Cell-Based Assays are Sensitive Tools for Quantifying the Cumulative Bioactivities of Endocrine-Disrupting and Polyaromatic Hydrocarbon Pollutants in Environmental Water Sources

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#18948

Abstract

Current protocols for water safety evaluation primarily focus on targeted chemical analysis, but this approach does not alert us to the presence of hazardous bioactivities from non-target chemical contaminants. Importantly, the cumulative bioactivities from low concentrations of numerous mixed chemical pollutants cannot be predicted using targeted analytical approaches. As a complement to analytical chemistry approaches, mammalian cell-based assays have been developed to provide a sensitive assessment of potential adverse effects from chemical contaminants in water such as endocrine-disruption (ED). Herein we demonstrate the utility of new cell-based luciferase reporter assays encompassing chemical-sensing receptors that are directly relevant to environmental biomonitoring applications. These include the polycyclic aromatic hydrocarbon (PAH) sensing Aryl Hydrocarbon Receptor (AhR), and the ED targets Androgen Receptor (AR), Estrogen Receptor (ER), Glucocorticoid Receptor (GR), and the Mineralocorticoid Receptor (MR). Environmentally relevant reference ligands for each receptor were tested in the assays, evaluated for sensitivity, and compared to current or suggested monitoring trigger levels (MTLs). Surface water samples collected from nine sites in the Spruce Creek watershed in Central Pennsylvania were tested in these assays. The assay data were compared to targeted chemical analyses of the sites to determine if the assays were able to detect biological activities that the chemical data would not have predicted. The results demonstrate that the limit of detection (LOD) for each assay is consistent with proposed MTLs presented in the literature. All samples exhibited detectable agonist activities for AhR, AR, ER, and MR, but minimal to no activity for GR. The comparison between bioassay and chemical analysis data demonstrated that using both methods together provide a better representation of the quality of the water samples, and that chemical analysis alone cannot predict the accumulated bioactivities revealed by the bioassays. These results demonstrate the utility and sensitivity of function-based assays for generating comprehensive water quality assessments. Bioassays provide reliable, actionable data that inform the decision-making process as to the need and extent of remediation required for a given water source.

Materials and Methods

Sample collection and processing: Water samples were collected on one day in August 2021 from nine sites in the Spruce Creek watershed in central Pennsylvania, plus one field blank. These groundwater collection sites represent mixed use water sources (forested 59%, agriculture 35%, developed 6%). Water samples were processed before bioassay analyses *via* solid-phase extraction (SPE) using 200 mg Oasis® HLB sorbents (Waters Corp.) and elution solvent mixes, as described by Vanden Heuvel, *et. al.* (Science of the Total Environment, manuscript in preparation).

Bioassay setup: Luciferase reporter assays were developed and optimized in mammalian cell lines for the following receptors: AhR, AR, ER, GR, and MR. Figure 1 depicts the setup and workflow of the five environmental bioassays.

Assay data analyses: For each assay a standard curve was generated using an environmentally relevant reference analyte. Each assay's limit of detection (LOD) was determined using an unpaired, two-tailed t-test to assess changes in RLU values between the vehicle-treated cells and the low-concentration reference agonist-treated cells. Significance was set at $p \leq 0.01$. Field Blank RLU values were used for background-subtraction from the Test Sample RLU values. For each Test Sample, values of Percent Relative Activation were calculated by normalizing RLU's to the maximal RLU value achieved by each receptor's reference agonist (= 100%). Respective standard curves were then used to interpolate bio-equivalent (BEQ) concentrations of each test sample.

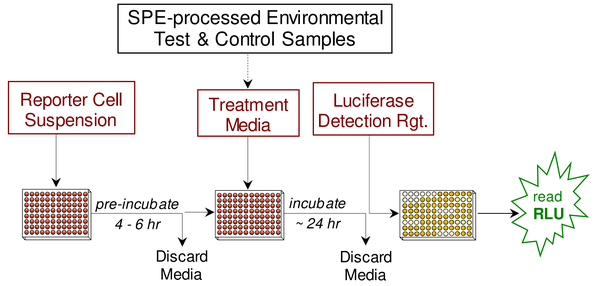

Figure 1: Bioassay setup and workflow. Assay protocols were performed as prescribed by INDIGO Biosciences (State College, PA, USA)

Chemical analyses: SPE-processed water samples were also analyzed for chemical contaminants including pharmaceuticals, pesticides, and personal care product components. Analyses were conducted using high-resolution accurate mass (HRAM) Q Exactive mass spectrometer interfaced with an ICS-5000+ chromatography system *via* a heated electrospray injection source, as described in Vanden Heuvel, *et. al.* (Science of the Total Environment, manuscript in preparation).

References: *California State Water Resources Control Board, Water Quality Control Policy for Recycled Water, Adopted December 11, 2018.
#Values summarized in Neale, *et. al.*, (2023) Effect-Based Trigger Values Are Essential for the Uptake of Effect-Based Methods in Water Safety Planning. Environmental Toxicology and Chemistry. 42: 714-726. Values depicted are those for Human EBT-BEQ (drinking and recycled water for indirect potable reuse).

Results and Conclusions

Figure 2

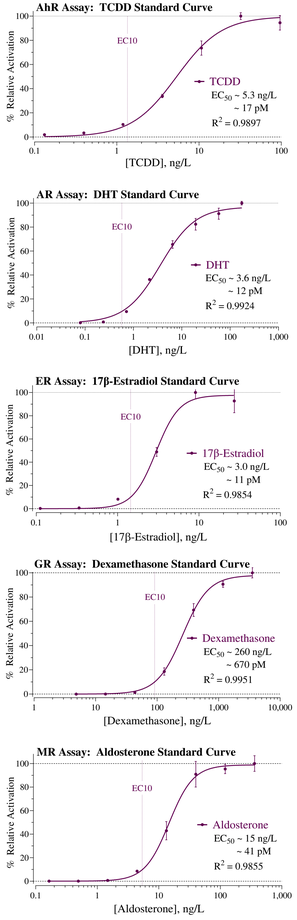

Figure 3

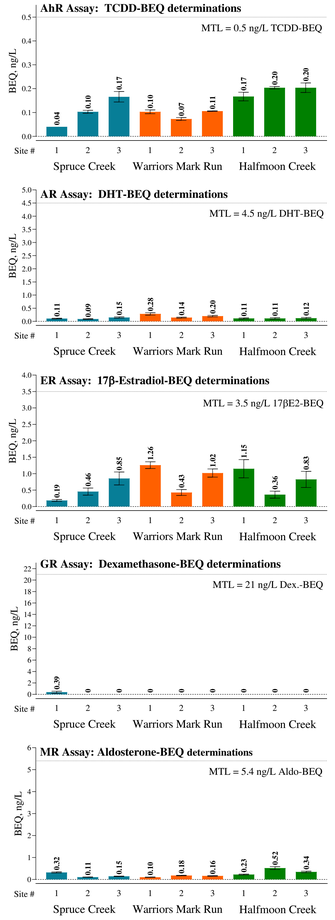

Figure 2: Standard Curves for the AhR, AR, ER, GR and MR bioassays. Dose-response curves are generated using environmentally-relevant reference agonists.

Figure 3: BEQ determinations for water samples using the AhR, AR, ER, GR, and MR bioassays. The Standard Curves depicted in Figure 2 were used to extrapolate respective BEQ values for each field sample tested. MTL values for each reference are depicted and summarized in Table 1. For the MR assay the EC_{10} value for Aldosterone is used as the MTL. Field sites that yield test sample BEQ values exceeding the MTL value require further investigation and potential remediation.

Unit of concentration		ng/L			
Assay	Reference Compound	EC_{50}	EC_{10}	LOD	MTL
AhR	TCDD	5.3	1.3	0.40	0.5*
AR	DHT	3.6	0.58	0.24	4.5 - 32#
ER	17β-Estradiol	3.0	1.4	1.0	3.5*
GR	Dexamethasone	260	90	15	21 - 150#
MR	Aldosterone	15	5.4	0.49	*na*

Table 1: Summary of Environmental Bioassay Metrics.

Assay metrics for the AhR, AR, ER, GR and MR assays are depicted using the reference agonists indicated.

na = not available
TCDD = 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
DHT = dihydrotestosterone

Stream Location	Field	Spruce Creek			Warriors Mark Run			Halfmoon Creek				
Site#	Blank	1	2	3	1	2	3	1	2	3		
Chemical name											Class	Type
Acetaminophen	0.0717	0.0059	0.0027	0.0010	0.0023	0.0066	0.0015	0.0031	0.0029	0.0008	Personal Care	NSAID
Benzophenone-1	0.0025	0.0001	0.0001	0	0.0001	0	0	0	0.0002	0	Personal Care	Sunscreen
Benzophenone-3	0.0106	0.0032	0.0001	0.0007	0	0.0001	0.0004	0.0001	0	0.0001	Personal Care	Sunscreen
Caffeine	0.0007	0.0061	0.0213	0.0124	0.0056	0.0063	0.0070	0.0298	0.0031	0.0217	Personal Care	Stimulant
DEET	0.0002	0.0004	0.0006	0.0013	0.0004	0.0011	0.0003	0.0017	0.0006	0.0016	Personal Care	Insect repellant
Ibuprofen	0	0.192	0.956	0	0.142	0.077	0	1.24	0	0.191	Personal Care	NSAID
Ketoprofen	0	0.0001	0	0.0009	0.0001	0	0.0001	0.0001	0.0001	0.0001	Personal Care	NSAID
Naproxen	0	0.0009	0.0005	0.0007	0.0003	0.0006	0.0011	0.0003	0.0009	0.0013	Personal Care	NSAID
Sucralose	0.0005	0	0.0004	0	0	0	0	0	0	0	Personal Care	Sweetener
Theobromine	0.0005	0.0034	0.0114	0.0075	0.0038	0.0042	0.0076	0.022	0.0036	0.0159	Personal Care	Cosmetics
Atrazine	0.0008	0.0691	0.0425	0.0357	0.0266	0.0248	0.0469	0.0203	0.0286	0.0213	Pesticide	Herbicide
Carbaryl	0	0.0036	0.0035	0.0041	0	0	0	0	0	0.0002	Pesticide	Carbamate insecticide
Chlorpyrifos	0.0146	0.0078	0.0040	0.0042	0.0024	0.0009	0.0012	0.0011	0	0.0007	Pesticide	Organophosphate insecticide
Clothianidin	0	0.0043	0.0033	0.0046	0.0045	0.0046	0.0073	0.0025	0.0087	0.0042	Pesticide	Neonicotinoid insecticide
Cyhalothrin	1.17	0	0	1.30	0	0	0	1.30	0	0	Pesticide	Pyrethroid insecticide
Esfenvalerate	0.991	0	1.02	1.02	1.02	1.02	0	1.02	1.02	1.02	Pesticide	Pyrethroid insecticide
Imidacloprid	0	0.0028	0.0052	0.0109	0.0019	0.0017	0	0.0091	0	0.0085	Pesticide	Neonicotinoid insecticide
Malathion	0.0008	0.0006	0.0001	0	0	0	0	0	0	0	Pesticide	Organophosphate insecticide
Permethrin	3.15	3.14	3.14	3.15	3.15	3.15	3.15	3.15	3.15	3.15	Pesticide	Pyrethroid insecticide
Simazine	0.0002	0.0029	0.0026	0.0032	0.0020	0.0025	0.0015	0.0019	0.0024	0.0022	Pesticide	Herbicide
Ampicillin	0	0	0	0.0025	0.0008	0	0	0.0075	0	0.0027	Pharmaceutical	Antibiotic
Atenolol-MRT	0	0.0115	0	0	0	0	0	0	0	0	Pharmaceutical	Beta Block
Buspirone	0	0.0129	0.0021	0.0014	0.0010	0	0	0	0	0	Pharmaceutical	Antianxiety
Carbamazepine-A	0.0004	0.0001	0.0076	0.0035	0.0016	0.0013	0	0.0298	0	0.0195	Pharmaceutical	Anticonvulsant
Chlortetracycline	0.0007	0.0021	0.0017	0.0016	0.0029	0.0014	0.0021	0.0018	0	0	Pharmaceutical	Antibiotic
Citalopram	0	0.0194	0.0001	0.0001	0.0003	0.0001	0.0006	0.0002	0.0001	0.0003	Pharmaceutical	Antidepressant
Clarithromycin	0.0003	0.0020	0	0	0	0	0	0	0	0	Pharmaceutical	Antibiotic
Erythromycin	0	0.0002	0	0	0	0	0	0	0	0	Pharmaceutical	Antibiotic
Fluoxetine	0.632	0.631	0.633	0.634	0.634	0.634	0	0.634	0.634	0.634	Pharmaceutical	Antidepressant
Metformin	0.0001	0.0007	0.0001	0.0002	0.0002	0.0001	0.0001	0	0	0	Pharmaceutical	Antidiabetic
Metoprolol	0.0001	0.0038	0.0018	0	0.0023	0.0030	0.0017	0.0037	0	0.0008	Pharmaceutical	Blood pressure
Ofloxacin	0	0.0239	0.0132	0.0017	0.0029	0.0014	0.0003	0.0009	0.0001	0	Pharmaceutical	Antibiotic
Oxytetracycline	0	0.0020	0.0013	0.0006	0.0016	0.0018	0.0033	0.0015	0.0015	0.0036	Pharmaceutical	Antibiotic
Sulfadiazine	0	0.0003	0	0	0	0	0	0	0	0	Pharmaceutical	Antibiotic
Sulfadimethoxine	0	0.0001	0	0	0	0	0	0	0	0	Pharmaceutical	Antibiotic
Sulfamethazine	0	0	0	0	0	0	0.0004	0	0	0	Pharmaceutical	Antibiotic
Sulfamethoxazole	0	0.0021	0.0005	0.0001	0	0	0.0001	0.0011	0	0.0007	Pharmaceutical	Antibiotic
Tetracycline	0	0.0012	0.0007	0	0	0	0	0	0	0	Pharmaceutical	Antibiotic
Trimethoprim	0.0001	0.0170	0.0027	0.0010	0.0009	0.0002	0.0004	0.0056	0.0003	0.0042	Pharmaceutical	Antibiotic
Venlafaxine	0	0.0057	0.0024	0.0014	0.0009	0.0005	0.0009	0.0046	0.0003	0.0034	Pharmaceutical	Antidepressant

Table 2: Chemical analyses of water test samples.

Concentrations of chemicals detected in the water samples are presented as mg/L; values are reported as ≤ 3 significant figures.

Also analyzed but not detected in any sample:

4-t-Octylphenol
β-cyfluthrin
Bifenthrin
Thiacloprid
Thiamethoxam,
Thiamethoxam-d3
Amoxicillin

Conclusions:

- Bioassays are sensitive tools for detecting synergistic activities of complex chemical mixtures in environmental water samples.
- Targeted chemical analysis alone cannot predict the activities observed in the bioassays.
- Combined data from cell-based bioassays and targeted chemical analysis provide a broader picture of water sample quality, leading to more effective decision making.