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# Current Status of Bioanalytical Methods

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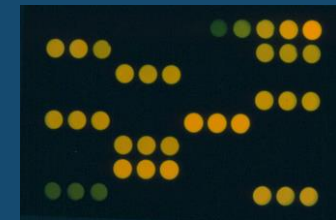
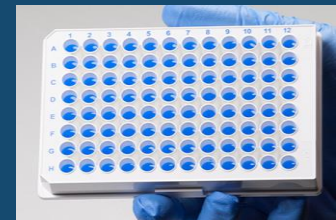
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# A 3-step process similar to targeted analysis

1. Extract water samples using solid phase extraction (SPE)
2. Perform bioanalytical (cell) assay
3. Analyze and report results: convert light intensity into a bioassay equivalent concentration (BEQ, ng/L)



# Standardized sample extraction methods are available

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- Consensus method is SPE using Oasis HLB (C-18)
- Standard protocols for targeted chemistry are sufficient
  - e.g. EPA Methods 1694 (PPCPs) (539 for hormones)
- Slight modifications include:
  - selected fortification (e.g. QA/QC matrix spike samples only)
  - final carrier solvent exchange to DMSO

# Standardized bioscreening methods for water quality

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- Commercially available technology have standard operating procedures (SOPs)
- Some assays have been validated in Europe (e.g. OECD, ISO)
- SOPs include detailed recommendations for
  - reference chemicals
  - vehicle solvent
  - plating instructions
  - incubation conditions, etc...

# Candidate ER- $\alpha$ transactivation assays

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- ER $\alpha$ -CALUX, BDS (Besselink 2015)
- ER $\alpha$  GeneBLAzer, LifeTechnologies (Mehinto 2016)
- BG1Luc ER TA assay (OECD TG455)
- HER $\alpha$  transactivation assay (ISO 19040-3)
- HER $\alpha$  assay, INDIGO Biosciences

# Candidate AhR transactivation assays

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- DR-CALUX, BDS (Besselink 2004)
- AhR CALUX, M. Denison (EPA Method 4435)
- AhR assay, INDIGO Biosciences

# Quality controls mirror that for targeted methods

- Living cells require an additional criterion (viability)

QA/QC parameter	Frequency of analysis	Acceptance Limits
Calibration	per batch	slope and EC50 within historical range; $R^2$ of sigmoidal curve > 0.95
Vehicle blank	per batch	vehicle-induced response within 25% RSD of response without vehicle
Precision	per sample	<30% RSD for triplicate measurements
Matrix spike	per batch	within 25% RSD of expected response
Cytotoxicity	per sample	>80% cell viability

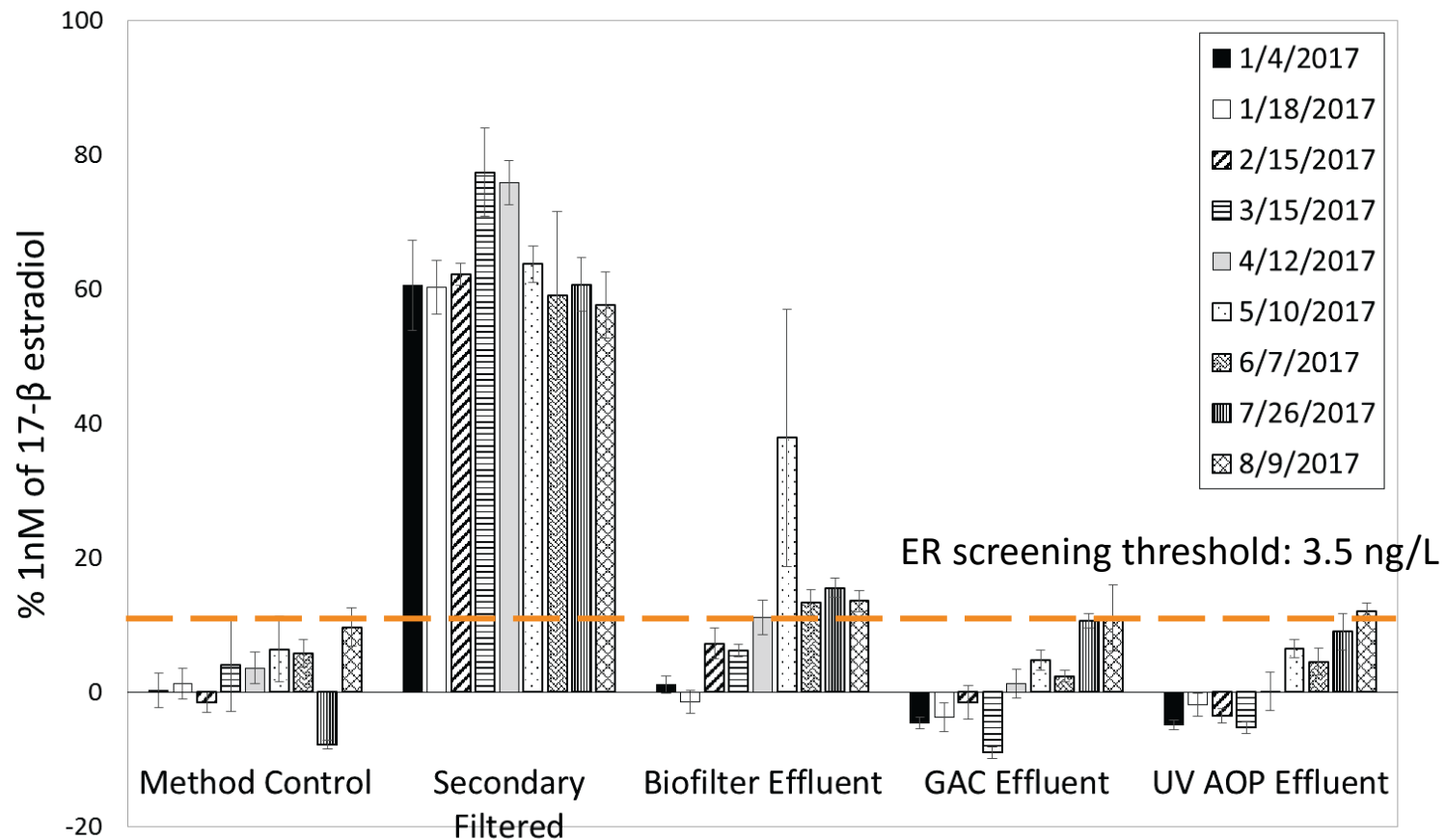
# Validation of ER- $\alpha$ and AhR for water quality

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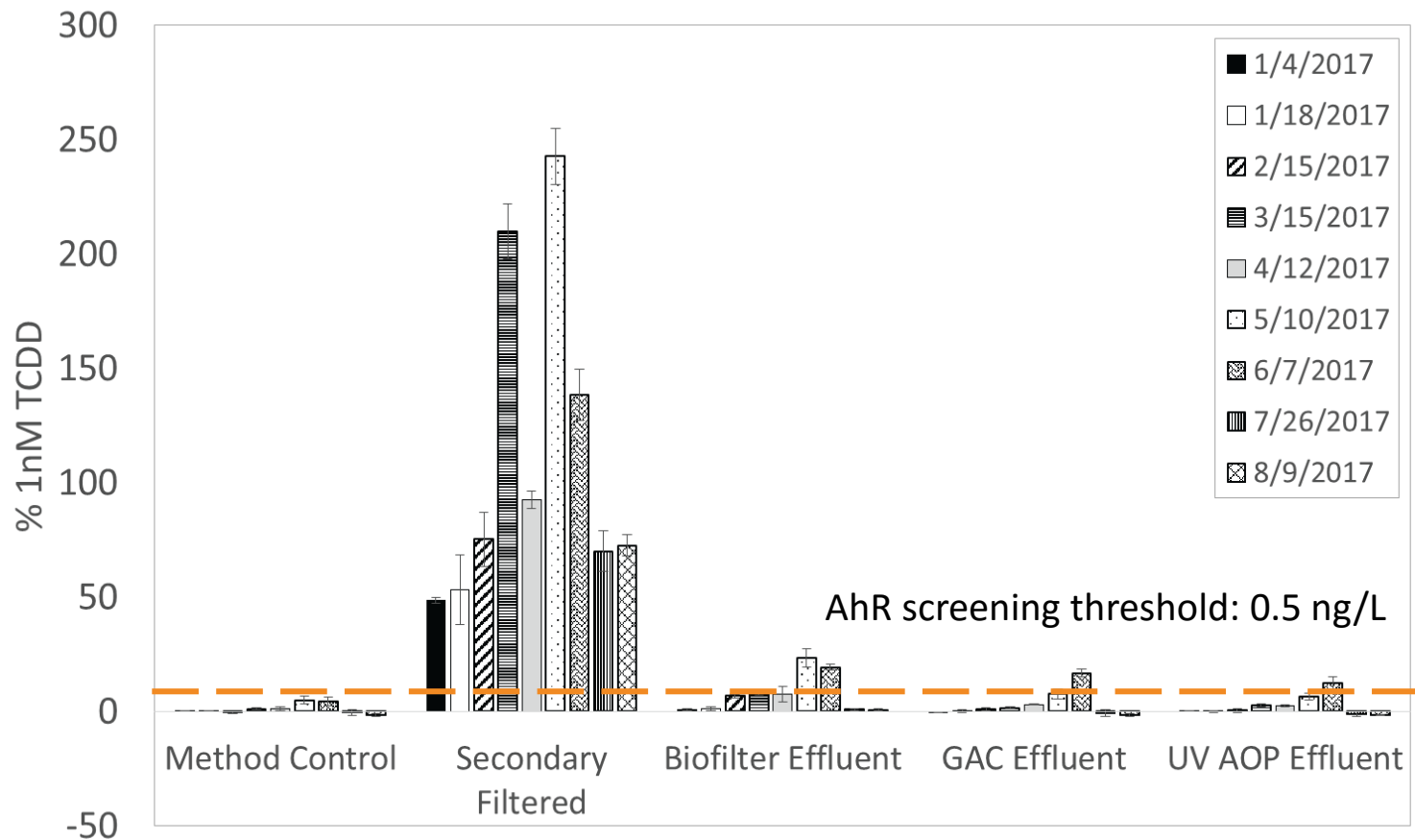
- Independent round-robin exercises
  - e.g. Besselink 2004, Escher 2014, Mehinto 2015, Kunz et al. 2017, Altenburger 2018
- Application - WWTP effluent, product water (RO, MF), surface water, drinking water
- ER- $\alpha$  and AhR results indicated adequate sensitivity and precision for benchmarking
- Comparability among different commercial cell lines/labs still needed



# Cell bioactivity reflects water quality / level of treatment



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# Implication / usage of bioscreening data

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- Bioscreening thresholds should be interpreted the same as MTLs for targeted CECs
- Full interpretive framework for bioscreening results is not ready for regulatory application
- Future development of bioanalytical monitoring should include rigorous evaluation of bioscreening thresholds

# Commercial services for bioanalytical monitoring

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- Limited for full service (sample extraction + analysis) – e.g. Biodetection System (BDS)
- More options using sequential (“2-lab”) approach
  1. Competent analytical lab for SPE extraction using modified EPA method
  2. Sample extracts shipped to cell assay lab – e.g. Life Technologies, INDIGOBiosciences, IonTox, BDS, etc.

# Guidance from technical experts

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- Advisory group recommended by the CEC Expert Panel to guide phased bioanalytical monitoring
- Can assist with:
  - selection of methods
  - identification of qualified service labs
  - validation and analysis of data

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# Questions?

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