SOUTHERN CALIFORNIA COASTAL WATER RESEARCH PROJECT AUTHORITY

THEMATIC RESEARCH PLAN

FOR

CONTAMINANTS OF EMERGING CONCERN

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Introduction

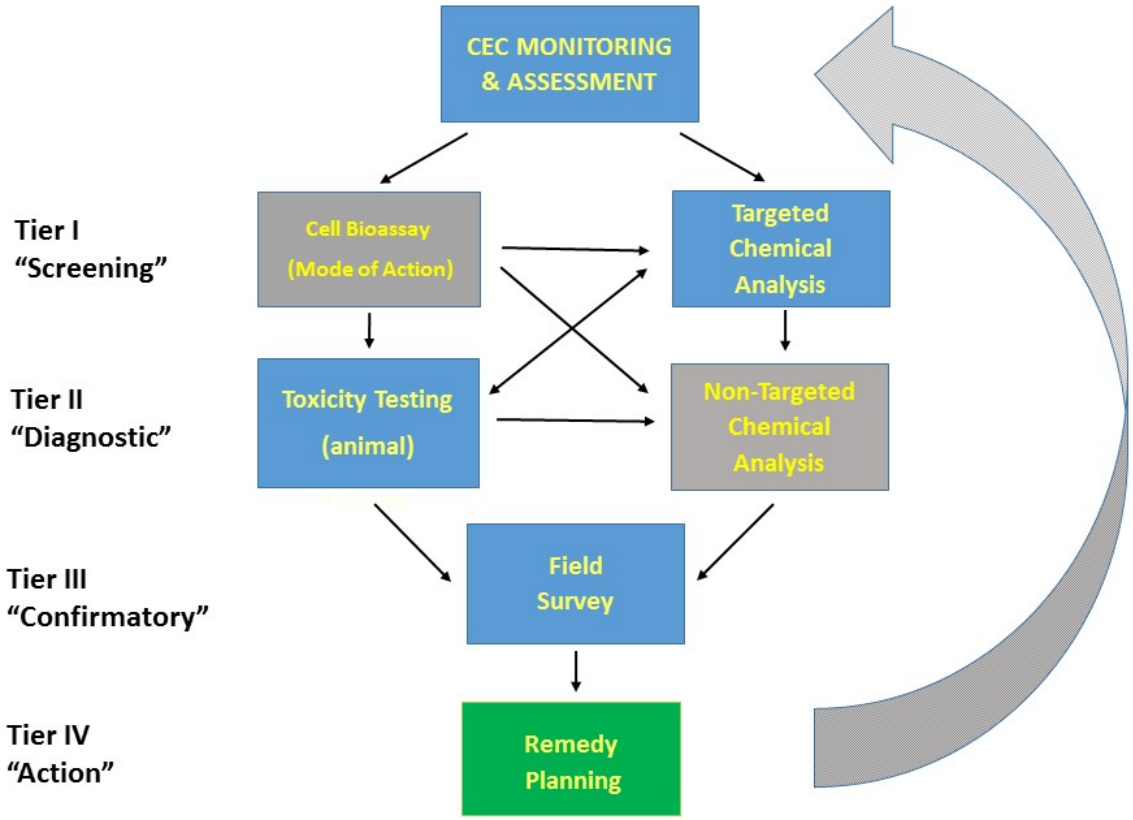
Contaminants of emerging concern (CECs) refer to the tens of thousands of chemicals that may be introduced to receiving waters through human activity that environmental managers are working to detect, understand and prioritize for monitoring. Although the knowledge base is limited, scientists are continually learning more about CECs' sources, pervasiveness, and effects. With literally tens of thousands of chemicals and their degradation byproducts to triage, the traditional chemical-by-chemical approach to monitoring, assessment and regulation has become unwieldy and obsolete. Moreover, constantly changing human activities have made CECs a "moving target," with new chemicals continually being substituted for ones being phased out. Confounding this challenge is that even if every CEC in every water sample could be identified, these chemicals come together as complex mixtures, exerting biological effects that cannot be determined through traditional assessment methods. Furthermore, in contrast to currently regulated chemicals, the potential for CEC impacts can occur at much lower levels and be manifested over longer periods of time. Recognizing these challenges, SCCWRP is working closely with experts in the field and the management community to develop and evaluate a comprehensive framework for improved monitoring and assessment of CECs in receiving waters statewide.

Conceptual Model

SCCWRP's conceptual model combines biological and chemical monitoring and assessment of CECs in a multi-tiered framework that is more efficient, relevant and comprehensive than the status quo and, perhaps most importantly, is adaptable to changes in contaminant source input (**Figure 1**, Maruya et al. 2016a). Chemical monitoring methods are necessary in characterizing the likelihood that humans and aquatic life will be exposed to harmful substances in the aquatic environment. Biological monitoring methods, meanwhile, are key to determining whether existing levels of chemical exposure are causing adverse impacts to wildlife and humans.

In **Tier I**, state-of-the-art bioanalytical tools (cell-based "*in vitro*" assays) complement traditional targeted chemical monitoring by screening for both known and unknown chemicals according to their mode of biological action (MOA). **Tier II** toxicity tests will provide a means for interpreting and validating Tier I screening results by measuring effects (e.g. on fish reproduction) induced by exposure to low-level CECs, coupled with chemical techniques that identify CECs exerting toxicity and that accumulate in wildlife (targeted and "non-targeted" chemical analysis). Confirmatory (**Tier III**) monitoring takes place in receiving water environments, e.g. via case studies and regional monitoring (e.g. the southern California Bight and Stormwater Monitoring Coalition programs) designed and carried out to serve as the reality check for monitoring results obtained via Tiers I and II. In **Tier IV** ("remedy planning"), monitoring tools developed in Tiers I-III are applied, as needed and in conjunction with predictive models, to identify CEC sources and to evaluate the effectiveness of management actions (e.g. best management practices, or BMPs). Models that incorporate relevant monitoring information to predict CEC input, occurrence, fate and effects round out the tools available to inform management actions.

Figure 1. Tiered biological and chemical monitoring to direct management action: SCCWRP's conceptual model for monitoring and assessment of CECs screens for unknown and known chemicals (Tier I), determines biological relevance and identifies problematic CECs (Tier II), investigates whether harm is occurring in receiving waters (Tier III) and informs management actions that address impacts on water quality (Tier IV).



SCCWRP's conceptual model was formulated in close collaboration with a panel of international experts (referred to hereafter as the "CEC Expert Panel" or "Panel") convened on behalf of the State Water Board to make recommendations on monitoring of CECs in aquatic ecosystems. After the Panel finalized its recommendations (Anderson et al. 2012), this conceptual model became the basis for design of a statewide CEC pilot monitoring program (Dodder et al. 2015), and subsequently, the major new elements (cell assays and non-target analysis) have been incorporated into the State Water Board's CEC Initiative. As an integral part of its formulation in these collaborative efforts, the model has been reviewed and endorsed by various stakeholder groups, including the discharger, regulator, public interest and commercial services communities. As a new construct, however, the framework must first be evaluated under simulated and/or real conditions to determine the utility of each successive tier. Moreover, flexibility must be retained in monitoring concurrently across the four tiers; if the results of Tier I

monitoring warrant immediate and/or urgent action, monitoring steps and/or entire tiers should be bypassed.

Tier I: Screening for a Broad Array of CECs

Traditional monitoring relies on sampling methodologies that require extensive pre-concentration of samples, followed by measurement of individual CECs using **targeted chemical analysis**, and subsequent comparison of measured occurrence to pre-established thresholds (e.g. measured vs. maximum allowable concentration). In contrast, **bioanalytical screening** using cell bioassays integrates the response of multiple chemicals present in a sample based on a common MOA (e.g. the sum total response of all estrogenic chemicals). Since it is impossible to use targeted chemical methods to monitor for all biologically active CECs, incorporation of bioanalytical screening tools serves as an important complementary approach that can provide information on bioactivity as well as occurrence of scores of CECs that may impact water quality. Moreover, **passive sampling methods (PSMs**) that rely on simple, cheap, pre-calibrated devices have evolved to the point where sampling of exceedingly low levels of CECs in water and sediment can be achieved in a more cost-effective manner than through conventional sampling. Each of these three screening tools has limitations, but when combined, they provide a robust CEC screening methodology.

- **Targeted chemical analysis** of known CECs utilizes standardized protocols for robust measurement of occurrence in water, sediment and/or tissue. Targeted measurements can be validated against well-established QA/QC criteria (e.g. accuracy, precision), and measured values can be directly compared to thresholds of interest, including monitoring trigger levels (MTLs) that the CEC Expert Panel convened by SCCWRP derived for water, sediment and tissue (Anderson et al. 2012); these thresholds were subsequently adopted by the State Water Board.
  Limitations: Targeted analysis excludes measurement of all bioactive chemicals not on the targeted list of chemicals, including transformation products of known CECs, naturally occurring chemicals, and unknown or unexpected chemicals. The availability of validated measurement methods, as well as scientifically credible, matrix-specific thresholds for individual CECs of interest, can also be a limiting factor. Establishing robust methods and credible thresholds for "new" CECs is costly and time-consuming.
- **Bioanalytical screening** of CECs utilizes engineered cell lines designed to respond to chemicals that act through a common MOA. An aliquot of a pre-concentrated sample extract is applied to test cells under controlled laboratory conditions, incubated overnight and analyzed using simple plate (light) readers. Bioassay results that are quantitative and translatable into an equivalent concentration can be compared to thresholds referenced to a known MOA-specific toxicant. Bioanalytical screening was the No. 1 recommendation made by the Panel to address unknown CECs (Anderson et al. 2012).
  Limitations: Since cell lines are specific in their biological response, a battery of cell bioassays is needed to detect exposure to CECs that exert toxicity via different biochemical pathways. Existing bioassays adapted for water quality monitoring cannot measure the effects of inorganic or unstable chemicals, and sample pre-concentration is still needed prior to bioanalysis. Presently, the number of commercially available cell bioassays is small, and unit costs are relatively high.
- **Passive sampling methods (PSMs)** utilize sorbents or exchange media that isolate and/or accumulate target CECs from the media of interest (e.g. seawater or contaminated sediment).

Passive samplers can detect extremely low levels of CECs in water and sediment (e.g. parts per quadrillion) and provide a time-weighted average concentration without the need to collect, filter and transport copious quantities of water and/or sediment in the field. PSMs can be applied under controlled lab conditions (*ex situ*) or in the field (*in situ*), e.g. where sentinel biota such as bivalves are not available. Moreover, PSMs target the freely dissolved, or bioavailable, fraction of a chemical, giving a measurement that is a better predictor of bioaccumulation and toxicity.
Limitations: PSMs must be pre-calibrated in the lab for the CEC of interest, and then standardized for widespread application by different entities. They also can be sensitive to changes in flow, and can require extended periods of exposure (weeks to months) for accurate measurement. Finally, passive samplers deployed *in situ* can be subject to damage and vandalism, and thus measures to protect their integrity are often necessary.

Tier II: Diagnosing the Likelihood of CEC Effects

The impact of CECs on wildlife and humans may differ profoundly from effects associated with historically regulated contaminants. CECs that affect endocrine function such as synthetic hormones, for example, are potent at low levels of exposure (e.g. at ng/L concentrations in water), and their biological impact may take weeks, months and sometimes years to manifest. Thus, **whole animal toxicity tests** are needed to measure endpoints that are relevant to and diagnostic for CECs, e.g. growth, fecundity and reproduction. Identifying the cause of toxicity in environmental samples, or toxicity identification evaluation (TIE), traditionally has relied on **targeted chemical analysis.** However, this approach often falls short since the number of potentially toxic CECs greatly exceeds the capabilities of targeted analytical methods. **Non-targeted chemical analysis** can enhance TIE by broadening the scope of contaminants subject to identification. As the main components of Tier II monitoring, whole animal toxicity tests and non-targeted chemical analysis can determine if bioactivity observed in Tier I monitoring is translated into higher-order effects, and can help focus the list of causative agents. Tier II monitoring also can inform which parameters are of greatest utility for confirmatory "field" monitoring (Tier III). These same diagnostic monitoring tools can be applied and integrated for remedy planning (Tier IV).

- **Whole animal toxicity testing** determines if exposure to CECs translates into higher-order effects on test species known to respond in a repeatable manner. Endpoints such as biomarker occurrence, growth, fecundity and reproductive success represent a continuum of responses whose relevance increases with increasing level of biological organization (i.e. molecule, tissue, organ, individual). Exposures range from single chemicals to full-strength receiving water samples. Multiple candidate tests utilizing fish and invertebrates exist and/or are under development, some of which may be appropriate for Tier II diagnostic purposes.
  Challenges: Determining how many different tests are needed for receiving waters of interest can be prohibitive. Test species must be compatible with fresh, brackish and marine water and sediment, and should represent different habitats (e.g. pelagic, epibenthic or infaunal) and life histories (e.g. short- and long-lived, sexually dimorphic) that will define the complexity of exposure, duration and ultimately the practicality of establishing a standardized test method. Data interpretation for multiple endpoints may present another challenge, and if toxicity is observed, additional steps are needed to identify the causes of toxicity (see Non-targeted chemical analysis).

- **Non-targeted chemical analysis** broadens the scope for identification of contaminants that are bioactive and/or toxic, including unexpected or previously unknown CECs that elude targeted chemical methods. This diagnostic technique captures mass spectral data on all compounds in a given sample, then identifies the compounds using mass spectral libraries and/or through manual interpretation. Non-targeted analysis allows for a focus on specific classes of CECs, as directed by the results of Tier I screening. For example, utilizing different instrumentation, hydrophobic and hydrophilic CECs can be targeted. As this technique matures, creation of non-targeted "fingerprint" libraries will be useful in diagnosing toxicity and in distinguishing between sources (e.g. wastewater treatment plant effluent vs. stormwater runoff).
  Challenges: Methods and databases for identifying CECs, particularly for water-soluble CECs, are scarce and must be developed. Indeed, positive identification of unexpected or previously unknown CECs is predicated on the availability of purified standards. Furthermore, managing and interpreting non-targeted data require specialized training and expertise. Also, capital equipment to perform non-targeted analyses is more expensive than instruments used for routine monitoring.

Tier III: Assessing CEC Impacts *In Situ*

Although screening-level (Tier I) and diagnostic monitoring (Tier II) can identify *potential* issues and narrow the field of possible stressors when responses/effects are observed, field conditions are never fully replicated. Thus, **monitoring of key parameters** in receiving water systems (i.e. *in situ*) remains an important component for determining whether CECs are impacting beneficial uses. Similar to monitoring parameters at the screening and diagnostic levels, *in situ* monitoring parameters are divided along chemical and biological lines. Biological condition is assessed at the molecular, or "biomarker," level for specific tissues (e.g. gonadal histopathology) and individuals within a given species (e.g. relative abundance in trawl surveys). If biological monitoring indicates impact at a level that is of concern or unacceptable, targeted chemical analysis can be performed to characterize exposure and to compare measured body burdens to pre-established thresholds or monitoring trigger levels (MTLs). If targeted chemical analysis falls short of identifying plausible stressor candidates, non-targeted chemical analysis can be initiated to broaden the scope of chemical exposure investigations.

A key issue for effective and informative *in situ* monitoring is the relevance and availability of biota targeted for investigation. Ideally, sentinel species should address all relevant paths of exposure, e.g. to CECs via the water column, by contact with contaminated sediments, and via consumption of contaminated prey. Additionally, the life history and toxicokinetics of **sentinel species** play important roles in designing field investigations. For example, monitoring of gonadal development and/or recrudescence in a sentinel fish species is performed during periods when the majority of the sampled population is expected to be at similar stages of reproductive development. **Case studies** are required to investigate the utility of proposed **monitoring parameters** and sentinel species. These field trials are performed under scenarios that are most susceptible to CEC impacts (e.g. effluent dominated inland waterways; coastal embayments; marine WWTP outfall zones), include specific habitats (e.g. pelagic vs. benthic), and that incorporate Tier I and II monitoring data to provide holistic context for *in situ* monitoring data.

- A list of **key monitoring parameters** is needed provide multiple lines of evidence for characterizing exposure to and effects from CECs. These parameters should represent physical,

chemical and biological characteristics that respond to CECs in a predictable and quantitative fashion. Chemical parameters may include concentrations of known and unexpected CECs in water, sediment and tissue. Biological parameters should represent responses and/or effects at different levels of organization, e.g. molecular biomarkers, tissue histopathology, and organism size/morphology, spatial/temporal abundance and/or population trends. A list of appropriate **sentinel species** should be identified to address the effects of CECs acting via different MOAs and that are exposed via different pathways (e.g. water column vs. sediment).
Challenges: Due to the co-occurrence of legacy pollutants and CECs, biological responses and effects observed *in situ* may not be specific or wholly attributable to CECs. Also, determining *in situ* conditions typically requires comparison to a reference (i.e. relatively undisturbed) condition, which can be difficult to identify in the Southern California coastal region. Lastly, appropriate sentinel species have not been well-established for all relevant scenarios (e.g. inland waters).

- **Case studies** provide opportunities to test and refine CEC monitoring methods, collect *in situ* monitoring data, and evaluate the effectiveness of *in situ* monitoring for protecting beneficial uses. By comparing measured CEC concentrations and biological parameters across different exposure scenarios and sentinel species, managers will be able to identify commonly observed responses and the most pervasive CECs. Case studies in multiple waterbodies representing different exposure scenarios can assist in standardizing *in situ* monitoring approaches.
Challenges: A challenge for case studies is the inherent high variability and resulting difficulty in interpreting *in situ* monitoring data. Seasonal, annual and decadal variability in climate as well as species mobility and adaptation create a "moving baseline" that must be understood and accounted for when interpreting these data.

Tier IV: Informing Management Actions

If monitoring results from Tiers I-III indicate that corrective action is warranted, additional tools and focused monitoring and/or modeling efforts will be needed to inform a directed response by managers and the selection of an effective solution. For example, identifying primary entry pathways for problematic CECs, also known as **source identification and tracking**, is a first step to reduce their input to impacted waterbodies. Green chemistry initiatives that serve to eliminate the source chemicals in consumer and commercial products *before* they are introduced to the environment can then target problematic CECs. If CEC elimination efforts fall short of their intended goal, implementation of **best management practices (BMPs)** may reduce concentrations of CECs *in situ* to acceptable levels, i.e. below established monitoring trigger levels (MTLs). Monitoring of receiving waters before and after BMP implementation is thus necessary to determine how management action correlates with a reduction in risk.

Although monitoring can provide data that are directly compared to thresholds established for receiving waters, collecting a sufficient amount of monitoring data is not always possible, nor is it typically the most cost-effective approach. Thus, as recommended by the CEC Expert Panel, **models that predict the input, occurrence, fate and potential for CEC effects** are an integral component for managing and mitigating the impact of CECs. Models developed to evaluate *a priori* the effectiveness of proposed BMPs can direct decision-makers toward the most cost-effective solution.

- **CEC source identification** allows managers to tailor corrective action, as warranted, against source inputs responsible for the impact in question. Cataloguing of CECs observed in receiving

waters impacted by different source inputs (e.g. discharge from WWTPs, industrial complexes and/or stormwater runoff) through targeted and non-targeted chemical analysis resulting in source-specific fingerprints serves as the basis for identifying problematic CEC source inputs.
Challenges: As with currently regulated contaminants, CECs may enter waterways of interest via diffuse pathways, e.g. atmospheric deposition. Thus, parsing the contribution of CECs among point (e.g. wastewater effluent discharge) and diffuse sources and among the various types of diffuse sources (e.g. stormwater runoff, atmospheric input) is a major challenge.

- **BMP effectiveness monitoring** entails an evaluation of conditions before and after implementation of corrective action. Tiered biological and chemical monitoring as depicted in the conceptual model (**Figure 1**) provides the data necessary to determine whether conditions downstream of the BMP are met, and can also characterize the degree of "removal" of CECs.
Challenges: BMPs targeting individual CECs or classes of CECs that share common MOAs require robust methods for bioanalytical, targeted and non-targeted chemical analysis to determine if the MOA and/or chemicals of interest are in fact being attenuated. Ensuring stable operation of BMPs is another challenge to their effectiveness.
- **Predictive modeling** is a complementary tool for data collection monitoring that estimates source input, occurrence, fate and effects. It is especially valuable for estimating input (i.e. loadings) and occurrence (i.e. concentrations) of discharged CECs when no other measurement methods are available. These models also are effective in planning and modifying future monitoring as needed, and in informing the selection and operation of BMPs.
Challenges: Developing models that are useful for management decision-making requires selecting an appropriate level of resolution that is achievable with existing support and that is practical to calibrate with existing monitoring data. Also, a number of diverse sub-models are needed to address CECs with different physicochemical properties and different MOAs in the multiple exposure scenarios of interest (i.e. inland waterways, coastal embayments, offshore marine outfall zones).

Research Directions

Tier I: Screening Tools and Monitoring

In response to recommendations of the CEC Expert Panel, SCCWRP and its collaborators have made significant strides in adapting existing biotechnology to screen for CECs in water. The successful development and implementation of a comprehensive toolbox of bioanalytical screening assays will allow for data on both known and unknown bioactive chemicals to be captured during routine receiving water monitoring. This screening paradigm, which also includes targeted chemical monitoring, has been vetted by scientists, engineers and water managers across the state, and has been i) incorporated into the monitoring design of a statewide pilot study to assess the sources and impacts of CECs (Dodder et al. 2015), ii) evaluated in several case studies (Mehinto et al. 2017; Maruya et al. 2018), iii) subject to laboratory intercalibration (Escher et al. 2014; Mehinto et al. 2015), and iv) has been touted by the State Water Board's CEC Initiative. SCCWRP remains focused on applying bioanalytical screening to receiving waters impacted by wastewater treatment plant (WWTP) and stormwater discharge, and on expanding the current bioassay toolbox to address all high-priority CECs and their relevant MOAs.

Targeted Chemical Analysis

Accomplishments

SCCWRP has a long history of developing and applying targeted analytical methods for characterizing the occurrence of contaminants, including several classes of CECs. Methods for a suite of hormones have recently been developed and applied to explain steroidal responses in inland waterways. The risk-based screening framework established by the Panel incorporated current information that has led to the addition of new CECs and removal of existing CECs from the monitoring list for recycled water (Drewes et al. 2018). A multi-agency partnership led by SCCWRP carried out a pilot monitoring study that screen a list of priority CECs, including pharmaceuticals, hormones, pesticides, PBDEs, and PFAs, in Region 1 and Region 4 ambient environments (Maruya et al. 2016b; Maruya et al. 2018).

Ongoing Research

The primary goal for ongoing research is to develop analytical methods for a list of priority cyanotoxins in water and tissue to provide quantitative occurrence and exposure results in support of regional and statewide monitoring efforts and investigative studies. To ensure collection of robust monitoring data, validated analytical methods for priority cyanotoxins in both matrices of interest are required.

> ***Project*: Method development for priority cyanotoxins**
> Analytical methods are being developed for priority cyanotoxins (e.g. microcystins, anatoxins, and cylindrospermopsin) to support field surveys. This project pays special attention to validation of performance (i.e. sensitivity, precision) for matrices of interest, including water and tissue.

Priorities for Future Research

The primary goal for future research is to develop targeted methods for CECs that exhibit activity via bioanalytical screening. Accurate quantification of bioactive CECs is needed to strengthen assessments linking exposure (screening results) to effects (diagnostic evaluation). Additional goals are to develop and/or derive targeted methods for known contaminants of interest, including pharmaceuticals and personal care products (PPCPs), per- and polyfluoroalkyl substances (PFAS) and microplastics. Robust, targeted methods for bioactive and known CECs are needed to support studies that address adverse health outcomes in humans and animals.

> ***Future focus area*: Targeted methods for bioactive CECs**
> Targeted methods will be needed to generate robust occurrence data for CECs that exhibit bioactivity using cell-based screening assays (see also Research Direction: Bioanalytical tools), and to characterize exposure for *in vitro* to *in vivo* linkage studies (see also Research Direction: *In vivo* toxicity testing: linkage to *in vitro* responses). This effort will put a premium on developing protocols that are transferable to the broader monitoring community.

> ***Future focus area*: Targeted methods for PPCPs, PFAS, and microplastics**
> Targeted methods will be needed to generate robust occurrence data for high priority CECs that have been closely linked with ecological and/or human health impacts. Upon successful method development, we will investigate the occurrence and fate of these high priority CECs in aquatic ecosystems where exposure may present elevated risk to wildlife and humans.

Bioanalytical Tools

Accomplishments

In vitro cell bioassays have shown promise as rapid bioscreening tools for water quality assessment (Escher et al. 2014, Mehinto et al. 2015). SCCWRP is part of an international group of researchers that have worked to optimize and standardize commercially available products for screening of endocrine active (i.e., estrogen receptor and glucocorticoid receptor assays) and dioxin-like (aryl hydrocarbon receptor assay) CECs (Mehinto et al. 2016). These protocols were used in pilot studies to screen water and sediment from the Russian River, a watershed with minimal urban impact, and Los Angeles River, an effluent-dominated watershed. Results of these studies revealed that bioanalytical screening is a cost-effective method to prioritize samples that contain bioactive CECs and require further chemical analyses (Maruya et al. 2018).

Ongoing Research

SCCWRP's priority is to expand the battery of cell bioassay endpoints and evaluate their ability to detect bioactive CECs in ambient matrices including fresh and brackish water, stormwater, sediment and fish tissues.

> ***Project*: Expansion of cell bioassay toolbox**
> The current toolbox consists of a handful of endpoints largely targeting endocrine-active CECs. For effective monitoring of known and unknown CECs, a more comprehensive set of cell bioassays for various MOAs are needed. SCCWRP is currently investing in the development of endpoints to monitor non-estrogenic MOAs including thyroid disfunction, immunosuppression and metabolic disorders. Protocols that are quantitative and include performance-based criteria will be optimized and evaluated during interlaboratory comparison exercises.

Priorities for Future Research

The majority of bioanalytical tools adapted for water quality monitoring have been applied to aqueous matrices including treated wastewater effluent, and their receiving waters. To date, only few studies have examined the bioactivity potential of stormwater runoff. The state of California is considering the use of stormwater capture for groundwater recharge. Therefore, assessing the occurrence of CECs and their potential for impact in captured stormwater runoff is a high priority.

> ***Future focus area*: Demonstration of bioanalytical screening for stormwater**
> Based on previous investigations, stormwater is expected to contain bioactive CECs that are measurable using *in vitro* screening assays and could potentially pose a risk to ecological health. Thus, research is needed to characterize and compare bioassay response in stormwater runoff from different landscapes. In this project, optimized cell bioassay endpoints (see Project: Expansion of cell bioassay toolbox) will be utilized to screen stormwater runoff samples for their potential of exerting toxic effects on aquatic life.

Passive Sampling Methods

Accomplishments

Passive sampling methods (PSMs) measure the freely dissolved concentration (C_{free}) of contaminants in water or sediment, a parameter that is superior to bulk or total (C_{total}) concentration as an exposure metric for observed bioaccumulation and toxicity (Parkerton and Maruya 2014). PSMs also represent a cost-effective sampling alternative for organic and metal contaminants of concern. To complement our PSMs that target C_{free} under field conditions ("*in situ*"), SCCWRP has developed a lab-based ("*ex situ*") PSM to quantify C_{free} for organic contaminants in sediments and applied this new protocol to assess sediment quality in San Diego Bay. We also demonstrated the benefits of standardizing our *ex situ* protocol in an international lab round-robin exercise (Jonker et al. 2018).

Ongoing Research

New classes of CECs, e.g. cyanotoxins, present different challenges for sample collection. Currently available PSMs for cyanotoxins are semi-quantitative at best, and thus cannot provide the exposure and occurrence data needed to assess risk. In response, SCCWRP is investigating the feasibility of developing improved PSMs which can quantitatively measure cyanotoxin concentrations in receiving waters.

> ***Project*: Development and evaluation of *in situ* PSMs for cyanotoxins**
> Various passive sampling configurations will be tested for measurement of high priority cyanotoxins in lab experiments. Those methods showing the best performance will then be evaluated in field trials, in conjunction with ongoing surveys in inland and coastal waterbodies. For this work, SCCWRP is considering both film- and resin-based sorbents, the latter of which are configured to minimize water flow impacts and have sampling rates predictable from physical-chemical properties of the compounds of interest (Challis et al. 2016; Stroski et al. 2018).

Priorities for Future Research

Future work will focus on the development, evaluation and application of PSMs for a broader range of CECs, e.g. PFAS, pharmaceuticals and personal care products, and current/future use pesticides, that pose a threat to ecological and human health, and that can be used to directly dose Tier I screening and Tier II diagnostic investigations. Successful development of this capability will lead to streamlined evaluation of stormwater runoff quality, recycled and potable water quality, as well as for media in receiving water environments, e.g. as depicted in **Figure 1**.

> ***Future focus area*: Development of PSMs for dosing toxicity testing**
> Current monitoring of CECs typically requires time-consuming and labor-intensive steps to isolate CECs from the sample matrix, an effort that may also miss the most relevant fraction of contaminant. PSMs that target C_{free} while at the same time rejecting co-occurring substances may provide utility in directly "dosing" targeted and non-targeted chemical analysis, as well as *in vitro* and (in some cases) *in vivo* toxicity tests. Research is needed to develop and optimize such methods, e.g. by comparing C_{free} measured by PSMs with traditional measures of exposure (e.g. bulk sediment concentrations).

Tier II: Diagnostic Tools and Monitoring

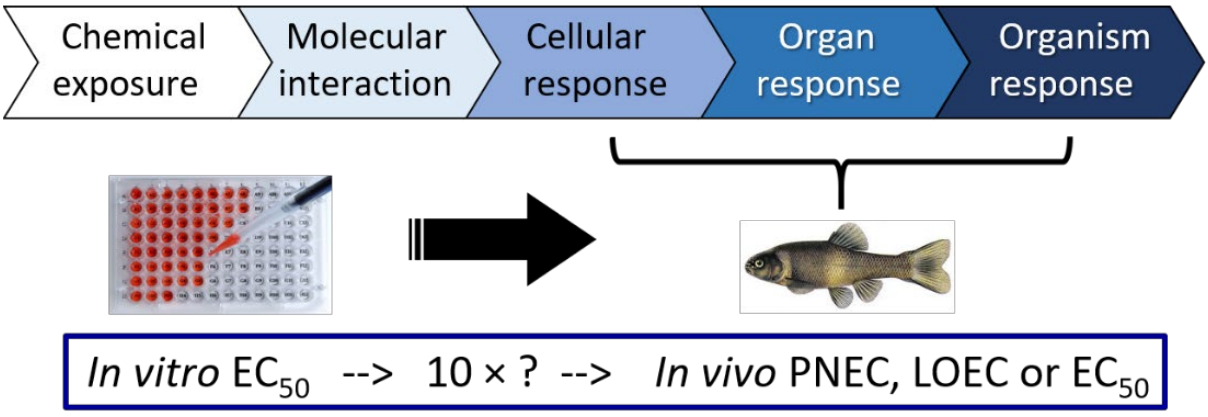
Currently available toxicity tests do not adequately address the action of many CECs, which can activate adverse outcome pathways at exceedingly low concentrations and over extended periods of exposure. Current targeted chemical analyses are limited to CECs for which robust methods exist. SCCWRP's research priorities for Tier II lie in developing (1) *in vivo* toxicity tests that capture the activity of CECs and can be linked to cell assay responses, and (2) non-targeted chemical analytical methods to enhance diagnostic capabilities for identifying causal toxicants.

In vivo toxicity testing: linkage to *in vitro* responses

Accomplishments

Historically, SCCWRP has provided methods to establish linkage between chemical exposure and biological effects through TIE. With the advent of cell bioassays as biomonitoring tools (see also Research Direction: Bioanalytical Tools), linkage of cellular level responses to *in vivo* effects represents a new challenge (**Figure 2**). In recent years, SCCWRP and collaborators have characterized adverse outcome pathways to improve our ability of linking molecular and whole organism responses (Martinovic-Weigelt et al. 2017). Our research has also demonstrated that it is possible to quantify the linkage between *in vitro* activity (e.g. for the estrogen receptor cell assay), altered gene expression (e.g. choriogenin, vitellogenin) and impaired gonadal development in the inland silverside *Menidia beryllina* (Mehinto et al. 2018). This study suggests that cell bioassay responses can provide a more sensitive and conservative screen than traditional toxicity tests.

Figure 2. Linking cell bioassay results to *in vivo* (whole animal) responses is key to develop bioscreening thresholds protective of aquatic life.



Ongoing Research

Lab studies have been initiated to characterize the relationship among cellular screening responses, gene expression and adverse outcomes in sentinel organisms. Due to the high potential for establishing linkage,

ongoing work is focused on evaluating the concordance between screening and diagnostic level responses for non-estrogenic MOAs.

> ***Project*: Linking biological responses for glucocorticoids in fish**
> This project will evaluate the utility of the glucocorticoid receptor (GR) assay in screening for molecular and organismal responses for fish exposed to selected GR-active chemicals found in receiving waters. The first step in establishing linkage will involve identifying appropriate animal life stages and endpoints that are responsive to changes in *in vitro* activity (e.g. organ development, metabolic rate, immune response). The second step will consist of dose-response exposures of GR active chemicals to *Menidia*. This study will help establish conservative bioscreening thresholds above which additional testing will be warranted.

Priorities for Future Research

Although estrogenic chemicals are a top priority due to their potency and pervasiveness, there are several other classes of CECs present in receiving waters. Future linkage studies will investigate these MOAs in concert with the addition of toolbox endpoints.

> ***Future focus area*: Linking biological responses for non-estrogenic CECs**
> This project will establish linkage for other classes of CECs that occur in receiving waters, e.g. androgens, dioxin-like and, eventually, thyroid-active chemicals. Linkage testing protocols for estrogens and glucocorticoids will be modified to include MOA-specific endpoints and response times. Establishing linkage with known toxicants will be followed by investigating linkage with receiving water samples.

Non-targeted chemical analysis

Accomplishments

For decades, SCCWRP and collaborators have developed and implemented targeted chemical monitoring methods. With the increased focus on CECs, however, these methods fall short of identifying all chemicals that may cause adverse effects. In response, SCCWRP has initiated research on non-targeted analytical methods that can detect unexpected and previously unknown chemicals. To catalog the diversity and abundance of halogenated organic compounds (HOCs) accumulating in the coastal marine food web, a method utilizing two-dimensional gas chromatography-time of flight mass spectrometry (GC×GC-TOF/MS) was applied to i) identify new DDT-related compounds (Mackintosh et al. 2016); ii) determine the best marine mammal sentinel species for the Bight (Cossaboon et al. 2019); and iii) compare region specific bioaccumulative contaminants with those being detected elsewhere (Alonso et al. 2017). The unifying feature among these studies was the identification of contaminants which are not detectable in regional monitoring efforts that utilize targeted methods.

Ongoing Research

> ***Project*: Identification of habitat-related chemical fingerprints in Bight sediments**
> This project will apply non-targeted chemical analysis to establish habitat-specific fingerprints using Bight'18 sediments. Complex CEC distribution plots ("heat maps") will be generated and compared across habitats to determine if distinct habitat-specific

fingerprints are observable. This project will also assist in identifying chemicals responsible for *in vitro* bioactivity.

***Project*: Source tracking and apportionment for impacted waterways**
This project will apply high-resolution mass spectrometry (HRMS) to establish source-specific signatures for dry and wet weather runoff as well as untreated sewage. Using a non-targeted HRMS workflow, we will search for clusters of chemicals that are source-distinct. Once source signatures have been established, this tool can be used to estimate and apportion source contributions, in conjunction with genetic markers used to track fecal contamination.

Priorities for Future Research

The goal for future research is to develop non-targeted chemical methods for TIE, e.g. starting with a focus on water-soluble CECs that exhibit *in vitro* bioactivity and/or *in vivo* toxicity. The newly developed TIE methods will be integrated into the CEC conceptual model (**Figure 1**) to identify and prioritize CECs for future monitoring and assessment.

***Future focus area*: Non-targeted methods for TIE**
This project will investigate and develop methods for HRMS analysis of water-soluble, bioactive CECs with different MOAs. SCCWRP and collaborators will optimize sample preparation methods, focusing on fractionation of complex mixtures, and to standardize workflows (including mass spectral databases) for environmental matrices of interest. The workflows can then be applied in case studies, e.g. to identify problematic CECs in future Bight surveys.

Tier III: Confirmatory Tools and Monitoring

In situ monitoring presents a challenge due to the dynamic nature of environmental conditions, juxtaposed onto the already complex biological and ecological interactions and processes that characterize receiving water ecosystems. Traditional tools available for *in situ* biological monitoring are largely inadequate for assessing CEC impacts on individual organisms. SCCWRP's research priorities are thus focused on defining key biological parameters and sentinel species that are linked to CECs' activity.

In situ biological monitoring

Accomplishments

Traditional field surveys that focus on exposure metrics (e.g. bioaccumulation via targeted chemical analysis) do little to assess biological integrity. Our previous RNA-based molecular studies (or "transcriptomics") have shown that gene biomarkers can serve as early indicators of sublethal effects in fish exposed to environmental CECs *in situ* (Martinovic-Weigelt et al. 2014).

Ongoing Research

***Project*: Impact of CECs discharged in urban runoff and effluent dominated rivers**
This project develops field-based toxicity tests to assess sublethal effects of CECs exposure in effluent-dominated rivers. In conjunction with bioanalytical screening and

targeted chemical analysis, cataloguing of gene expression change profiles is needed to (1) identify CECs present *in situ* and (2) infer potential adverse outcomes that will form the basis of a predictive toxicity model.

Priorities for Future Research

Assessing CEC impacts on biological health *in situ* remains a challenge, and monitoring of sublethal effects in the field is difficult. Changes in molecular biomarkers (e.g. gene or protein) may be useful for identifying adverse outcomes before they become irreversible. Informed application of gene expression tools, however, requires knowledge of the life history and population genetics of sentinel organisms. Such information needs to be gathered for species of interest to SCCWRP and its member agencies.

> ***Future focus area*: Identifying sentinel species for CEC *in situ* assessments**
> This project will identify the most appropriate resident vertebrate and invertebrate species for *in situ* assessment of CEC impacts in both freshwater and marine receiving water environments. Species with available baseline genetic and site fidelity information will be assessed relative to their counterparts residing in reference ("cleaner") areas in order to determine relevant health criteria. This will aid in identifying potential biomarkers in relevant species.

> ***Future focus area*: *In situ* application of molecular biomarkers**
> This project will evaluate the utility of molecular biomarkers to predict toxicity in field-oriented case studies. Biomarkers selected during method development (see previous project) will be measured in tissues of field-collected sentinel species. Gene expression profiles will be compared with CEC occurrence *in situ* as validation of gene fingerprinting documented during method development and lab evaluation.

Implementation support and case studies

Accomplishments

SCCWRP has applied the CEC monitoring framework in several case studies to evaluate the utility of the new CEC monitoring tools, and to assess linkage among chemical and biological components for receiving water applications (Mehinto et al. 2017; Maruya et al. 2018; Mehinto et al. 2018). The SCCWRP-led CEC Expert Panel updated CEC monitoring recommendations for recycled water, concluding that two bioanalytical tools (ER and AhR) were ready to screen for unknown CECs (Drewes et al. 2018). As a result, the State Water Board amended their policy to require monitoring of these two endpoints in recycled water applications.

Ongoing Research

SCCWRP continues to evaluate the utility of cell bioassays for screening of bioactive CECs in ambient and recycled water, focusing on case studies that provide a range of expected response and/or impact, and that address a variety of receiving water habitats. Both small- and large scale case and/or special studies are underway, with the combined expectation of demonstrating the utility of the new tools and enhanced framework. Concurrently, SCCWRP will facilitate transfer of the most promising technologies and provide guidance on their application and interpretation to water-quality monitoring agencies.

***Project*: Large scale evaluation of CEC screening tools in Bight region**
This project involves evaluating elements of the proposed CEC monitoring framework (**Figure 1**) to new matrices (sediments and fish tissue) in five different marine habitats. Bioanalytical screening and non-targeted chemical methods will be applied to screen for bioactive CECs and identify habitat-specific chemical fingerprints. This project will serve as a seed for other regional pilot projects, expanding the opportunities needed to validate and establish consensus on the utility of these tools and of the overall framework.

***Project:* Transferring new CEC monitoring technologies**
This project will provide hands-on training on the use of bioanalytical tools for the water quality community. This training will inform laboratory set up, assist with proper conductance of these tests, and provide background and context for interpretation of results. This project will allow labs to demonstrate proficiency for bioanalytical screening of water quality, consistent with anticipated requirements specified in state certification programs.

Priorities for Future Research

As individual CEC monitoring tools are validated, the focus will shift to integrating monitoring results to inform management decisions. Moreover, technologies developed and/or adapted by scientists need to be transferred to water-quality agencies and their contractors prior to widespread implementation.

***Future focus area*: Case studies for using bioanalytical screening for marine outfalls**
This project will examine the fate and impact of CECs found in treated wastewater and discharged in marine habitats. This effort will demonstrate the robustness of assays in protecting against unacceptable consumer and aquatic health risks while affording managers a practical tool that can be incorporated into monitoring. This project will represent an opportunity for SCCWRP and member agencies to collaborate and generate CEC occurrence data for the Panel update of CEC monitoring recommendations in ambient waters.

Tier IV: Remedy Planning

The final tier in the conceptual model for CEC management is remedy planning. Issues arising from monitoring in Tiers I-III that warrant management action will require additional tools and assessment in some cases. In cases where reduction of CEC input is needed to protect beneficial uses, **source tracking and identification** will allow managers to tailor a cost-effective solution that addresses the issue. If implementation of best management practices (BMPs) offers the best solution, the utility of BMPs will need to be demonstrated by **evaluating the effectiveness of BMPs** through monitoring inlet/outlet conditions and before/after conditions. Adapting to future changes in chemical use and technological advancement also may be required, particularly in the face of severe competition for monitoring resources. To respond to changes in water quality, an integrated, **predictive model of CEC input, fate and effects** will be needed that allows managers to adjust monitoring designs and/or implement effective BMPs.

Accomplishments

SCCWRP and collaborators have recently shown that fingerprinting of wastewater and urban runoff via non-targeted chemical analysis is possible (Peter et al. 2018). Researchers have made significant accomplishments in developing bioanalytical tools, and have already initiated research to provide diagnostic capability for positive screening results.

Ongoing Research

As research continues to provide diagnostic capability for identifying and distinguishing between major sources of CECs in receiving waters, targeted and non-targeted analytical methods are concurrently being developed and standardized to perform source fingerprinting.

> ***Project:*** **CEC source tracking and identification**
> This project will assess whether non-targeted chemical fingerprinting can assist in identifying the origin of contamination in a waterway with known human (sewage) impact. Successful fingerprinting of different sources (e.g. wastewater vs. runoff) will allow for possible source apportionment using a flow and mass balance approach.

Priorities for Future Research

As issues associated with the impacts of CECs arise, SCCWRP will shift focus to applying bioanalytical, in vivo toxicity and non-targeted chemical analysis for (1) TIE in impacted (e.g. 303d listed) coastal watersheds and (2) evaluating the effectiveness of management actions, including BMPs.

> ***Future focus area*****: Evaluating the effectiveness of BMPs**
> This project will apply the latest targeted and non-targeted chemical analytical methods in concert with in vitro screening and in vivo toxicity testing to evaluate the effectiveness of BMPs. SCCWRP will apply these tools opportunistically in habitats and watersheds that have been (or are in danger of being) impacted by chemical contaminants.

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