Workplan for SWAMP project "Screening for Emerging Contaminants in the Santa Ana Region"

Alvina Mehinto (Southern California Coastal Water Research Project)

Project Scope and Objectives

Constituents of emerging concern (CECs) are a diverse group of unregulated and unexpected chemicals (including pharmaceuticals, household products, pesticides, and their metabolites) for which limited chemistry and toxicity data are available. Therefore, assessing the effects of the exposure to CECs on overall biological conditions can be challenging. Bioanalytical screening tools, also known as cell bioassays, have been proposed in water quality assessment frameworks to monitor known and unexpected chemicals exerting a common biological activity (Poulsen et al. 2011, Leusch and Snyder et al. 2015, Maruya et al. 2016). This novel approach could enhance current assessment practices by providing an integrated measure of all bioactive chemicals that may impact aquatic health. Previous studies in southern California habitats demonstrated that cell bioassays can serve as rapid screening tools to identify contaminated sites and infer toxicity potential in aquatic organisms (Crago et al. 2016, Mehinto et al. 2017, Maruya et al. 2018).

The objectives of this study are to (a) measure bioactivity levels of CECs in surface water from sites within the Santa Ana region using a series of cell bioassays; and (b) determine concentrations of known bioactive CECs in the water column.

Experimental Approach

Study sites and Sampling protocols

Candidate sites (20 - 25) for this study are listed in Table 1. The study sites were selected to reflect varying water qualities in Region 8. Each site will be sampled twice - once during dry season (May-Sept 2021) and once during wet season (Nov 2021-Feb 2022). Wet season samples will not be collected during storm events. For each site, GPS coordinates, water temperature, dissolved oxygen, pH, and salinity will be recorded.

Water samples will be collected in areas with good flow and minimal suspended solids by directly immerging the sampling container below the surface. In area with reduced flow, a collection vessel, previously cleaned with methanol and deionized water, will be used to collect the samples. The containers will be filled to the top, inverted 2-3 times to ensure that any preservative agents are completely dissolved, and stored immediately on ice and in the dark. For cell assay, 2-L water samples will be collected at all sites. A predefined subset of sites will be sampled for chemical analyses (~10 sites). A summary of the sampling procedure is provided in Table 2.

SCCWRP will coordinate with relevant programs for sampling and provide all necessary equipment (incl. pre-labelled sampling bottles, coolers for storage and shipping, field sheets).

Table 1. Candidate study sites prioritized by Santa Ana RWQCB (Region 8)

Waterbody_Name	StationCode	Latitude	Longitude	Description of location
San Diego Creek	801SDC-	33.65556	-117.845	San Diego Creek @Campus -above
Santa Ana Delhi Channel	801RB8549	33.66013	-117.881	Reach 1; at Irvine Ave
San Jacinto Wildlife Preserve		33.89727	-117.108	near pond E-1
Cucamonga Creek	801S15677	33.9545	-117.604	SB Flood; Helman ave W side does not have fencing
San Jacinto River	802S35837	33.7364	-116.826	SJR, R7; near 802SJCREF San Jacinto River - Reference Site for SPoT program
San Jacinto River	802S29064	33.7956	-116.743	San Bernardino NF;Take HWY 243 from Pine Cove, no sign of road closures
Strawberry Creek	802S11394	33.71296	-116.768	near Hwy 74
Temescal Creek	801S18169	33.8918	-117.572	Temescal Creek Reach 1a - Lincoln Ave. to Arlington Channel confluence
Temescal Creek	801RB8400	33.83012	-117.509	Temescal Creek, Reach 2
Temescal Creek	801RB8439	33.86874	-117.534	Temescal Creek, Reach 1b
Santa Ana River	801PFB019	33.92417	-117.598	Santa Ana River at River Rd; SAR, R3
Big Bear Lake	801BBL673	34.26009	-116.885	Big Bear Lake, Stanfield Cutoff northern end
Big Bear Lake	801BBL678	34.24529	-116.973	Big Bear Lake, Pine Oak Lane North Beach
Barton Creek	801WE1008	34.18128	-116.912	SBNF; Glass Rd to 1N86; ~1.4 mi. NW of Barton Flats campground
Mill Creek	801S00135	34.07819	-116.879	San Bernardino NF; Falls Rd near Vivian Crk trail
Mill Creek Canyon	801M15591	34.08539	-117.055	access from Mill Crk Rd HWY 38, trails and dirt rds
City Creek	801WE1043	34.1729	-117.181	If parking difficult, use 34.171431, -117.181252; City Creek (Mtn reach)
Santa Ana River	801S02059	34.06182	117.30727	SAR, R4; SB Flood; public access santa ana river trail, park at mt. vernon ave
Chino Creek	801M15560	33.943881	-117.659452	Prado Regional parks/golf course;Chino Creek Reach 1B; w/in Prado Basin Management Zone
Little Chino Creek	801M15581	33.986338	-117.713613	Not a named creek in our BP; San Bernardino Flood; Access from W side of Pipeline and no ladder needed; unavoidable chainlink
Plunge Creek	801S00375	34.10902	-117.15272	SB Flood control; off Greenspot Rd, short barbwire fencing
Fredalba Creek	801FDCCCR	34.17	-117.12977	Fredalba Creek above City Creek Road
Mountain Home Creek	801MHC219	34.11304	-116.986588	at junction of Mtn Home Creek, East Fork

Table 2. Sampling equipment and procedures for water samples collection

Sample type	Unfiltered grab sample from area with good flow and minimal suspended particles. Sample shall not be collected during rain event/storm.
Bottle/volume	For cell assay: 2 L in amber glass bottle previously rinsed with methanol and ultrapure water. Preservatives added include 1 g/L sodium azide to prevent microbial activity and 50 mg/L sodium thiosulfate if sample contains chlorine residues. For chemistry: 250-500 mL bottle provided by Eurofins Eaton Analytical, LLC. Bottle type and preservatives will vary depending on analytes targeted. All bottles must be labeled with site code/name, date & time, sampling agency name and staff initials.
Direct sampling (preferred method)	Stand down-current and fill sample bottle by immersing just below surface (be careful not to flush out the preservatives). Fill bottle completely to the top to exclude air. Cap tightly and invert bottle several times to dissolve the preservative agents.
Indirect sampling (if applicable)	Collection equipment (e.g. bucket, funnel) must be cleaned 2-3 times with milliQ water and protected with foil or plastic during transport. At collection site, equipment must be rinsed with site water, and final sample must be collected upstream of the 'cleaning' site. Fill bottle completely to the top to exclude air. Invert bottle several times to dissolve the preservative agents.
QA samples	2 L field blank by transferring ultrapure water in the bottle using appropriate collection equipment. Collect one blank per agency or one blank for every 15 samples. One duplicate sample and one matrix spike (spike will be added in the lab).
Field measurements	Record GPS coordinates, time/date of sampling, water quality parameters (pH, temperature, dissolved oxygen, salinity when applicable). Record streamflow when applicable.
Storage condition	Immediately place sealed sample at 1 - 4°C on ice in the dark. Transfer to the refrigerator at 4°C in the lab.
Holding time	For cell assay, sample should be processed by SPE within 72 hours. For chemistry, holding time will vary between 1 and 4 weeks depending on the analyte of interest.

Note: Field crew should **avoid** contact with products that contain known CECs such as **detergents, fragrances, sunscreen, and pharmaceuticals**. Sampling bottle shall be handled with gloves at all time and opened only for sample.

Cell Bioassay Screening

Water samples will be processed within 72 hours post collection using solid phase extraction (SPE) protocols described in Denison et al. (2020). Briefly, water samples will be pre-filtered using 1.6 µM glass-fiber filters and passed through pre-conditioned Oasis HLB 6cc cartridges (200 mg). After elution of the cartridges using 10 mL of methanol and 10 mL of acetone:hexane (1:1, v/v), the sample extracts will be concentrated, and solvent exchanged to 0.5 mL DMSO. Final extracts will be stored in amber glass vials at -20C until subsequent analyses.

Cell bioscreening will be performed using the GeneBLAzer estrogen receptor alpha (ERa), glucocorticoid receptor (GR) and aryl hydrocarbon receptor (AhR) cell bioassays as described in Mehinto et al. (2021). These endpoints were selected based on their ability to screen priority CECs and/or their potential to screen for adverse effects in aquatic life (Table 3). Briefly, division-arrested HEK 293T cells (for ERα, GR) or LS180 immortal cells (for AhR assay) will be seeded into 96-well plates and exposed to serial dilutions of water extracts. After 16 h incubation at 37°C and 5% CO2, LiveBLAzer-FRET loading substrate and PrestoBlue solution will beaded to each well, and assay data will be collected using a Synergy H1 Hybrid plate reader (BioTek). Receptor bioactivity will be measured at 409/460 nm and 409/530 nm (excitation (Ex)/emission (Em) wavelength), and cell viability at 560 Ex/590 Em nm. Assay-specific calibration curves will be generated and used to convert the results in 17β-estradiol equivalent (ng E2/L) for ERα, dexamethasone equivalent (ng DEX/L) for GR, and 2,3,7,8-tetrachlorodibenzodioxin equivalent (ng TCDD/L) for AhR bioassays.

Table 3: Cell bioassays of interest

Endpoint activity	**Relevant CEC classes**	**Potential biological impact**
Estrogen receptor (ER)	Estrogens, bisphenols, alkylphenols	Impaired reproduction
Aryl hydrocarbon receptor (AhR)	Dioxin-like chemicals, pesticides	Tumor, tissue lesions
Glucocorticoid receptor (GR)	Anti-inflammatory drugs	Compromised immune system

Analytical Chemistry

Water samples (0.25-1 L depending on the analytes of interest) will be shipped on ice to Eurofins Ltd and analyzed for a suite of contaminants including pharmaceuticals and personal care products, pesticides, and surfactants. The volume of water and extraction procedure will depend on the analytes targeted. Chemical analyses will be conducted using a combination of in-house protocols and standardized EPA methods whenever available. Table 4 below provides the list of analytes of interest and analytical method parameters including and extraction, instrument and method detection limit.

Table 4. Analytical chemistry methods to measure target chemicals

Analyte	Extraction method	Instrument used	Method name	RL Water
17a ethinyl estradiol	SPE	LC/ESI-MS/MS	Hormones - EPA 539	10 ng/L
17b estradiol	SPE	LC/ESI-MS/MS	Hormones - EPA 539	10 ng/L
Estrone	SPE	LC/ESI-MS/MS	Hormones - EPA 539	10 ng/L
Estriol	SPE	LC/ESI-MS/MS	Hormones - EPA 539	10 ng/L
Testosterone	SPE	LC/ESI-MS/MS	Hormones - EPA 539	10 ng/L
Bifenthrin	SPE	GC/MS/MS	PBDEs & Pyrithroids	5 ng/L
Chlorpyrifos	SPE	GC/MS/MS	PBDEs & Pyrithroids	10 ng/L
Fipronil	SPE	GC/MS/MS	PBDEs & Pyrithroids	2 ng/L
Galaxolide	SPE	GC/MS/MS	PBDEs & Pyrithroids	10 ng/L
Permethrin (total)	SPE	GC/MS/MS	PBDEs & Pyrithroids	10 ng/L
PBDE-47	SPE	GC/MS/MS	PBDEs & Pyrithroids	5 ng/L
PBDE 99	SPE	GC/MS/MS	PBDEs & Pyrithroids	5 ng/L
4-Nonylphenol (semiquant)	Online SPE	HPLC/ESI-MS/MS	PPCPs	400 ng/L
Bezafibrate	Online SPE	HPLC/ESI-MS/MS	PPCPs	5 ng/L
Bisphenol A	Online SPE	HPLC/ESI-MS/MS	PPCPs	10 ng/L
Carbamazepine	Online SPE	HPLC/ESI-MS/MS	PPCPs	5 ng/L
Diclofenac	Online SPE	HPLC/ESI-MS/MS	PPCPs	5 ng/L
Fluoxetine	Online SPE	HPLC/ESI-MS/MS	PPCPs	10 ng/L
Gemfibrozil	Online SPE	HPLC/ESI-MS/MS	PPCPs	5 ng/L
Ibuprofen	Online SPE	HPLC/ESI-MS/MS	PPCPs	25 ng/L
Ketoprofen	Online SPE	HPLC/ESI-MS/MS	PPCPs	5 ng/L
Naproxen	Online SPE	HPLC/ESI-MS/MS	PPCPs	20 ng/L
Progesterone	Online SPE	HPLC/ESI-MS/MS	PPCPs	10 ngL
Triclosan (semiquant)	Online SPE	HPLC/ESI-MS/MS	PPCPs	30 ng/L
Perfluorooctanoic acid	SPE	LC/MS/MS	PFAS - EPA 537.1	2 ng/L
Perfluorooctane sulfonate	SPE	LC/MS/MS	PFAS - EPA 537.1	2 ng/L
Copper	Acid Digestion	ICP/MS	Metals - EPA 200.8	2 ug/L
Lead	Acid Digestion	ICP/MS	Metals - EPA 200.8	0.5 ug/L
Zinc (dissolved only)	Filtration + Acid Digestion	ICP/MS	Metals - EPA 200.8	20 ug/L
Mercury	Acid Digestion	CV/AA	Mercury - EPA 245.1	0.2 ug/L

Quality Assurance/ Quality Control Measures

A performance-based QA/QC approach, adapted from the Statewide CEC Pilot Study Guidance (Dodder et al. 2015), will be followed to ensure analytical and bioanalytical data of the highest quality. These quality control measures are consistent with those described in the NWRI Bioanalytical Implementation guidance document (Denison et al. 2020). Data quality for cell bioassays will be validated against criteria for calibration, blank, matrix spikes, DMSO control, cytotoxicity (cell viability) and sample dose response (see Table 5). Targeted chemistry data will be validated against criteria for instrument calibration, analysis of blanks, matrix spikes and duplicate samples (see Table 6)

Table 5. Quality objectives for cell bioassays (adapted from Dodder et al., 2015 and Denison et al. 2020)

Measurement Parameter	Frequency of Analysis	Control Limits
Extract Cytotoxicity	Per sample extract	Dilutions of the extract shall not cause > 20% cell mortality
Cell-Free Media Blank	Per assay plate	Average response for media only wells shall be < 15% of sample response RSD of replicate wells shall be < 15%.
Vehicle Blank Response	Per assay plate	Average response of cells exposed to vehicle shall be within 25% RSD of response for unstimulated cells
Initial Calibration	Per bioanalytical batch	Linear dose-response curve for reference toxicant; $r^2 > 0.95$. Minimum of 9 points per curve (one of them at or below sensitivity threshold).
Calibration Verification	Per subsequent assay plates within a bioanalytical batch	Continuing calibration shall remain within 20% of mean response for initial calibration.
Repeatability	All samples	Triplicate measurements shall be within 20% RSD

Table 6. Quality objectives for target analytes in water (adapted from Dodder et al., 2015)

Measurement	Frequency	Control Limit
Initial Calibration	A new response factor or calibration curve should be established for each instrumental batch.	Correlation coefficient $r^2 \geq 0.95$ for linear and non-linear curves.
Continuing Calibration Verification	Per 24 hours	Expected concentration ± 20%.
Method Blank	5% of total no. samples (1 per batch of 20 samples)	Less than the RL for target analytes.
Sample Duplicate	5% of total no. samples (1 per batch of 20 samples)	RPD ≤ 35%.
Certified Reference Material or Laboratory Control Sample	5% of total no. samples (1 per batch of 20 samples)	70-130% recovery if certified; otherwise, 50-150% recovery.
Spiked Standard Recovery	All field and QC samples	50-150% or based on historical laboratory control limits.

References

Crago J, Xu EG, Kupsco A, Jia F, Mehinto AC, Lao W, Maruya KA, Gan J, Schlenk D (2016). Trophic transfer and effects of DDT in male hornyhead turbot (Pleuronichthys verticalis) from Palos Verdes Superfund site, CA (USA) and comparisons to field monitoring. Environmental Pollution 213: 940-948.

Denison M, Mehinto A, Olivieri A, Plumlee M, Schlenk D, Thompson S, Waggoner C (2020). Bioanalytical tools for detection and quantification of estrogenic and dioxin-like chemicals in water recycling and reuse. Guidance document for developing a standard operating procedure. National Water Research Institute, prepared for Water Reuse California.

Dodder NG, Mehinto AC, Maruya KA (2015). Monitoring of constituents of emerging concern (CECs) in California's aquatic ecosystems - Pilot study design and QA/QC guidance. Southern California Coastal Water Research Project Authority, Technical Report 854.

Leusch FDL, Snyder SA (2015). Bioanalytical tools: Half a century of application for potable reuse. Environmental Science: Water Research and Technology 1: 606-621.

Maruya KA, Dodder NG, Mehinto AC, Denslow ND, Schlenk D, Snyder SA, Weisberg SB (2016). A tiered, integrated biological and chemical monitoring framework for contaminants of emerging concern (CECs) in aquatic ecosystems. Integrated Environmental Assessment and Management 12: 540-547.

Maruya KA, Mehinto AC, Lao W, Sutton R, Jabusch T, Sun J, Lin D, Davis J, Fadness R (2018). Pilot monitoring of constituents of emerging concern (CECs) in the Russian River watershed (Region 1). Technical Report 1020. Southern California Coastal Water Research Project. Costa Mesa, CA.

Mehinto AC, VanDervort DR, Lao W, He G, Denison MS, Vliet SM, Volz DC, Mazor RD, Maruya KA. 2017. High throughput in vitro and in vivo screening of inland waters of Southern California. Environmental Science: Processes & Impacts 19: 1142-1149.

Mehinto AC, Schoenfuss HL, Wenger E, Diehl D, Bay SM (2021). Application of an effects-based monitoring strategy to assess the impact of contaminants on fish health in an urbanized watershed. Environmental Toxicology and Chemistry 40: 402-412.

Poulsen A, Chapman H, Leusch F, Escher B (2011). Application of bioanalytical tools for water quality assessment. Urban Water Security Research Alliance Technical Report No. 41.