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Refocusing Mussel Watch on contaminants of emerging concern (CECs): The California pilot study (2009–10)

Keith A. Maruya [a,*], Nathan G. Dodder [a], Rebecca A. Schaffner [a], Stephen B. Weisberg [a], Dominic Gregorio [b], Susan Klosterhaus [c,1], David A. Alvarez [d], Edward T. Furlong [e], Kimani L. Kimbrough [f], Gunnar G. Lauenstein [f], John D. Christensen [f]

[a] Southern California Coastal Water Research Project Authority, 3535 Harbor Boulevard Suite 110, Costa Mesa, CA 92626, USA
[b] California State Water Resources Control Board, 1001 I Street, Sacramento, CA 95814, USA
[c] San Francisco Estuary Institute, 4911 Central Avenue, Richmond, CA 94804, USA
[d] U.S. Geological Survey, 4200 New Haven Road, Columbia, MO 65201, USA
[e] U.S. Geological Survey, Denver Federal Center, Denver, CO 80225, USA
[f] National Oceanic and Atmospheric Administration, 1305 East West Highway, Silver Spring, MD 20910, USA

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ABSTRACT

To expand the utility of the Mussel Watch Program, local, regional and state agencies in California partnered with NOAA to design a pilot study that targeted contaminants of emerging concern (CECs). Native mussels (*Mytilus* spp.) from 68 stations, stratified by land use and discharge scenario, were collected in 2009–10 and analyzed for 167 individual pharmaceuticals, industrial and commercial chemicals and current use pesticides. Passive sampling devices (PSDs) and caged *Mytilus* were co-deployed to expand the list of CECs, and to assess the ability of PSDs to mimic bioaccumulation by *Mytilus*. A performance-based quality assurance/quality control (QA/QC) approach was developed to ensure a high degree of data quality, consistency and comparability. Data management and analysis were streamlined and standardized using automated software tools. This pioneering study will help shape future monitoring efforts in California's coastal ecosystems, while serving as a model for monitoring CECs within the region and across the nation.

1. Introduction

To characterize the spatial extent and temporal trends in contaminant levels in the coastal ocean and Great Lakes, the National Oceanic and Atmospheric Administration's National Centers for Coastal Ocean Science Mussel Watch Program ("Mussel Watch") has collected and analyzed bivalves and sediments since 1986 (http://ccma.Nos.noaa.gov/stressors/pollution/nsandt). Representative samples of locally abundant bivalve species have been collected from more than 200 stations across the nation on a fixed, biennial schedule, e.g. during the winter months in California. To date, bivalve tissue samples have been analyzed for more than 100 trace metal and semi-volatile organic constituents and for overall condition using histopathology. After more than 20 years of assessment, a downward trend in levels of persistent organic pollutants (POPs) that have been phased out or severely restricted, such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane and its derivatives (DDTs) and chlordanes, is apparent nationwide (Kimbrough et al., 2008a). No such trend is discernable for other contaminant classes whose usage and discharge into the environment continues, such as total polycyclic aromatic hydrocarbons (PAHs), a product of fossil fuel combustion, and trace metals such as arsenic, copper, nickel, lead and zinc.

Since most of the currently targeted POPs have been banned for use in the U.S., these trends are expected to continue into the foreseeable future. Thus, the value of continuing to analyze these contaminant classes via Mussel Watch is decreasing from the perspective of local and regional aquatic resource managers. In response to a waning demand for legacy contaminant data, NOAA held a workshop in 2009 with personnel from local, state, regional and federal agencies to identify the most relevant information emanating from the Mussel Watch Program. The workshop participants concluded that information on chemicals that are expected to increase in production and usage, whose discharge and fate characteristics favor environmental "persistence", and that are currently not routinely monitored for and/or regulated, so-called "contaminants of emerging concern" (or CECs), was lacking (California Ocean Science Trust, 2009). The recommendation was made

* Corresponding author. Tel.: +1 714 755 3214; fax: +1 714 755 3299.
E-mail addresses: keithm@sccwrp.org (K.A. Maruya), gglauenstein@gmail.com (G. G. Lauenstein).
[1] Present address: Cradle to Cradle Products Innovation Institute, San Francisco, CA 94108, USA.

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that Mussel Watch would be an excellent platform for examining CECs.

A wide variety of chemicals including pharmaceuticals and personal care products (PPCPs), flame retardants, contemporary use pesticides (CUPs) and even food additives (e.g. caffeine) are considered CECs. Except for those most recently formulated, many of these chemicals have likely been present in aquatic ecosystems for years and perhaps decades, but were not previously targeted or detectable using available monitoring methods. Public awareness and recent advances in analytical chemistry have since resulted in widespread detection of many CECs in the environment. Moreover, CECs possess a wide range of physicochemical properties, and thus exhibit differential behavior once discharged into the aquatic environment. Some, like polybrominated diphenyl ether (PBDE) flame retardants, are hydrophobic and display persistence and bioaccumulative potential (Kimbrough et al., 2008b; Meng et al., 2009). Others, such as DEET, sulfamethoxazole and other PPCPs are water soluble and are rapidly transformed in surface waters (Boreen et al., 2003; Guo and Krasner, 2009). Whereas bivalves or other aquatic species may be appropriate monitoring sentinels for bioaccumulative CECs, alternative approaches including the use of passive sampling devices (PSDs) that target water soluble compounds (Petty et al., 2004) as well as hydrophobic pollutants (Zeng et al., 2004) show promise for monitoring of CECs in natural waters.

A consortium of research, monitoring and regulatory agencies in California seized the opportunity to serve as an initial test bed to facilitate this transformation. During the 2007–08 Mussel Watch collection cycle, the Southern California Coastal Water Research Project Authority (SCCWRP), the Multi-Agency Rocky Intertidal Network (MARINe) and the Ocean Unit of the State Water Resources Control Board (SWRCB) teamed with NOAA to increase spatial coverage of Mussel Watch by doubling the number of existing Mussel Watch stations in California. In contrast to the original, overarching Mussel Watch strategy of selecting stations with no obvious anthropogenic perturbation, the new stations were selected to address differences in land use and the impact of point and non-point source discharge, including several that were located in areas of special biological significance (ASBS), defined by State law as those areas devoid of permitted or regulated discharge (http://www.waterboards.ca.gov/water_issues/programs/ocean/).

A steering committee was established for this "California pilot study", with representatives from SCCWRP, the SWRCB, the San Francisco Estuary Institute (SFEI), NOAA and the U.S. Geological Survey (USGS), to design a two-year pilot study that addressed the following questions:

1. What is the occurrence (frequency of detection, concentration) of CECs in the coastal California environment?
2. How does CEC occurrence vary with land use?
3. How does CEC occurrence vary with proximity to discharge of treated municipal wastewater effluent and storm water runoff?
4. Which CECs are detectable in the water column using passive sampling devices (PSDs)?
5. What is the relationship between CEC accumulation by PSDs and bivalve tissue?

The steering committee identified a list of high priority CEC classes based on the state of the science and availability of robust analytical methods, and designed a field study to address the above questions. This paper describes the process used to select target CECs, the field sampling design, analytical requirements including data quality objectives and quality assurance/quality control (QA/QC) provisions and strategies for data management and analysis. The results of the pilot study, which are documented in a series of papers also appearing in this special issue, will provide the basis for development of a robust comprehensive monitoring and assessment program for contaminants that will inform future management decisions concerning the quality of the California coastal environment.

2. Approach

2.1. Sampling locations

A total of 68 stations were identified for this study (Fig. 1). From 1986–2006, NOAA established 36 Mussel Watch stations in California, with 21 located in southern California (south of Point Conception) and the remainder in central and northern California, including San Francisco Bay (SFB) (Lauenstein et al., 1997). To increase coverage and to include stations that are subject to discharge from different and/or changing land uses, 32 new stations were identified in collaboration with MARINe, a consortium of local, State, and federal agencies, universities and private organizations whose members perform long term monitoring of rocky intertidal habitat along the California coast, including the Channel Islands. Ten new stations were located in ASBS, whereas five new stations were located in urbanized and/or agriculturally-impacted embayments, including two in the Los Angeles/Long Beach Harbor complex, and one each in Newport Harbor, Mugu Lagoon and Agua Hedionda Lagoon in southern California. Five new stations were established in agriculturally dominated coastal watersheds. Lastly, a station was added in 2007–08 at the NOAA Tijuana River National Estuarine Research Reserve near the international border with Mexico. A complete listing of stations is given in Supporting Information (Table SI-1).

2.2. Stratification by land use and proximity to known discharges

2.2.1. Land use

Land cover surrounding each station was determined by a GIS-based analysis with four classifications (http://www.mrlc.gov/nlcd_definitions.php): (1) urban; (2) low density; (3) undeveloped (open space characterized by barren, grass and forested land and wetlands); and (4) agricultural (cultivated crops and pasture land). Based on the conservative conveyance of chemicals discharged into coastal California waterways from WWTPs and in storm water runoff (Lyon and Stein, 2009), the influence of adjacent land use on a given station is expected to extend over a much larger distance compared to, for example, microbial pollutants where any association with local source contributions rapidly diminishes at distances of tens to hundreds of meters (Kelsey et al., 2004). As a result, the percentage of land cover within these classifications was calculated for three radii of increasing distance (2, 5, and 10 km) from the GPS coordinates corresponding to the center of each station. Because the distance between stations was much greater than 10 km in most instances, percentages for the 10 km radius were adopted. In addition, many stations faced the open ocean; therefore, the percentage of land use was normalized to the land area within the specified radius, i.e. the area associated with water was not considered [land area = (total area within radius) – (open water area)].

Each station was then assigned to one of four mutually exclusive categories: urban, mixed development ("Mixed Dev"), low development ("Low Dev"), or agricultural ("Agr") based on principle components analysis (PCA) of the land cover percentages. This analysis showed three distinct clusters of stations corresponding to the urban, mixed development, and low development categories (Fig. 2). The PCA assignment resulted in a clustering of stations by land cover percentage as follows: urban (sum of urban and low density land cover >50%); low development (sum of low

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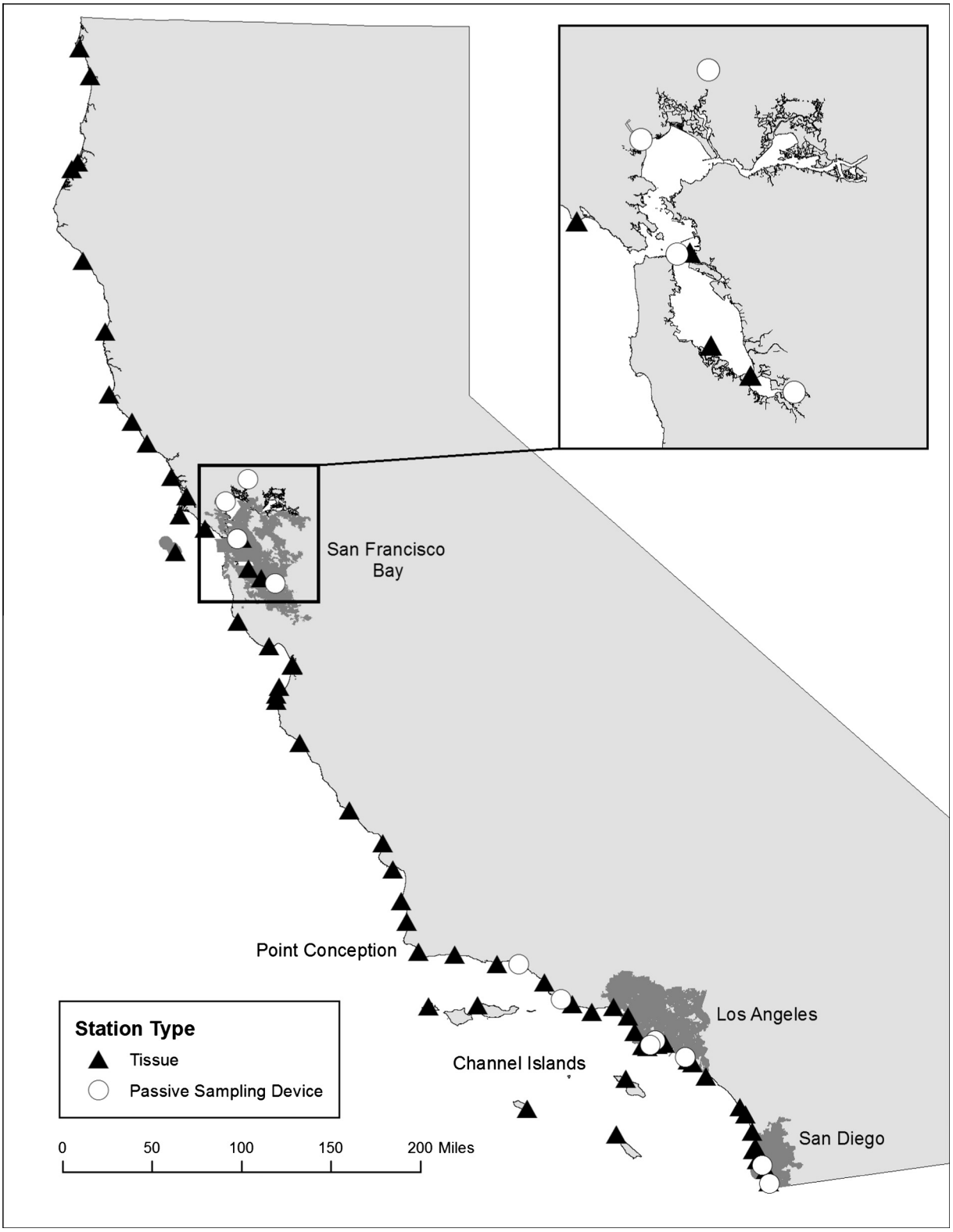

Fig. 1. Mussels (*Mytilus* spp.) were collected at 68 stations for the 2009–10 CEC pilot study along the California (USA) coastline. Passive sampling devices (PSDs) were deployed at 11 stations.

density and undeveloped land >75%); agricultural (>10% agricultural land cover); and mixed development as the remaining stations not classified as urban, low development or agricultural. Seven of the eight agricultural stations clustered among the mixed development stations, with the remaining agricultural station clustered among the low development stations. The land cover profile for each station was plotted to verify the assignments within each land use category (Fig. S1).

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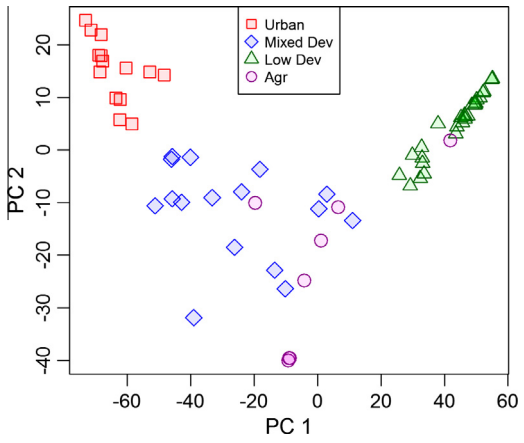

Fig. 2. Mussel (*Mytilus* spp.) collection stations were grouped into four categories (Urban, mixed development, low development and agricultural) based on principal components analysis (PCA) of land cover within 10 km of each station. There are three overlapping agricultural stations at PC 1 ≈ −10 and PC 2 ≈ −40.

2.2.2. Proximity to regulated discharge

To protect the beneficial uses of receiving waters within California, the SWRCB and nine Regional Water Quality Control Boards have the authority to regulate discharges from potential point sources of contaminants (e.g. municipal and industrial treatment facilities) as well as in storm water runoff in accordance with the National Pollutant Discharge Elimination System (NPDES). On an annual basis, the loading of priority pollutants (e.g. PAHs, legacy organochlorines and trace metals) into the coastal zone from these two major sources are roughly equivalent in southern California (Lyon and Stein, 2009); storm water has a somewhat larger contribution in central and northern regions of the State. Regardless, both treated municipal wastewater effluent and storm water discharge are primary sources of anthropogenic contaminants found in coastal waters.

Publicly owned treatment works (POTWs) that discharge treated effluent in coastal waters of the State were identified based on the NPDES GIS outfall layer obtained from the U.S. Environmental Protection Agency (Region 9). Stations were categorized as influenced by a POTW if a sewerage facility (as opposed to one that processes >20% by volume of industrial waste) identified by its Federal Standard Industrial Code (SIC) was found to be:

(1) Within 2 km of small POTWs (<100 MGD average daily discharge).
(2) Within 5 km of large POTWs (>100 MGD average daily discharge).

Station proximity to permitted storm water discharges were based on Phase I and II NPDES-permitted storm water discharge regions, classified by the size of the urban area covered by each NPDES permit. A station was categorized as influenced by storm water discharge if it was within 1 km of a Phase I or Phase II boundary.

There are 34 areas of special biological significance (ASBS) along the California coast (http://www.waterboards.ca.gov/water_issues/programs/ocean/asbs_map.shtml). These areas support a variety of aquatic life, and often host unique individual species, and are managed by the State as basic building blocks for a sustainable, resilient coastal environment and economy. From a water quality perspective, no permitted discharges (i.e. POTW effluent or storm water) are allowed within ASBS. Twenty-two (22) stations were categorized as within 1 km of an ASBS boundary (http://app.databasin.org/app/pages/datasetPage.jsp?id=e1711fc704314b10ae34532e4341422b).

In contrast to land use, the three discharge categories (POTW, STORM WATER, ASBS) were not mutually exclusive; as a result, stations could be assigned into one or more of these categories. Using the above approach and criteria, stations were equally divided among Urban/Mixed Dev (45%) and Low Dev (44%) land use categories with a smaller proportion of agricultural stations (11%) (Table 1). Roughly half (51%) of the stations were categorized as directly influenced by storm water, another third protected as ASBS (32%) and a smaller percentage (16%) influenced by POTWs (Table 1).

As expected, stations in the metropolitan areas of Los Angeles, San Diego and SFB were classified as urban and/or influenced by POTWs (Table SI-1). In contrast, most stations located along the central and north coastal regions were classified as low development. The agriculturally influenced stations were clustered along the central coast in the Pajaro and Salinas River watersheds draining into Monterey Bay, and further south in Ventura and Santa Barbara counties. Stations categorized as influenced by storm water were spread across the State.

2.3. Sampling and analytical protocols

Native mussels (*Mytilus* sp.) were collected by hand at low tide from November 2009 through April 2010 following protocols established by NOAA (Lauenstein and Cantillo, 1998). Permission to collect samples in restricted access areas (e.g. ASBS) was obtained from the appropriate authorities prior to scheduled collection visits. As many as 160 individual mussels were collected by hand from three sub-locations (30–50 mussels per sub-location) for each station, placed into plastic bags, and shipped on ice by overnight courier to TDI Brooks (College Station, TX) for further processing. After shucking, soft tissue was combined and homogenized into three composites per station and frozen in pre-cleaned glass jars, prior to overnight shipping to participating analytical labs.

Passive sampling devices (PSDs) consisting of polar organic chemical integrative samplers (POCIS), low density polyethylene film devices (PED) and solid phase microextraction (SPME) fibers were co-deployed at 11 stations, four in SFB and the remainder in southern California (Table SI-1) (Alvarez et al., in press). Each PSD array was anchored sub-tidally within 500 m of the corresponding mussel collection station for a minimum of 28 days (Zeng et al., 2004). Deployment of PSDs occurred within ± 3 weeks of the corresponding mussel station collection date. Mussels (*Mytilus* spp.) collected from Bodega Head (CA) and acclimated in 15 °C seawater for 7 days, were co-deployed in cages with PSDs at two depths, near the bottom (9 m) and 2 m below the surface at the Los Angeles Harbor Terminal Island station (LATI) during the summer months for a period of 90 days. Upon retrieval, PSDs were stored and transported to the lab in the dark and on ice. Caged mussels were processed as described above for native mussels. POCIS samplers were shipped to the USGS (Columbia, MO) for subsequent processing and analysis. PEDs and SPME samplers were kept frozen until analysis at SCCWRP.

For this effort, target analyte selection was based on three main criteria: (1) the compound was known or suspected to occur in sediments and/or tissue from previous surveys in California or other regions; (2) the compound was known or suspected to occur in the aqueous phase of receiving waters based on traditional or alternative (i.e. passive sampling) methods; and (3) robust analytical methods for the analyte in tissue or PSD were available (Dodder et al., in press; Alvarez et al., in press). Six classes of chemicals were targeted, three each classified as CECs or legacy contaminants (Table 2). Classes of CECs targeted included PPCPs, CUPs, PBDEs and other flame retardants (OFRs), alkylphenols/alkylphenol ethoxylates (APs/APEs), perfluorinated compounds (PFCs) and single

Table 1
Distribution of stations by land use category and discharge scenario.

Land use	Urban	Mixed development	Low development	Agricultural	Total
No. stations	14	16	30	8	68
%	20	24	44	12	100
Discharge[a]	POTW	Storm water	ASBS		Total
No. stations	11	35	22		
%	16	51	32		100

POTW – publicly owned treatment works discharging treated municipal wastewater.
ASBS – areas of special biological significance.
[a] Not mutually exclusive.

Table 2
Classes and numbers of CEC analytes targeted for analysis in bivalve tissue in this study. Not all analytes were analyzed at all 68 stations.

Analyte class	Examples	No. analytes	No. stations
Pharmaceuticals and personal care products (PPCPs)	DEET, erythromycin, fluoxetine, ibuprofen, triclosan	88	All
Industrial and commercial CECs[a]	4-Nonylphenol, BDE47, HBCD, PFOS	52	Partial
Current use pesticides (CUPs)	Chlorpyrifos, dachthal, permethrin, simazine	27	All
Legacy organohalogens and butyltins	Chlordanes, DDTs, endosulfan, PCBs, TBT	74	Partial
Polycyclic aromatic hydrocarbons (PAHs)	Phenanthrene, benzo[a]pyrene, C1-fluorenes	66	Partial
Trace metals	Ag, Cu, Ni, Pb, Zn	14	Partial
Total		321	

[a] Includes alkylphenols/alkylphenol ethoxylates, polybrominated diphenyl ethers, other flame retardants, perfluorinated compounds, and single-walled carbon nanotubes.

walled carbon nanotubes (SWNTs). Legacy organic and metal analytes were chosen from the current Mussel Watch list (Kimbrough et al., in press). Including SWNTs, the number of individual compounds analyzed in mussel tissue was 321:167 CECs, 140 legacy organic and 14 metal analytes (Table SI-2). The identity and number of analytes for PSDs varied by device, with POCIS targeting a combination of hydrophobic, semi-polar and polar analytes, and PED and SPME targeting hydrophobic analytes only (Table SI-3).

Detailed sample processing and analytical protocols for tissue CECs (Dodder et al., in press) and legacy organics/trace metals (Kimbrough et al., in press) are documented elsewhere. Tissue analyses were performed by AXYS Analytical (Sidney, BC, Canada), TDI Brooks (College Station, TX), Dr. M. LaGuardia at the Virginia Institute of Marine Science, Dr. K. Kannan at the New York State Department of Health Wadsworth Research Center, and Dr. P.L. Ferguson at Duke University. POCIS were analyzed by USGS, and PED and SPME analysis was performed at SCCWRP, the details of which are documented elsewhere (Alvarez et al., in press).

2.4. Performance-based quality assurance/quality control

A performance-based QA/QC approach was adopted by all project analytical participants. Participating laboratories utilized analytical methods of their choosing, as long as they met a comprehensive set of performance-based data quality objectives (DQOs), including criteria for instrument calibration, procedural blanks, matrix spike and surrogate recoveries, analyte-specific reporting limits, and where available, agreement with certified/standard reference materials (Table 3). Raw data for field and QA/QC samples (i.e. blanks, spikes, duplicates, and SRMs) from each of the participating laboratories were delivered to a central node using a standardized spreadsheet format (Fig. 3). Submitted QA/QC data was checked for completeness and compared to specified guidelines using a combination of automated and manual checks, with the automated checks programmed in R (R Core Team, 2012). Data for analytes, samples, and/or batches of samples that failed the general criteria specified in Table 3 were further scrutinized by the project QA Manager and submitting laboratory personnel to determine the likely cause for the exceedance. Specific analyte/station pairs that did not meet the QC criteria were considered "not sampled". Detailed results of the QA/QC data evaluation are given elsewhere (Alvarez et al., in press; Dodder et al., in press; Kimbrough et al., in press).

2.5. Data management and analysis

Analytical data that passed the QA/QC evaluation and filtering process were assembled into a master database in a format

Table 3
Data quality objectives (DQOs) for contaminants of emerging concern (CECs).

Measurement	Frequency	Control limit
Initial calibration		Relative standard deviation (RSD) within ± 25% for 80% of the analytes
Sample batch	N/A	20 samples (max)
Calibration verification	1 set/batch	Performed at beginning and end of each batch. Relative percent difference (RPD) compared to initial calibration <25% for 80% of analytes
Method blank	1/batch	<Reporting limit (RL) for all target analytes
Sample duplicate	1/batch	RPD <30% for all target analytes> RL
Matrix spike/duplicate	1 set/batch	70–130% recovery of spike
(MS/MSD)		RPD <30% between MS/MSD for >80% of target analytes within each class
Surrogate spikes	1/sample	50%< surrogate recovery <150%
Certified reference material	1/batch	Measured value within ± 40% of certified value for >70% of target analytes

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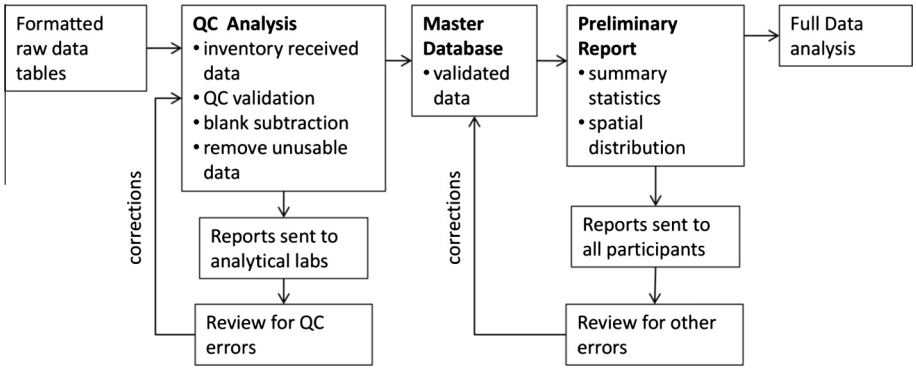

Fig. 3. Data management and validation sequence. Formatted measurement data was entered into a validation system with three primary outputs: (1) a quality control report ("QC Analysis"); (2) a master database of validated data; and (3) preliminary reports made available to all participants for final verification prior to data analysis.

consistent with current Mussel Watch data compilations and with the State of California Surface Water Ambient Monitoring Program (SWAMP). Subsequent data analyses were focused on answering the five primary study questions listed in the Introduction. Tables were generated to show the frequency of detection, minimum, maximum, median and mean concentrations for each analyte. Box plots showing summed classes of contaminants (e.g. PPCPs or PAH) were created to compare concentrations by land use and discharge category. Linear regression analyses were performed for analytes that were detected in both *Mytilus* tissue and PSDs. Details of the above analyses and graphics are available in the supporting manuscripts (Alvarez et al., in press; Dodder et al., in press; Kimbrough et al., in press).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2013.04.027.

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