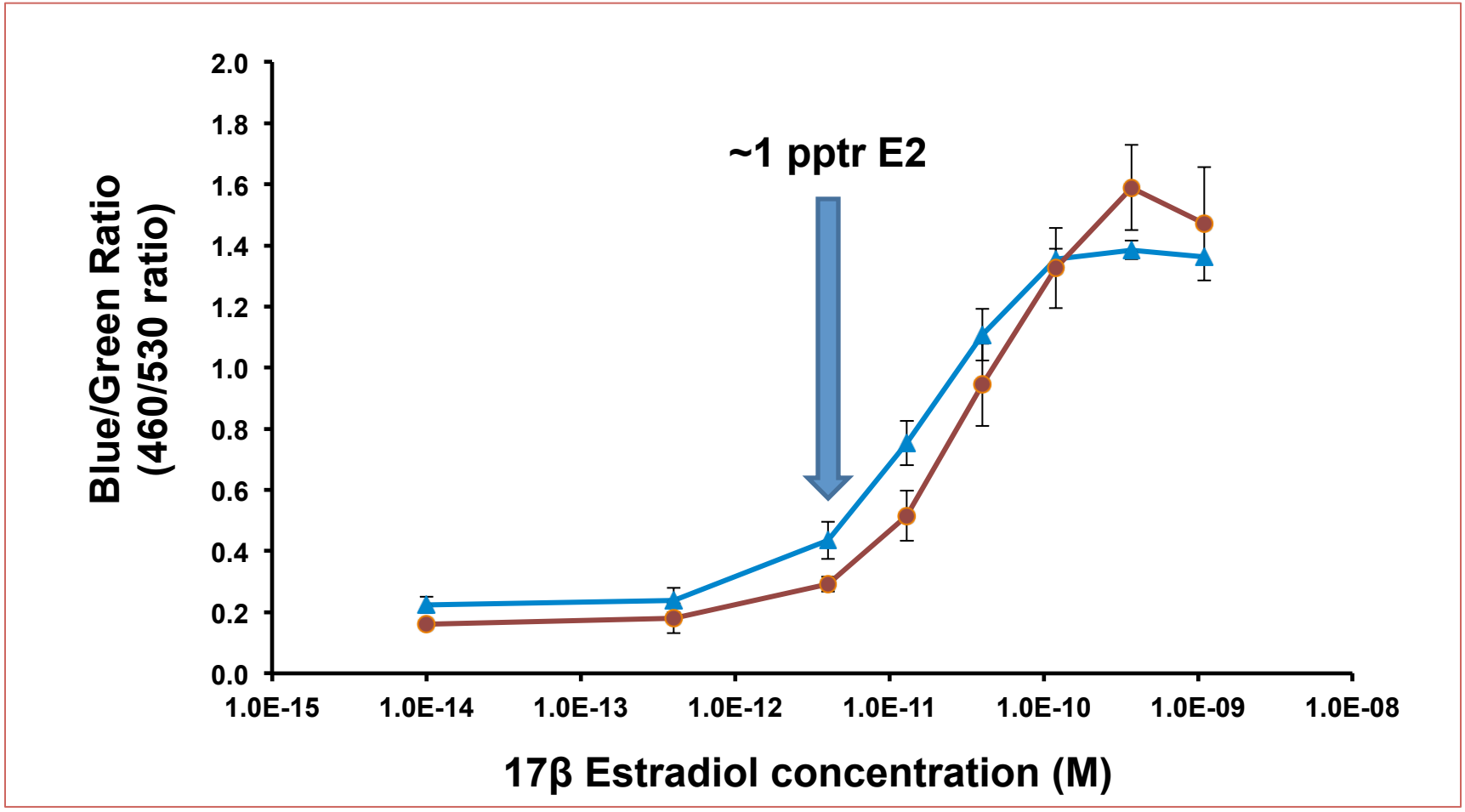
BIOASSAY OPTIMIZATION

- **Optimize/verify basic bioassay parameters (no matrix)**
  - growth media, time, incubation conditions
  - evaluate dose-response relationships using spiked reference compounds
  - lab/field collection blanks & handling considerations
- **Test & validate bioassay response with water samples**
  - Range of water qualities
  - supporting CEC concentration data (see WATER CHEMISTRY)
  - document standardized operating protocols
- **Conduct interlaboratory round-robin testing**
  - All PI labs to participate; SCCWRP as model “utility” lab

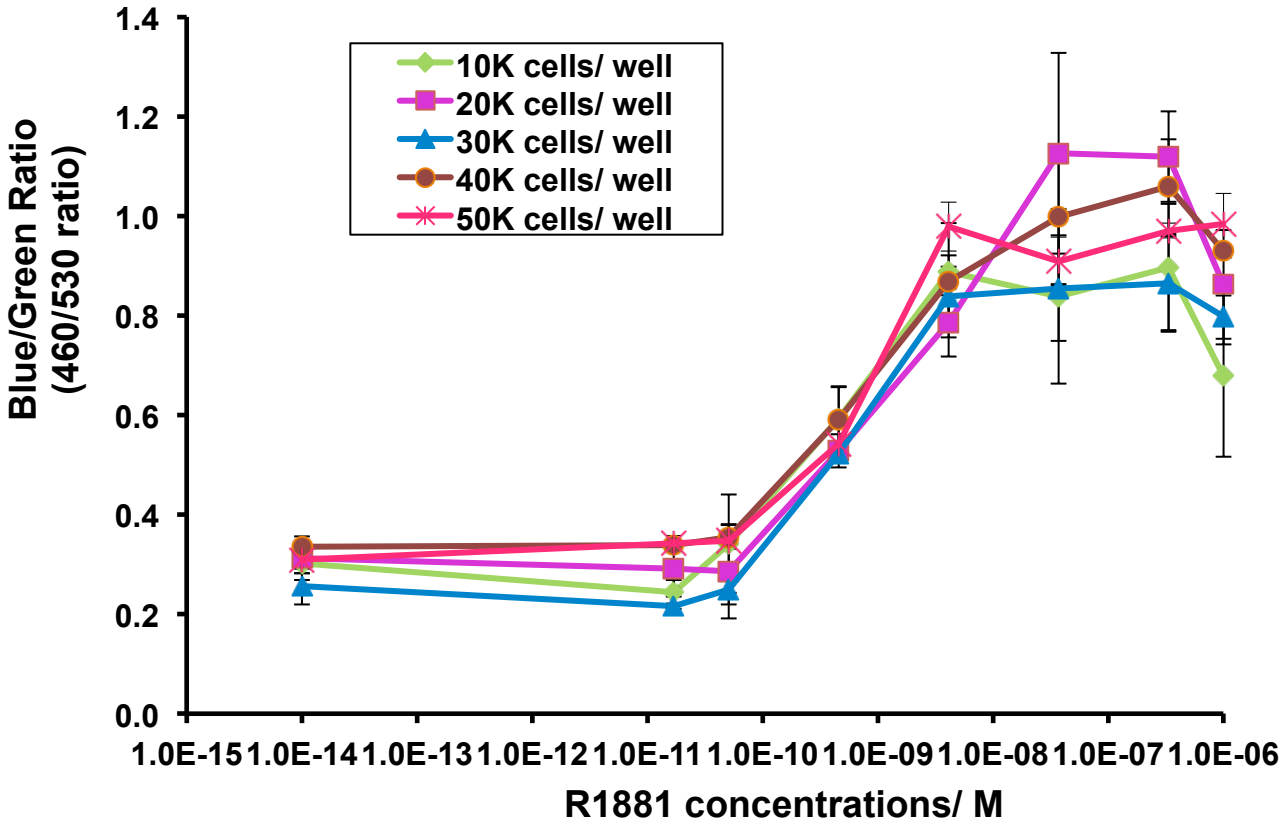
PRIORITY ENDPOINTS (REVISED)

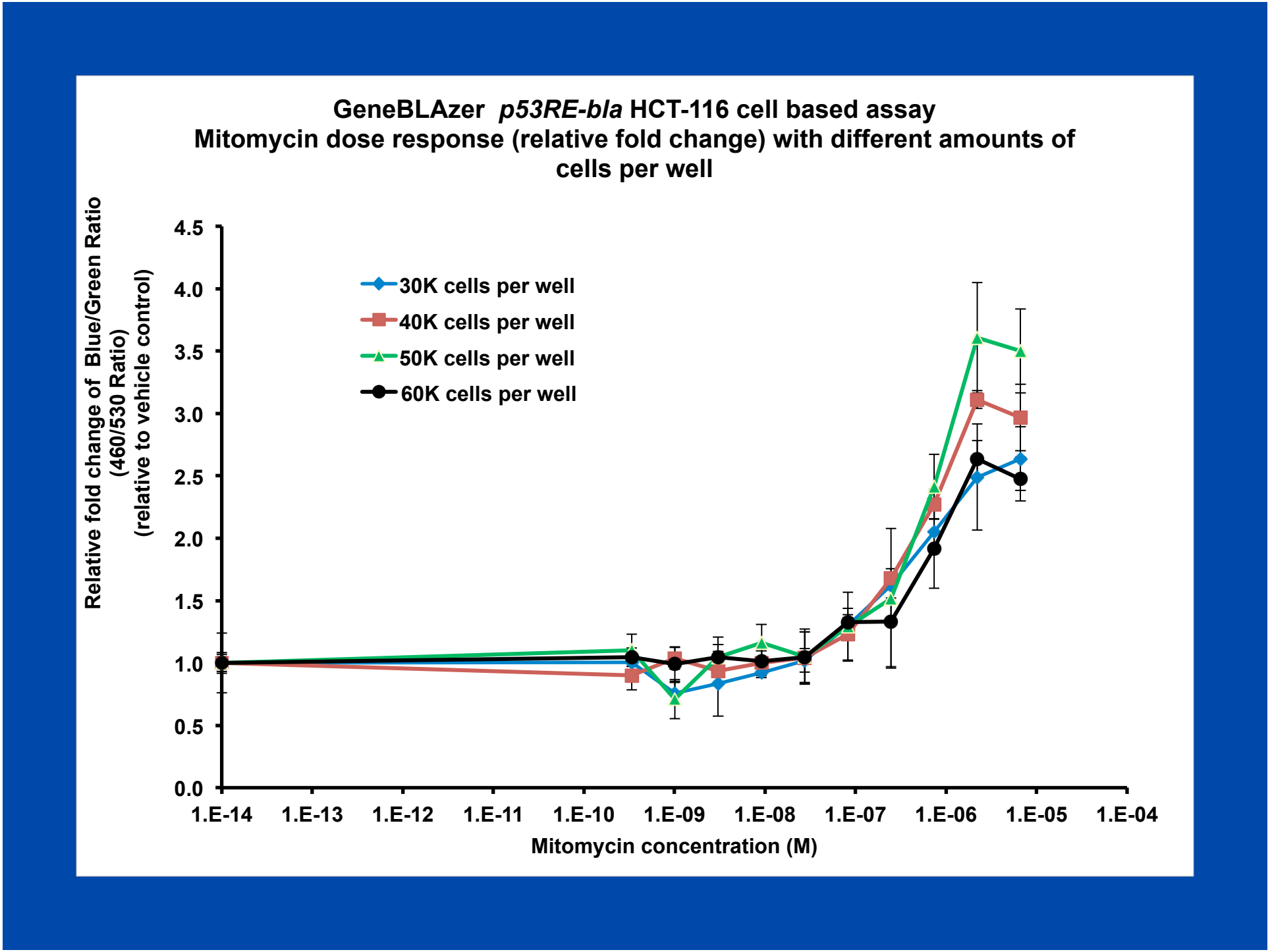
ENDPOINT	INDICATOR CEC	REF TOXICANT	CANDIDATE BIOASSAY(S)
estrogenicity	17β-estradiol	17β-estradiol	estrogen receptor (ER)
androgenicity		testosterone & derivatives	androgen receptor (AR)
progesterone activity		Progesterone	progesterone receptor (PR)
glucocorticoid activity		Dexamethasone	glucocorticoid receptor (GR)
genotoxicity	NDMA	benzo[a]pyrene	UMU, p53 reporter
aryl hydrocarbon activity	caffeine	TCDD; PCB126; benzo[a]pyrene	Aryl hydrocarbon receptor (AhR)
CEC-specific response	gemfibrozil	gemfibrozil	Peroxisome Proliferator Activated Receptor (PPAR)

Dose response for GeneBLAzer ERα Assay 17β-estradiol ("E2")

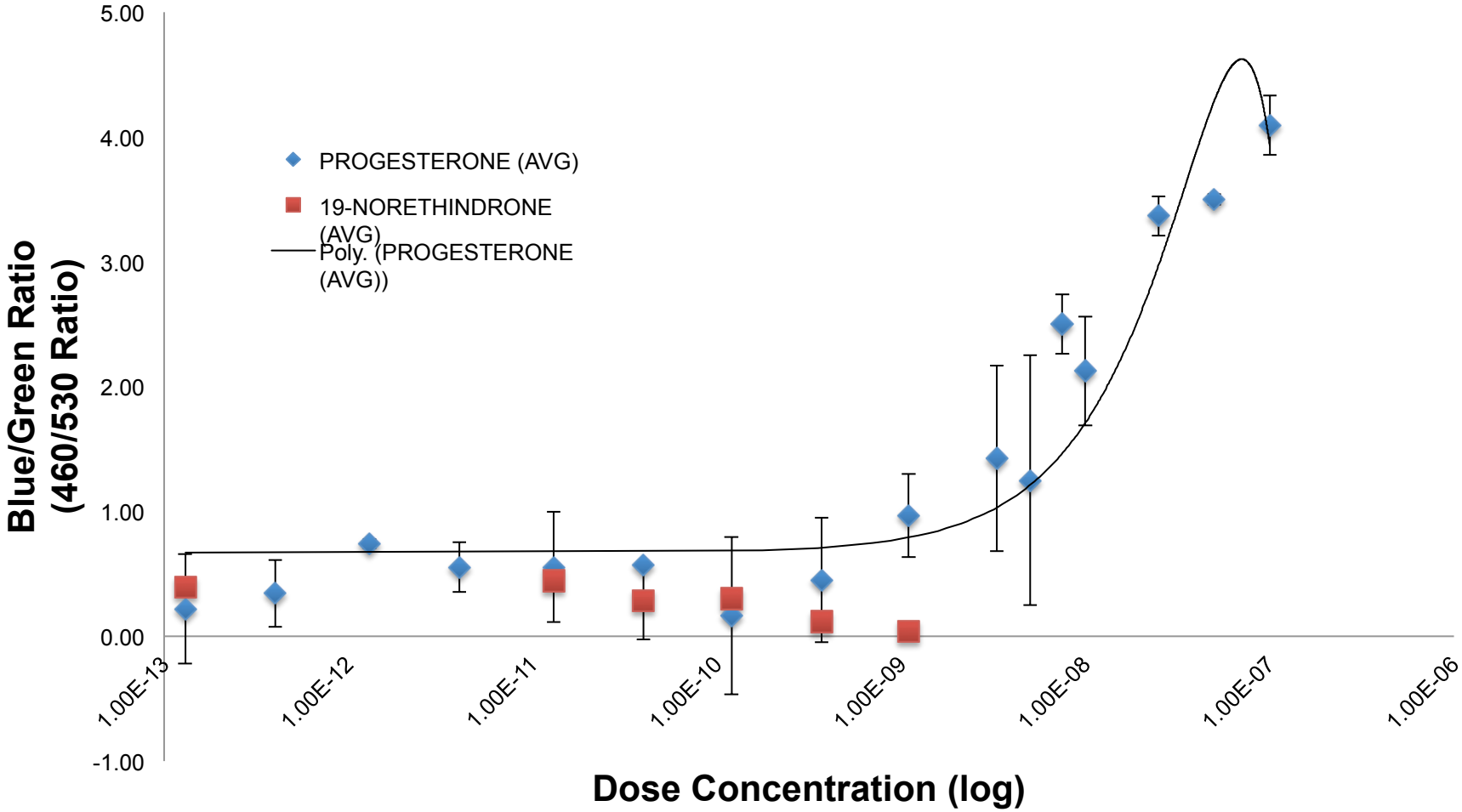


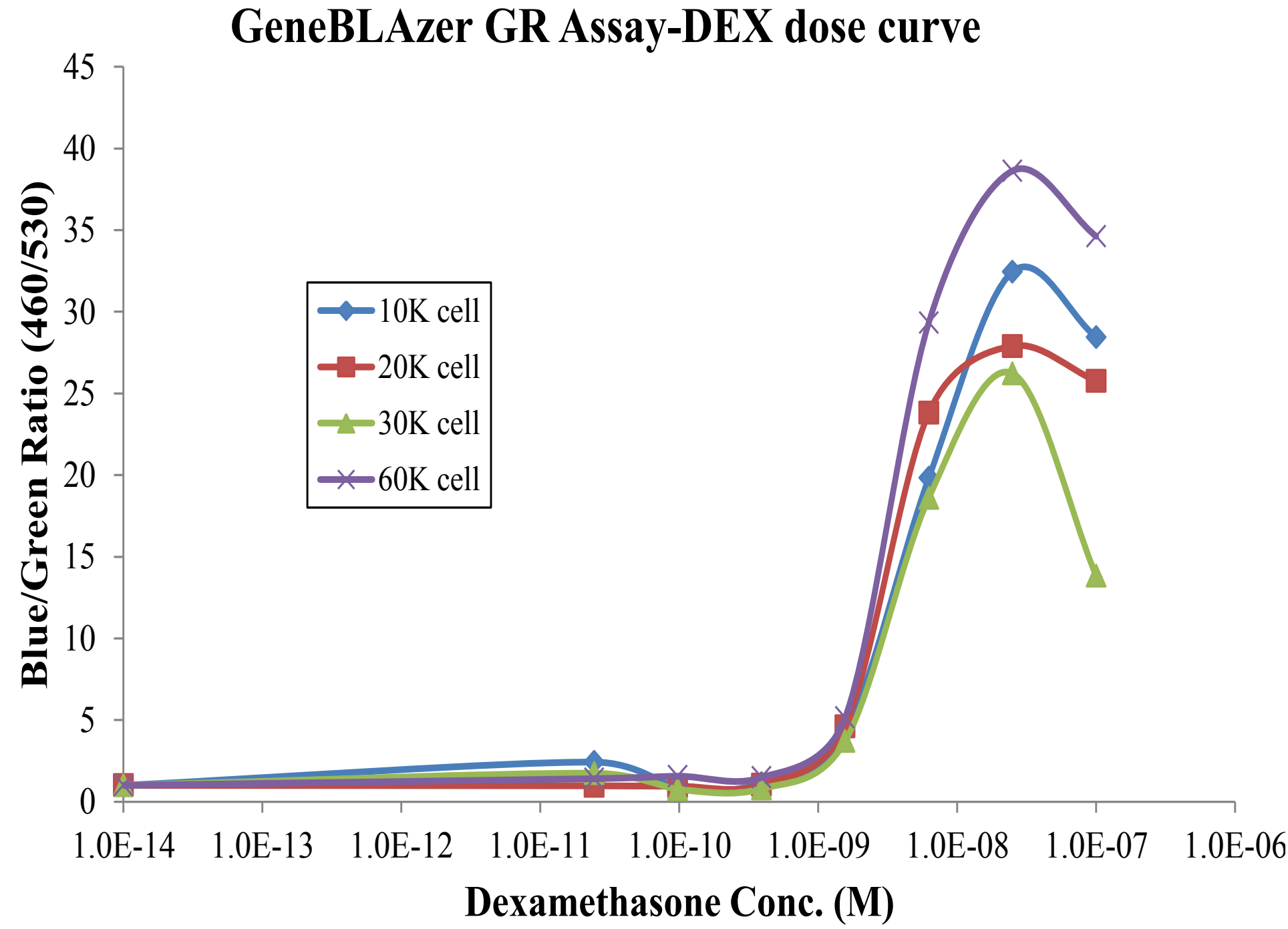
GeneBLAzer AR Assay - Synthetic androgen methyltrienolone (R1881) (AR Agonist) dose response with different amounts of cells per well



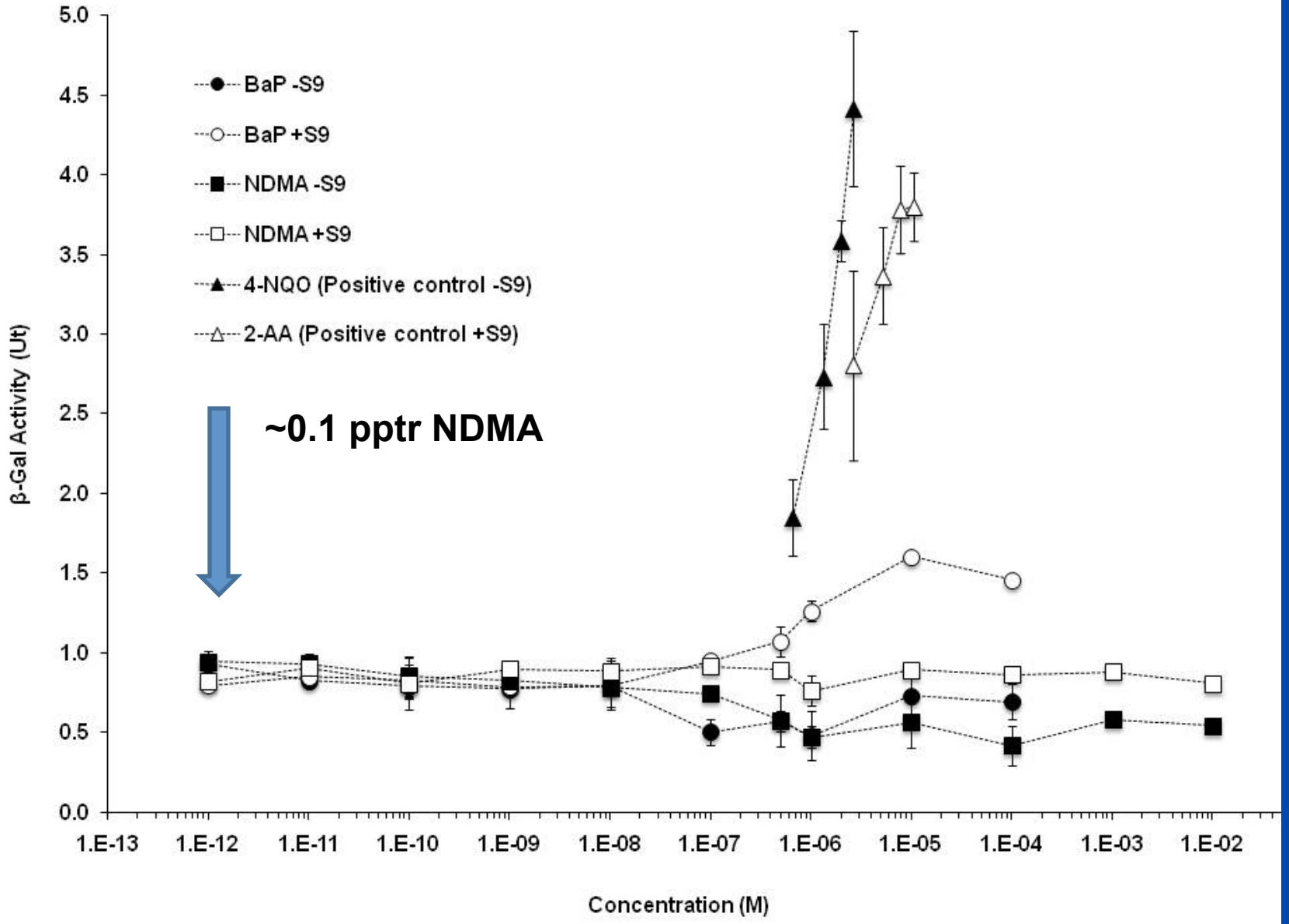

GeneBLAzer p53RE-bla HCT-116 cell based assay
Mitomycin dose response (relative fold change) with different amounts of cells per well
Relative fold change of Blue/Green Ratio (460/530 Ratio) (relative to vehicle control)
30K cells per well
40K cells per well
50K cells per well
60K cells per well
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1.E-14
1.E-13
1.E-12
1.E-11
1.E-10
1.E-09
1.E-08
1.E-07
1.E-06
1.E-05
1.E-04
Mitomycin concentration (M)

GeneBLAzer PR assay – Invitrogen
Progesterone and 19-norethindrone dose response

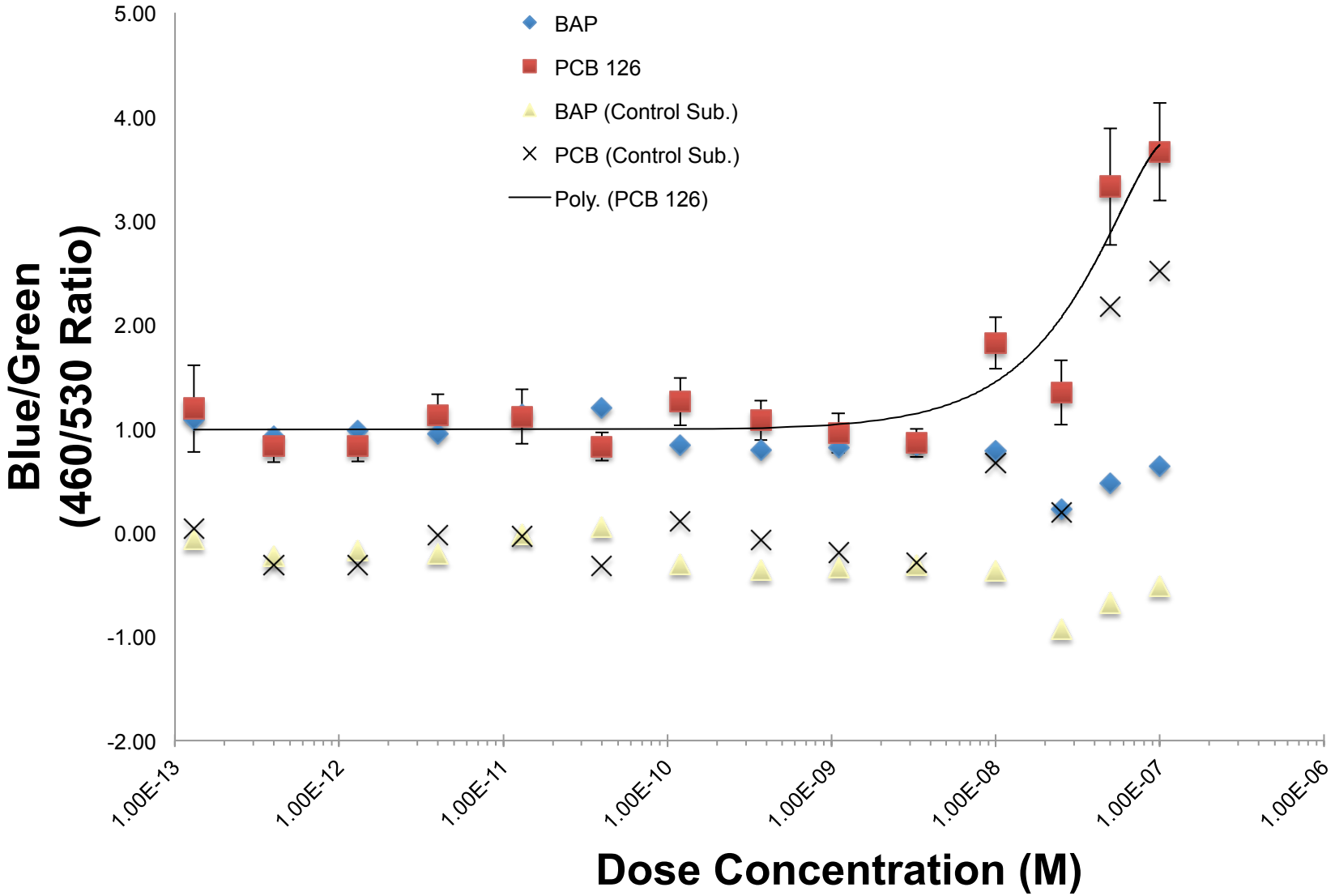



GeneBLAzer GR Assay-DEX dose curve
Blue/Green Ratio (460/530)
45
40
35
30
25
20
15
10
5
0
10K cell
20K cell
30K cell
60K cell
1.0E-14
1.0E-13
1.0E-12
1.0E-11
1.0E-10
1.0E-09
1.0E-08
1.0E-07
1.0E-06
Dexamethasone Conc. (M)

Dose response for genotoxicity (UMU) assay



GeneBLAzer AhR assay – Invitrogen
Benzo[a]pyrene and PCB 126 dose response



Next steps
DATA INTERPRETATION & GUIDANCE

- **Test Water extracts and Intercalibrattion**
- **Translate bioassay results into quantifiable threshold**
  - total equivalent concentrations or quotients (TEQs)
- **Investigate relationship to CEC concentrations & health based monitoring trigger levels**
  - compile reference doses or “TTCs” for known/measured CECs
- **Develop tiered framework that best utilizes bioassay results**
  - first tier screening tool
  - bioassay threshold exceedances that trigger appropriate response
- **Conduct workshop for stakeholders**
  - appropriate role, implementation and use of bioassay results

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