A Tiered, Integrated Biological and Chemical Monitoring Framework for Contaminants of Emerging Concern in Aquatic Ecosystems

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ABSTRACT

The chemical-specific risk-based paradigm that informs monitoring and assessment of environmental contaminants does not apply well to the many thousands of new chemicals that are being introduced into ambient receiving waters. We propose a tiered framework that incorporates bioanalytical screening tools and diagnostic nontargeted chemical analysis to more effectively monitor for contaminants of emerging concern (CECs). The framework is based on a comprehensive battery of in vitro bioassays to first screen for a broad spectrum of CECs and nontargeted analytical methods to identify bioactive contaminants missed by the currently favored targeted analyses. Water quality managers in California have embraced this strategy with plans to further develop and test this framework in regional and statewide pilot studies on waterbodies that receive discharge from municipal wastewater treatment plants and stormwater runoff. In addition to directly informing decisions, the data obtained using this framework can be used to construct and validate models that better predict CEC occurrence and toxicity. The adaptive interplay among screening results, diagnostic assessment and predictive modeling will allow managers to make decisions based on the most current and relevant information, instead of extrapolating from parameters with questionable linkage to CEC impacts. *Integr Environ Assess Manag* 2015;X:000–000. ©2015 SETAC

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INTRODUCTION

Tens of thousands of chemicals are produced and/or imported into the United States annually, some in large quantities (e.g., high production volume chemicals >1 million pounds/y) (http://www.epa.gov/hpv/). Although many of these are reaction intermediates, a significant fraction winds up in consumer and commercial products that are inevitably discharged into the environment via treated municipal wastewater effluent, land-based runoff, or atmospheric emission. The composition of the discharged chemical mixture can vary depending on local or regional chemical usage, land use, climatic conditions and patterns, and the efficacy of management intervention. The resultant mixtures can be complex, consisting of inorganic and organic substances that span a wide range of physicochemical properties, chemical functionalities and resistance to transformation under ambient conditions. Moreover, as the production and/or discharge of chemicals deemed problematic are eliminated, replacement chemicals are identified to take their place that may also become new environmental contaminants.

The traditional approach to assessing likely effects of chemicals in aquatic ecosystems relies on 1) chemical-specific measurements to characterize exposure to priority chemicals ("targeted chemical analysis"), and 2) standardized toxicity testing with invertebrate or vertebrates species (in vivo toxicity testing) to identify levels at which those targeted chemicals will likely lead to a biological response. However, this approach is not well-suited to chemicals that are not routinely or widely monitored, so-called contaminants of emerging concern (CECs), that include pharmaceuticals and personal care products (PPCPs), newly registered pesticides, and commercial and industrial chemicals for which analytical methods do not exist or are just now coming on line. Standardized targeted monitoring methods exist for upward of 1000 chemicals, but developing and validating chemical methods for the ever-changing list of priority CECs is costly and takes too long. Moreover, targeted methods rarely capture the occurrence of transformation products, which if bioactive, can be more relevant than parent CECs in the aquatic environment (Cwiertny et al. 2014). Similarly, in vivo toxicity testing is limited because the approved methods focus on lethality, growth, and/or development of animals that are easy to maintain and that respond to chemical stress in a relatively

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short time (days to weeks). Alternative testing protocols that use mode of action (MOA) linkages to in vivo adverse outcomes of regulatory concern affected by environmental contaminants have been proposed by national and international agencies (Ankley et al. 2010; OECD 2013). These protocols, termed Adverse Outcome Pathways (AOPs), rely on defining the molecular initiating event for the chemical of interest and then linking this event with an adverse outcome to the organism through a series of linked key events that must occur through different levels of biological complexity. One of the goals of using AOPs is to infuse weight of evidence into ecological risk assessments, enhance predictive ecotoxicology, and eventually reduce in vivo toxicity testing.

One of the key components of AOPs are the development of high throughput in vitro bioassays (IVBs) that has provided an opportunity to broaden the scope of chemical-specific monitoring. Taking ligand-binding assays to the next level, receptor-based transactivation assays now allow for MOA screening of chemical mixtures, for example, those occurring in environmental media. Such assays have been developed and applied for screening of dioxin-like activity in water (Addeck et al. 2012), with a more recent focus on endocrine disrupting chemicals (EDCs), a subset of CECs, culminating in the ongoing effort to standardize and implement IVBs for identifying problematic estrogenic and androgenic chemicals (USEPA, 2009). Significant progress has been made in adapting IVBs for screening of EDCs in wastewater (van der Linden et al. 2008), in surface waters receiving discharge from wastewater treatment plants (WWTPs) (Leusch et al. 2010; Jarosova et al. 2012), and in storm water runoff (Tang et al. 2013; Scott et al. 2014). These bioanalytical tools have also been applied to screen for bioactive chemicals in sediments (Koh et al. 2001; Hamers et al. 2010) and biological tissue (Suzuki et al. 2011). Because of their sensitivity and potential for robust measurement, IVBs have garnered attention in screening for residual chemicals in recycled water for eventual potable use (Escher et al. 2014; Leusch et al. 2014). Despite their ability to focus and streamline monitoring, for example, as the screening component in effects directed analysis (EDA), IVBs have yet to be widely incorporated into routine monitoring programs, largely due to a lack of 1) a standardized set of test products and protocols, and 2) a consensus approach for interpreting bioassay results (Escher et al. 2014).

Concurrently, the advent of sophisticated analytical instrumentation has allowed for identification of previously unidentified and/or unknown chemicals in the environment. Early application referred to as nontargeted chemical analysis, was performed for chemicals occurring at μg/L concentrations in surface waters (Bobeldijk et al. 2001). Ibáñez et al. (2005) were among the first investigators to use LC-quadrupole time-of-flight mass spectrometry (QTOF-MS) to tentatively identify unknowns in water. Tandem mass spectrometry (MS/MS) has been used in EDA to identify bioactive chemicals in water (Furuichi et al. 2004) and sediment (Schmitt et al. 2012). Hoh et al. (2012) used 2-dimensional GC-TOF-MS to catalog hundreds of individual compounds in marine mammal tissue as a means for identifying bioaccumulative and persistent chemicals missed by targeted monitoring. The utility of nontargeted methods is hampered by the lack of mass spectral libraries for polar chemicals, whereas their broad application is limited by relatively high capital costs and level of expertise required to perform and interpret the output from such analyses.

Recognizing the need for inclusion and integration of a more comprehensive and relevant set of monitoring tools is on the rise (Snyder 2014). In Europe, Water Framework Directive (WFD) monitoring requirements describe a toolbox of biological and chemical testing methods, including early warning bioanalytical screening systems and whole organism bioassay testing (Allan, Mills et al. 2006; Allan, Vrana et al. 2006). This was followed by a recent initiative from European Union (EU) researchers to develop a framework (called SOLUTIONS) that integrates chemical and effect-based screening tools to inform policy decisions regarding water quality (Brack et al. 2015). The tools to be developed under the SOLUTIONS project would be integrated with ecological assessment data and models to assess risks of complex, chemical mixtures found in receiving waters. Similarly, Cwiertny et al. (2014) recently highlighted the need for an integrated approach for assessment of environmental mixtures, which would improve our ability to identify problematic contaminants. A combination of bioanalytical screening, complementary chemical analysis and computational modeling was proposed as the best approach for triaging among the thousands of possible CECs, including transformation products generated in both natural and engineered systems (Cwiertny et al. 2014). With their generic and adaptable components, these strategies can apply to aquatic systems, including freshwater, brackish, and marine environments.

In California, the need to modernize monitoring and address gaps in available ambient water quality data for CECs resulted in a series of recommendations by a panel of scientific experts (Maruya et al. 2014). This panel recognized early on that current monitoring practices address a finite number of well-studied, problematic chemicals and does a poor job at guarding against effects that may result from most CECs and their mixtures, not to mention the impacts from changing chemical use and/or occurrence into the future. In response, the Southern California Coastal Water Research Project (SCCWRP) and collaborators, which included the panel members and representatives from discharger and regulatory agencies in southern California and across the state, co-developed an enhanced monitoring framework to comprehensively screen for a wide variety of contaminants, including CECs, and to better identify bioactive contaminants with the potential to impact the quality of receiving water environments. This framework was developed with a wide range of impacted waterbodies, including inland freshwater, coastal and/or estuarine, and open ocean scenarios as defined previously (Maruya et al. 2014).

CONCEPTUAL FRAMEWORK

Our conceptual model combines biological and chemical monitoring and assessment of CECs in a multitiered framework that expands on the current approach (represented by the blue boxes in Figure 1). In Tier I, state-of-the-art cell bioassays (IVBs) complement traditional targeted chemical monitoring by screening for both known and unknown chemicals according to MOA. Tier II toxicity tests provides a means for interpreting and validating Tier I screening results by assessing whether deleterious effects (e.g., altered fish reproduction) are induced by exposure to individual CECs and/or environmental mixtures, coupled with nontargeted chemical techniques that identify CECs exerting toxicity and that accumulate in wildlife. Confirmatory (Tier III) monitoring takes place in situ, for example, via case studies designed and carried out in actual

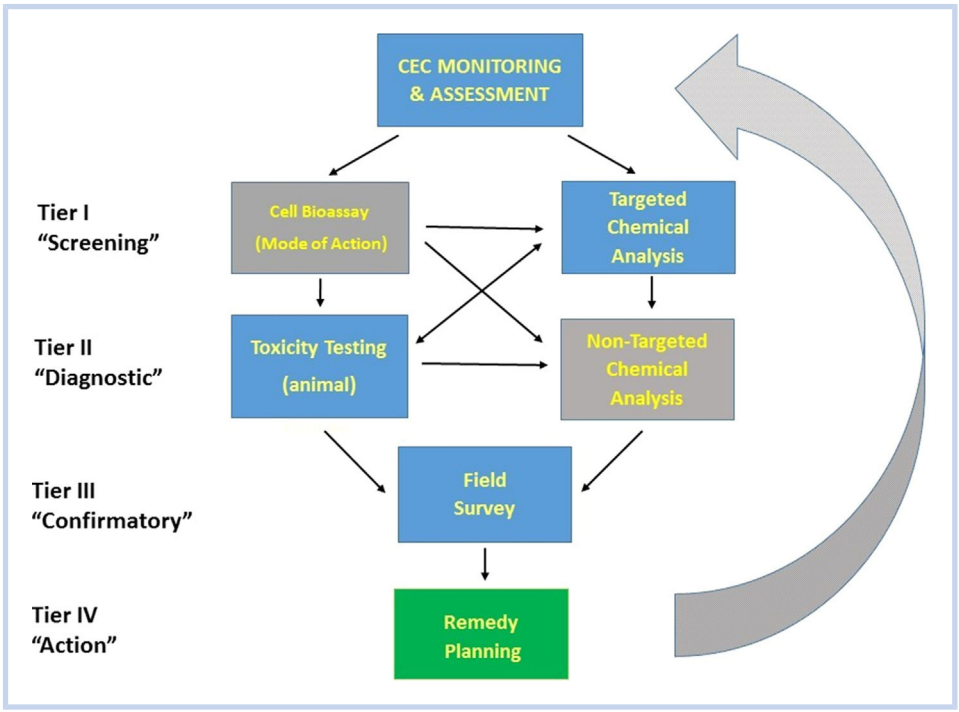

Figure 1. A new conceptual model for monitoring of contaminants of emerging concern (CECs) screens for unknown and known chemicals (Tier I), determines biological relevance and identifies problematic CECs (Tier II), investigates whether harm is occurring in receiving waters (Tier III), and informs management actions that address water quality impacts (Tier IV).

receiving water environments 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 to 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). In concert with high quality monitoring data, models that incorporate relevant monitoring information to predict CEC input, occurrence, fate, and effects round out the tools available to inform management actions. Although represented as a linear, sequential process, flexibility in sequencing of the monitoring and assessment activities described in the tiered framework should be afforded to address site- or region-specific needs. For example, in the case where Tier I trigger levels are grossly and persistently exceeded, managers may make the decision to undertake both Tier II and Tier III activities in parallel (i.e., at the same time).

Tier I—Bioanalytical tools to enhance screening of CECs

Current monitoring of aquatic ecosystems relies on extensive preconcentration of field-collected samples, followed by targeted chemical analysis and subsequent comparison of measured occurrence to pre-established thresholds, for example, monitoring trigger levels (MTLs) derived from toxicity information (Maruya et al. 2014). The availability of robust measurement methods as well as scientifically credible MTLs for individual CECs of interest limits the practicality and scope of targeted chemical screening for CECs. Targeted chemical monitoring may, however, be the only viable option for screening of certain matrices (e.g., tissues), and for CECs that bioaccumulate or whose MOAs are unknown or nonspecific.

Cell bioassays complement targeted monitoring by integrating the response of multiple chemicals present in a sample that act via a common MOA, for example, the net total response of all estrogenic chemicals. In contrast to nonspecific assays (e.g., cytotoxicity), dozens of cell lines have been developed that are able to discriminate among chemicals that initiate AOPs leading to reproductive, developmental, immunosuppression and cancer (Allan, Vrana et al. 2006; van der Linden et al. 2008; Escher et al. 2014). Of particular interest for environmental monitoring are IVBs that screen not only for exposure and uptake by a cell (e.g., cell or cell-free binding assays), but more importantly for the chemical's (or mixture's) ability to initiate an AOP (Figure 2).

Because it is not feasible to monitor for all biologically active CECs using targeted methods, incorporation of IVBs at the first ("screening") level of monitoring provides information on bioactivity regardless of the identity of the bioactive components present. An aliquot of a preconcentrated sample extract is applied to test cells under controlled laboratory conditions, incubated overnight and analyzed using simple plate (light) readers (der Linden et al. 2008; Leusch et al. 2010; Tang et al. 2013). Because transactivation IVBs tend to be specific in their response, a battery of such assays is needed to screen for CECs capable of exerting toxicity via different biochemical pathways. As with targeted analysis, sample extraction and preconcentration is required prior to performing these bioassays. Because development, validation, and application of a comprehensive suite of IVBs is years (perhaps decades) away, bioanalytical monitoring is complementary to targeted chemical screening methods. At some point in the future, however, it may be possible that a comprehensive, robust set of bioanalytical screening tools will reduce the need for extensive screening level (Tier I) chemical monitoring.

In one of the first studies to compare the screening response among labs using a standardized protocol for commercially available IVB test kits, responses were detectable for 2 of the 4 endpoints evaluated (ER and GR) in samples representing a gradient of water quality, ranging from secondary WWTP effluent to highly polished recycled water (Mehinto et al.

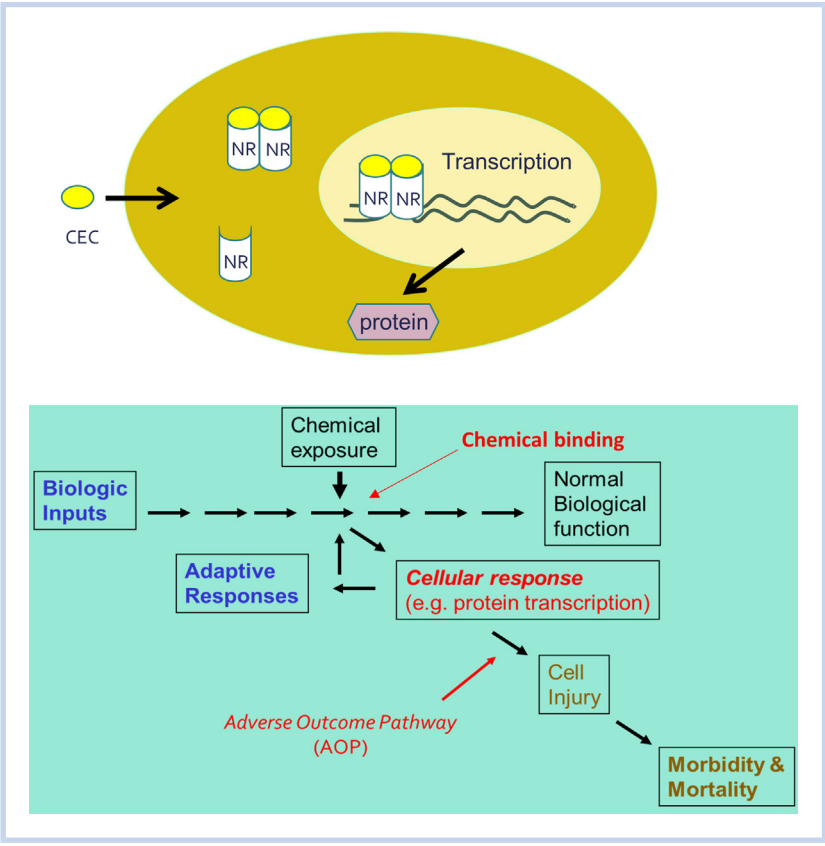

Figure 2. Commercially available in vitro transactivation bioassays developed for high throughput screening of chemicals have been adapted for water quality monitoring. Receptor-based assays that respond to contaminants of emerging concern (CECs), including endocrine active and genotoxic compounds, acting via a common mode of biological action are part of the biochemical cascade that make up adverse outcome pathways (AOPs). NR = nuclear receptor. Adapted with permission from Anderson et al. (2005) with permission from Elsevier.

2015). Moreover, the ER and GR results correctly ranked the samples according to their water quality, based on treatment level and in concordance with available targeted analytical chemistry data (Figure 3). To facilitate future application, it is important to note that these IVBs use a reference toxicant (e.g., 17β-estradiol for ER) that enable bioassay responses to be translated into an equivalent concentration (Escher et al. 2008) that can then be compared to thresholds referenced to the assay-specific reference toxicant.

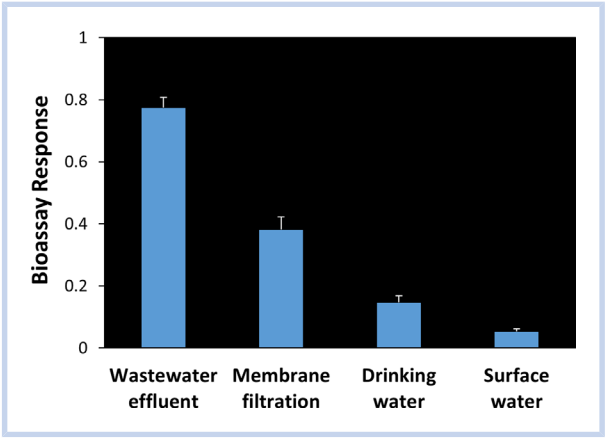

Figure 3. A commercially available receptor-based transactivation bioassay that targets estrogenic chemicals was adapted to successfully distinguish among samples representing a wide range of water quality (adapted from data published in Mehinto et al. 2015).

Although much progress has been made, the application of IVBs for water quality monitoring remains hindered by several factors. The number of commercially available assays that have been adapted and validated for water quality analysis is limited. Because there is little to no market currently for such analysis, unit costs for existing products remain high. Collection and pre-analytical sample preparation protocols would be virtually identical, minus the addition of surrogates, to protocols used for conventional (targeted) chemical analysis (Vanderford et al. 2014) and thus remains time consuming and costly. In addition, no IVBs are currently available for measuring inorganic or unstable organic chemicals, and information needed to establish bioassay thresholds is scarce. Another issue is the specter of false positive responses that may occur with increasingly sensitive bioassays. A rigorous quality assurance/quality control (QA/QC) program that includes performance-based criteria to guard against unacceptably high false positive and negative rates, such as the one outlined by Mehinto et al. (2015), is needed. One of the most daunting challenges, however, is the critical need to understand the linkage among multiple lines of evidence for chemical exposure (e.g., as represented by environmental concentrations), screening-level in vitro bioactivity and in vivo effects (http://ehp.niehs.nih.gov/123-A95/). Accurate and thorough delineation of AOPs will serve as the basis for such linkage (Ankley et al. 2010; Hutchinson et al. 2013). Clearly, future research and evaluation of IVBs in a routine monitoring context is needed to better characterize their full potential (see *Research and technology transfer needs*).

Tier II—Nontargeted chemical analysis for enhancing diagnostic monitoring

A positive IVB screening test identifying possible exposure of a CEC leads to 2 activities in response in this framework. The first is implementation of whole animal toxicity tests that are used to determine if bioactivity observed in Tier I monitoring is translated into higher-order effects measure endpoints relevant for CECs, for example, growth, fecundity, and reproduction (Figure 1). Many such examples have been developed and/or adapted for monitoring and assessment of freshwater systems, including the fathead minnow (*Pimephelas promelas*) 21-day recrudescence assay (USEPA 2007) and Japanese medaka (*Oryzias latipes*) short-term reproduction and multigenerational reproduction assays (OECD 2012; USEPA 2013). However, far fewer standardized tests are available for estuarine and marine environments (Table 1).

The second component is use of nontargeted chemical analysis (NTA), which provides enhanced capability for identifying likely causative agents (Figure 4). This diagnostic technique can be applied to broadly scan for multiple classes of nonvolatile (LC-based) or semi- to volatile (GC-based) chemicals, or more narrowly to specific classes of CECs as directed by Tier I screening results (Figure 5). As NTA matures, creation of nontargeted "fingerprint" libraries will be useful in diagnosing toxicity and in distinguishing between sources (e.g., wastewater treatment plant effluent vs storm water runoff). Diagnostic NTA can also inform which contaminants are of greatest use for confirmatory "field" monitoring (Tier III) and can be applied and integrated into remedy planning (Tier IV), for example, for source identification or assessment of planned or recently implemented best management practices (BMPs).

Although not diagnostic in nature, a second useful application of NTA is for longer-term periodic monitoring of unexpected or previously unknown CECs. For example, GC × GC-TOF/MS analysis of biological samples from sentinel aquatic species can be used to screen for new or unexpected chemicals and/or spatial and temporal changes in exposure (Hoh et al. 2012; Shaul et al. 2015). This is particularly important for interpreting linkage between chemical exposure and biological effects, where NTA can reveal the co-occurrence of hundreds of unmonitored chemicals, sometimes of similar, unknown, or even greater intrinsic toxicity than routinely monitored pollutants. This strategy can be applied to other matrices (Figure 5), such as sediments impacted by known point sources or by runoff from contrasting land uses (e.g., urban vs agricultural).

Similar to IVBs, several challenges remain that presently limit application of NTA for informing ambient monitoring. Methods and mass spectral databases for identifying CECs are limited and thus need to be developed. Development has been hindered for water soluble CECs by fragmentation settings and hardware differences among instruments that result in incompatible libraries. For GC/MS-based NTA, the commonly used NIST Electron Impact database is more comprehensive and can be applied across instruments, but it still may not contain a majority of observed contaminants (Shaul et al. 2015). Managing and interpreting nontargeted data currently requires specialized expertise. Positive identification of unexpected or previously unknown

Table 1. Candidate estuarine and marine fish species for in vivo testing of reproductive effects

	Sheepshead minnow	Atlantic killifish	Inland silverside
	Cyprinodon variegatus	*Fundulus heteroclitus*	*Menidia beryllina*
Test duration	180 d	15 d	15–20 d
Endpoints	Fecundity, fertility, GSI	Plasma sex steroid	Fecundity, fertility
	Plasma sex steroids and vitellogenin	Vitellogenin	Molecular markers
	Hatching success	GSI	Hatching success
	Larval morphology		Gonad histology
Strengths	USEPA validated protocol	Killifish species are widespread	USEPA validated species
			Found in state waters
Limitations	Long test duration	Adapted to polluted environments	Reproductive endpoints have not been validated
	Less responsive to CECs than other fish	No egg output endpoint	
References	Raimondo et al. (2009)	MacLatchy et al. (2003)	Personal communication (S. Brander, UNCW)

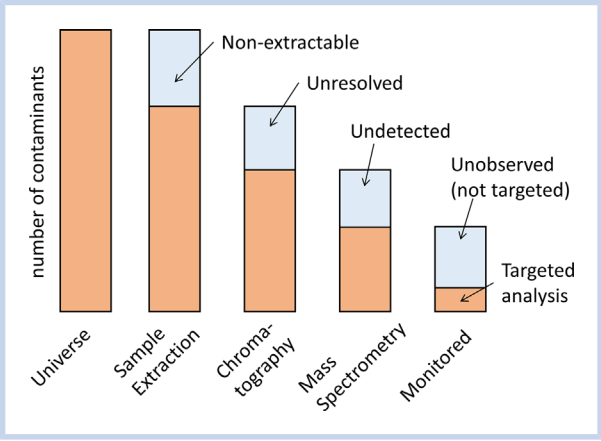

Figure 4. Nontargeted chemical analysis aims to expand the number of contaminants that can be identified in a sample by minimizing compound loss at each step of the analysis.

CECs is predicated on the availability of purified standards, which is largely controlled by free market forces and/or individual initiative and innovation. Last, the capital and/or recurring cost for NTA is currently more expensive than that needed for routine targeted monitoring. Future research as well as collaboration and communication among the research, regulatory, and commercial sectors is key in moving forward with the development and application of NTA methods (see *Research and technology transfer needs*).

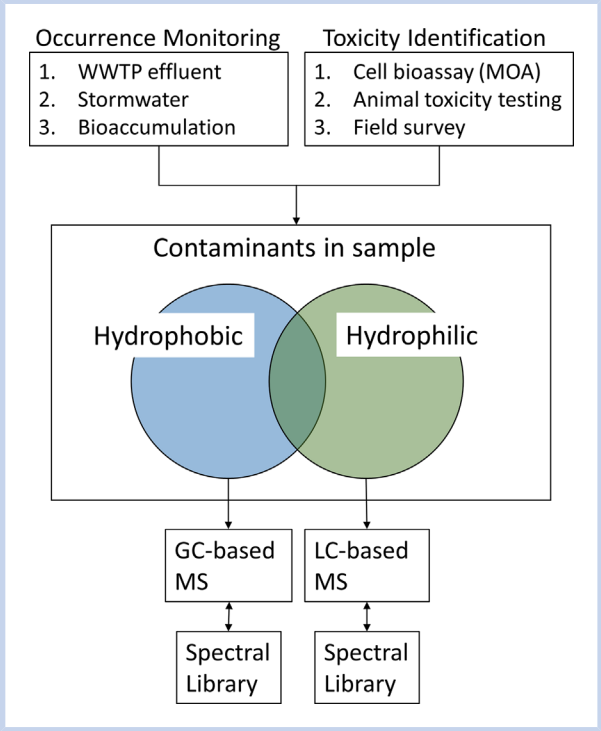

Figure 5. Nontargeted chemical analysis can inform monitoring of receiving waters in multiple ways: 1) To periodically screen for unexpected or previously unidentified chemicals in various environmental matrices such as wastewater treatment plant (WWTP) effluent, stormwater runoff, and tissues; 2) To identify bioactive chemicals, which are missed by targeted chemical analysis through cell bioassay guided fractionation coupled with analytical chemistry. MOA= mode-of-action measured by the cell bioassay.

Tiers III and IV—Confirmatory monitoring and management response

Like Tier 1 targeted chemical screening and Tier II whole animal toxicity testing, field-based ("in situ") monitoring is not a new concept or addition to monitoring frameworks, nor is the need for remedial solutions should monitoring data compel decision makers into action (Tier IV). Periodic evaluation of conditions in situ, however, is a key "ground-truthing" component of the CEC monitoring conceptual framework (Figure 1). Field evaluation of water–sediment quality, contaminant exposure, effects to indigenous biota, and population–stock assessment constitute critical lines of evidence that when assessed in total, help prevent unacceptable degradation of beneficial uses of the resource. When triggered by Tier I and II responses, parameters that quantify exposure and/or biological responses in situ that are similar or identical to Tier I and II parameters and/or endpoints should be identified, developed (as necessary) and incorporated into Tier III field monitoring. Moreover, biological parameters at increasingly relevant levels of biological organization (i.e., molecular → tissue → individual → population) for sentinel species representing key exposure scenarios (i.e., benthic and pelagic habitats) for CECs will be particularly informative.

Models that simulate chemical input using GIS layers and fate (dilution, transformation, partitioning) in aquatic systems (Gobas et al. 1995) can be used to estimate concentrations for CECs which lack robust measurement methods. In silico tools that use quantitative structure activity relationships (QSARs) and adverse outcome pathway (AOP) analysis to link exposure, initiation of toxicity (e.g., in vitro transactivation screening assay response), effects in vivo and/or impacts in situ will provide the necessary linkage information to inform decisions on management of specific CECs and/or waste streams (Worth et al. 2011).

Often, additional diagnostic assessment is needed to assist with Tier IV remedy planning. For example, targeted screening and whole animal toxicity testing can be combined to determine the extent of source contamination of a CEC (or class thereof) in a given watershed or region. Conversely, if toxicity is consistently observed with samples associated with a given source or land use, NTA can be implemented to identify additional candidate toxicants when targeted analysis fails to shed light on potential causative agents. As new management practices come on line, such as BMPs meant to limit or remove the occurrence of problematic CECs, the tools contained within the new framework (Figure 1) can be applied to characterize the effectiveness of the management action.

Periodic assessment of CEC monitoring data is an essential component in maintaining a relevant, up-to-date framework. Annual reviews of CEC monitoring data will allow managers to assess current conditions and the effectiveness of management actions. Comparison of occurrence data to pre-established monitoring thresholds will inform the continued relevance of specified monitoring parameters, for example, the list of priority CECs measured (Maruya et al. 2014; Sengupta et al. 2014). Monitoring thresholds (e.g., MTLs) should be re-assessed and updated as necessary based on the most current toxicological information. In some cases, such as tissue monitoring for high trophic level species, longer assessment periods may be needed to discern temporal trends. In all cases,

review of collected monitoring data and a reassessment of the monitoring design (including list of analytes, robustness of methods and monitoring thresholds) should be planned, scheduled, and performed on a regular basis (e.g., every 5–10 y).

RESEARCH AND TECHNOLOGY TRANSFER NEEDS

The utility and ultimate success of our proposed framework for the management community is clearly dependent on continued advancement of the key monitoring technologies described herein. At the Tier I screening level, a comprehensive battery of bioanalytical screening tools is needed to address exposure to CECs that present the highest risk in aquatic ecosystems, and as a corollary, for MOAs that are critical to maintaining ecosystem health. Although perhaps not an exhaustive listing, prime examples of CECs (and their respective MOAs) that warrant screening include steroid hormones and synthetic phenolic compounds (estrogenicity, anti-androgenicity), aromatic hydrocarbons (carcinogenicity), PBDEs and triclosan (growth and thyroid regulation, neurotoxicity, immunosuppression), and pesticide-specific MOAs (Maruya et al. 2014). Moreover, it is imperative that linkage between IVB endpoints (Tier I) and in vivo toxicity and/or in situ effects (Tiers II and III) be established, ostensibly via delineation and analysis of CEC or CEC-class specific AOPs. Once appropriate IVBs have been identified, they will need to be optimized, standardized, and validated for water quality applications. A terminal step in realizing the monitoring utility of IVBs is to provide training and guidance on proper bioassay setup, performance, and interpretation of results for investigation versus regulatory applications to a broad audience of practitioners within the water quality monitoring community.

For Tier II diagnostic methods, we need to first develop and validate NTA methods for identification of unexpected and/or previously unknown CECs in aqueous, sediment, and biological tissue matrices. Once vetted, we can then apply NTA for identification of bioactive CECs in samples exhibiting responses that exceed thresholds of concern in vitro (Tier I), in vivo (Tier II), and/or in situ (Tier III). In addition, we should incorporate NTA to periodically screen for unmonitored CECs in tissues of sentinel species and sediments collected from locations of management interest, for example, near WWTP outfalls; urban river mouths; stormwater management or agricultural zones. For higher trophic species like marine mammals or birds, a larger regional perspective is likely needed (Shaul et al. 2015). In parallel, we need to focus on adapting and, as necessary, developing in vivo toxicity tests that better address the effects of CECs. Careful consideration of appropriate test species and/or models for the different aquatic habitats (i.e., fresh, brackish, and marine; pelagic vs benthic; invertebrate vs vertebrate) is needed. Once adapted and/or developed, the response of candidate tests should be characterized against CECs of known activity, and protocols standardized, ideally across multiple organizations (e.g., ASTM, EPA, OECD).

Because monitoring can never fill all possible data gaps, it is important to codevelop predictive models to inform management action on mitigation of CEC impacts in Tier IV of the monitoring framework. Such models should provide characterization of sources, mass loading, fate, and potential for effects due to CECs, in representative aquatic receiving water scenarios. Before widespread adoption by the management community, the compatibility of data across monitoring Tiers I to IV and the ultimate utility of the framework should be tested in case studies. Such "test drives" would consist of collecting monitoring data at the local, watershed, or regional scales to verify compatibility of framework components and adequacy of available data management tools. As all tools will not be developed and validated concurrently, piloting of the framework could take a stepwise approach, first starting with a subset of screening IVBs whose MOAs are better understood and thus developed (e.g., the response and impacts of estrogenic chemicals). Selection of multiple watersheds for pilot scale monitoring would help determine the relevance of the framework and individual monitoring tools across areas of diverse biogeography and land use. Fine tuning of the framework and its components could result in standardization of monitoring at larger scales, for example, to form the basis of CEC monitoring statewide. Information and lessons learned from regional case studies can then serve as the basis for workshops for dischargers, regulators, and laboratory services personnel addressing the relationships among the framework components and the appropriate use of the framework for making informed management decisions.

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