

Monitoring Strategies for Chemicals of Emerging Concern (CECs) in California's Aquatic Ecosystems

Recommendations of a Science Advisory Panel

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Southern California Coastal Water Research Project

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by the

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PREFACE

In October 2009, the Science Advisory Panel for Chemicals of Emerging Concern (CECs) in California's Aquatic Ecosystems ("CEC Ecosystems Panel") was convened at the request of the State Water Resources Control Board (SWRCB) to provide unbiased science-based recommendations for monitoring of chemicals of emerging concern in oceanic, brackish and fresh waters across the State that receive discharge of treated municipal wastewater effluent and stormwater. Initiation of this effort coincided with the final deliberations of the Science Advisory Panel for CECs in Recycled Water Applications in California, made up of 6 of the 7 members of the CEC Ecosystems Panel, and whose final recommendations were published in June 2010. Funding for the CEC Ecosystems Panel effort was provided by the SWRCB and the David and Lucile Packard Foundation.

The Southern California Coastal Water Research Project (SCCWRP) was selected to convene the Panel, facilitate and manage their activities, provide access to data from local, regional and State monitoring programs and investigations, and to coordinate the writing and submission of this report. Dr. Keith Maruya served as the Project Manager and Lead Facilitator for SCCWRP. Mr. Rick Moss (2009-2011) and Mr. Gary Dickenson (2011-present) served as Contract Managers for the SWRCB. A group of 6 advisors representing the discharger, NGO, regulator and resource communities was established to provide stakeholder input to the process and to assist the Panel Members in understanding water quality issues and in gathering information. The initial charge to the Panel was focused on coastal and marine receiving waters; however, in late 2011, their charge was expanded to inland freshwater bodies. A series of 6 agendaized meetings were held over a 2-year period (the final meeting scheduled for March 22-23, 2012) for the Panel to formulate their recommendations. This report, targeted to the stakeholder audience described above, represents the culmination of the CEC Ecosystems Panel's work.

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Members of the Panel and SCCWRP would also like to acknowledge members of the Stakeholder Advisory Committee formed to assist with selection of the Panel experts, and to serve as a state-wide liaison for their respective constituencies. These advisors are:

Chris Crompton, Orange County Watersheds

Jim Colston, Orange County Sanitation District

Mark Gold, Heal the Bay

Amber Mace, California Ocean Science Trust

Rick Moss, SWRCB

Gary Dickenson, SWRCB

Linda Sheehan, California Coastkeeper Alliance

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Special appreciation goes to Dr. Stephen Weisberg, Executive Director of SCCWRP, and Dr. Keith Maruya for providing invaluable feedback and advice, and for masterminding the collegial environment present at Panel meetings. The Panel team appreciated the opportunity to serve in this capacity, and once again collectively express their heartfelt gratitude to all parties named above, as well as those not specifically acknowledged who contributed to this report.

EXECUTIVE SUMMARY

Although thousands of substances can now be detected in the environment, a small percentage of known chemicals – approximately 200 -- are currently regulated and/or routinely monitored in California receiving waters. The much larger group of chemicals that remain largely unregulated and/or unmonitored in the aquatic environment, known as chemicals of emerging concern (CECs), may originate from a wide range of point and non-point sources. Upon discharge to receiving waters, CECs that are readily soluble in water will remain in the dissolved (aqueous) phase and provide a route of exposure to aquatic life. A smaller subset of CECs that are hydrophobic will associate with particles, where they may remain suspended in the water column or accumulate in sediments and ultimately in tissues of aquatic and terrestrial biota. The larger concern is that exposure to aqueous, sediment and tissue CECs may affect wildlife and humans.

In response, the California Water Resources Control Board (State Water Board) in conjunction with the David and Lucile Packard Foundation and a group of stakeholder advisors tasked a group of leading scientists to address the issues associated with CECs in the State's aquatic systems that receive discharge of treated municipal wastewater effluent and stormwater. The group was charged to identify potential sources and evaluate the fate and effects of CECs, and ultimately to provide guidance for developing monitoring programs that assess those chemicals with the highest potential to cause effects in the State's receiving waters. Seven experts in chemistry, biochemistry, toxicology, chemical and risk assessment, engineering and coastal and marine environmental health science were convened as the CEC Ecosystems Panel ("Panel") in October 2009. The Panel held six in-person meetings to formulate their approach and recommendations, while soliciting input from stakeholders and the public. This report provides the results from the Panel's deliberations, including four products intended to assist the State in developing a monitoring process for CECs based on sound, up-to-date scientific principles.

Product #1: A conceptual, risk-based approach to assess and identify CECs for monitoring in California receiving waters

Given the thousands of chemicals that are potentially present in the aquatic environment and that information about CECs is rapidly evolving, the Panel created a transparent approach to focus the universe of CECs based on their potential for health effects and their occurrence in waters receiving discharge of municipal wastewater treatment plant effluent ("WWTP effluent") and stormwater. The health and environmental risk for individual CECs within this select group was then assessed to guide prioritization of chemicals which should be included in monitoring programs both now and in the future. The Panel adopted a risk-based screening framework, which includes four primary steps:

1. Develop monitoring trigger levels (MTLs) for CECs that pose the greatest potential risk to aquatic systems based on published effects concentrations.
2. Compile measured or predicted environmental concentrations (MECs or PECs) for CECs for which MTLs could be estimated.
3. Identify those CECs that have the greatest potential to pose a risk by comparing MECs (or PECs) to MTLs. CECs with a monitoring trigger quotient (MTQ = MEC(or PEC)/MTL) greater than "1" were identified for monitoring. (Note that an MTQ of greater than 1.0 does not indicate a risk is present, only that sufficient potential for a risk exists that the chemical should be considered for inclusion in a monitoring program.)
4. Apply the risk-based screening framework (steps 1-3) to each of three representative scenarios

that capture the key types of exposure (sources and fate) to CECs in the State's inland, coastal and marine receiving water systems.

The risk based screening framework focused on CECs for which occurrence and toxicity information could be obtained, giving priority to those data representing California sources and receiving waters. Priority was also given to CECs for which adequate quality assurance/quality control (QA/QC) information was available. Occurrence data were obtained for WWTP effluent and stormwater (where available), and in relevant receiving water matrices (i.e., water, sediment and biological tissue). Toxicological information was obtained for the most sensitive aquatic species based on expected mode of action, which included organisms across a wide spectrum of trophic levels (i.e., microbes, invertebrates, fish, birds and marine mammals).

Product #2: Application of the risk-based screening framework to identify a list of CECs for initial monitoring

Several conservative assumptions were used in the risk-based screening framework (Product #1) to identify appropriate CECs for monitoring. The framework was applied to three representative receiving water scenarios:

Scenario 1: a WWTP effluent-dominated inland (freshwater) waterway;

Scenario 2: a coastal embayment that receives both WWTP effluent and stormwater discharge; and

Scenario 3: offshore ocean discharge of WWTP effluent.

For each scenario, MECs were compiled from the literature and from the most recent studies in California. The maximum MEC was selected for use in the risk-based screening framework. In cases where MECs were not available, PECs were employed. To derive MTLs the toxicological literature was reviewed to identify lowest observed effect concentrations (LOECs) and no observed effect concentrations (NOECs) from studies of reproduction, growth or survival of fish and invertebrates. LOECs and NOECs were also identified for antibiotic resistance (ABR). MTLs were derived by adjusting LOECs and NOECs by safety factors ranging from 1-1,000 to account for several sources of uncertainty including extrapolation of toxicity data across species and differences in receiving water environments. Monitoring trigger quotients (MTQs), equal to the MEC or PEC divided by the MTL, were estimated for aqueous, sediment and tissue matrices for each scenario when data were available.

For effluent dominated freshwater systems (Scenario 1), 10 compounds [*17-beta estradiol, and estrone (hormones); bifenthrin, permethrin, and chlorpyrifos (insecticides); ibuprofen, bisphenol A, galaxolide, diclofenac, and triclosan (pharmaceuticals and personal care products)*] were identified for aqueous phase monitoring based on MTQs exceeding unity. For coastal embayments (Scenario 2), 8 of the 10 compounds identified in Scenario 1 were identified for monitoring (diclofenac and ibuprofen were the exceptions). No aqueous phase CECs were identified for monitoring near WWTP ocean outfalls (Scenario 3).

For sediments in coastal embayments, *bifenthrin, permethrin* and two flame retardants (*PBDEs 47 and 99*) were identified for monitoring. For ocean sediments, the high production volume chemicals, *bis (2-ethylhexyl) phthalate, butylbenzyl phthalate, p-nonylphenol* and polybrominated diphenyl ether (*PBDE*) flame-retardants (*PBDEs 47 and 99*) were identified for monitoring. For tissue monitoring, *PBDEs 47 and*

99 and perfluorooctane sulfonate (PFOS), a perfluorinated chemical used in consumer product manufacture, were prioritized for monitoring. The Panel emphasizes that these CECs represent an initial prioritization list based on available data and a number of qualifying assumptions. While their identification at this time represents a conservative screening of “CECs at large”, the information available for performing such screening continues to grow rapidly. The Panel thus urges the State to consider this an initial list that will evolve over time, to which more CECs may be added and others removed (see also Product #3).

Product #3: An adaptive, phased monitoring approach with interpretive guidelines that direct and update actions commensurate with potential risk.

The Panel recommends an adaptive, four-phase approach for implementing CEC monitoring programs for WWTP effluent and stormwater discharges to receiving waters of the State.

- In Phase 1, the Panel reduced the universe of chemicals to an initial list of CECs based on available toxicity and occurrence information. This list was evaluated following the risk-based screening framework resulting in 16 CECs recommended for initial monitoring (Table ES-1). The Panel recommends adopting this list for any initial CEC screening efforts. Any additional chemicals should only be added to this list upon screening through the risk-based framework.
- In Phase 2, guidance is provided for development and implementation of pilot monitoring studies with the objective to generate data needed to assess the occurrence and potential effects of the 16 CECs identified in Phase 1. This initial list of CECs is not intended to represent a target list for statewide compliance monitoring, but to execute focused regional monitoring efforts that will result in the development of a final list of CECs (see Phase 3). Further, to assist with assessment and update of the monitoring information collection during Phase 2, the Panel strongly recommends development or adaptation of environmental fate models (e.g., such as the 1-Box source and fate model utilized by the Panel for PBDEs in Section 3) as tools for summarizing and synthesizing existing knowledge including CEC production and usage, loading and loss rates in receiving water scenarios and environmental compartment transfer rates. In addition, the Panel recommends development and testing of a pilot screening-level mass-based model that would be used to assist in estimating the predicted environmental concentrations (PECs) in effluents coupled with structure-based toxicity assessments.
- In Phase 3, monitoring data from Phase 2 are evaluated to refine their potential to pose adverse human health and ecological effects. Based on the initial screening efforts, the ultimate goal is to derive a final list of CECs that are recommended for routine monitoring as part of discharge permits. During this process, CECs may be added to or removed from the list based on the trends in production/use, occurrence, or findings of directed effects studies.
- In Phase 4, action plans are developed, if warranted, to respond to conditions identified in Phase 3.

Table ES-1. CECs recommended for initial monitoring (Phase 2) by scenario and environmental matrix (i.e., aqueous, sediment, tissue). M = include in monitoring program (discharges to: E = embayments, F = freshwater, O = ocean waters); NA = not applicable.

Compound	Scenario 1 Inland Waters Aqueous	Scenario 2 Embayment Aqueous	WWTP Effluent	FW Stream - Storm- water (Aqueous and Sediment)	Scenario 2 Embayment Sediment	Scenario 3 Marine Sediment	All Scenarios Tissue
Bis(2-ethylhexyl) phthalate	NA	NA	M-O	NA	NA	M	NA
Bisphenol A	M	M	M-E/F	M	NA	NA	NA
Bifenthrin	M	M	M-E/F	M	M	NA	NA
Butylbenzyl phthalate	NA	NA	M-O	NA	NA	M	NA
Permethrin	M	M	M-E/F	M	M	NA	NA
Chlorpyrifos	M	M	M-E/F	M	NA	NA	NA
Estrone	M	M	M-E/F	M	NA	NA	NA
Ibuprofen	M	NA	M-F	M	NA	NA	NA
17-beta estradiol	M	M	M-E/F	M	NA	NA	NA
Galaxolide (HHCB)	M	M	M-E/F	M	NA	NA	NA
Diclofenac	M	NA	M-F	M	NA	NA	NA
p-Nonylphenol	NA	NA	M-O	NA	NA	M	NA
PBDE -47 and 99	NA	NA	M- E/F/O	M	M	M	M
PFOS	NA	NA	M- E/F/O	M	M	M	M
Triclosan	M	NA	M-F	M	NA	NA	NA

Incorporation of this phased approach allows for a logical, sequential course of action to develop new information utilizing state-of-the-art monitoring and modeling tools. These include:

- *non-targeted analyses* using advanced bioanalytical and chemical methods;
- *confirmatory biological investigations* linking chemical and bioassay screening data with higher order effects (i.e., at the organism and population level);
- *environmental fate models* and screening-level mass-based model can assist in estimating the *predicted environmental concentrations (PECs)* in effluents coupled with structure-based toxicity assessments to determine the source, occurrence, fate and effects of CECs; and
- *baseline monitoring for antibiotic resistance* in WWTP effluent

The Panel urges the State to incorporate CEC monitoring into the various existing statewide, regional and local monitoring programs (e.g., California Surface Water Ambient Monitoring Program or SWAMP,

San Francisco Bay regional monitoring and the southern California Bight regional monitoring programs), taking maximum advantage of regional differences and uniform statewide guidelines for data collection and monitoring designs. The Panel also developed guidelines for designing monitoring plans and for sampling and laboratory measurements to ensure collection of data that address the questions of water safety. The Panel strongly recommends that it be consulted on the design of the proposed work plans. Lastly, the Panel recommends a three-year re-evaluation of this conceptual approach, which would include updating the risk-based screening process and the CEC monitoring lists. After this interval there will undoubtedly be new tools to assess toxicity and occurrence which should be thoroughly evaluated (see Product #4); it will also be important to fully assess the effectiveness of control actions (if any) that have been undertaken by the State at periodic intervals. The Panel recommends that after two to three years of implementation, the Panel, or a similar entity, be reconvened to evaluate the results of the initial monitoring.

Product #4: Research needs to develop bioanalytical screening methods, link molecular responses with higher order effects, and fill key data gaps

The science of CEC investigation is still in its early stages. The Panel recommends that the State promote and support research initiatives in three broad categories to improve the scope and performance of monitoring and data interpretation for waters receiving WWTP effluent and stormwater discharge.

1. *Development of bioanalytical screening tools.* High-throughput *in vitro* bioassays with endpoints that respond to CEC exposure in ecological receptors (e.g., endocrine disrupting activity) can screen for multiple CECs, reducing the need for chemical-specific monitoring and shifting us away from the expensive and time-consuming chemical-by-chemical risk-screening paradigm. Research is also needed to identify adverse outcome pathways at the molecular level that are linked to higher order effects (e.g., fish reproduction). Further development and application of the latest genetic microarrays and targeted toxicity testing for species of highest relevance in California receiving waters is recommended to establish this linkage.
2. *Filling data gaps on CEC sources, fate, occurrence and toxicity.* Information on occurrence and toxicity (e.g., MECs and NOECs) are needed for CECs for which there is currently little or no data for California's aquatic systems. Candidate classes of CECs in this category are newly developed pharmaceuticals, replacement flame retardants and recently registered pesticides. In addition, the Panel recommends development and/or refinement of environmental fate models to predict environmental concentrations of CECs based on their production volume, use and environmental fate, as a means for prioritizing chemicals on which to focus method development and toxicological investigation.
3. *Assessing the relative risk of CECs and other monitored chemicals.* The Panel urges the State to compare the potential risks associated with CECs with the potential risks posed by other, currently monitored environmental stressors. This assessment is essential for directing future monitoring investments toward those stressors that present the highest potential risk to the beneficial uses of the State's receiving waters.

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ACRONYMS

ABR	Antibiotic Resistance
ADI	Allowable Daily Intake
AEQ	Androgen Equivalency
AR	Androgen Receptor
ASBS	Areas of Special Biological Significance
BMP	Best Management Practice
BOD	Biochemical Oxygen Demand
BSAF	Biota-Sediment Accumulation Factor
CCL3	USEPA Candidate Contaminant List 3
CECs	Chemicals of Emerging Concern
CMP	Coordinated Monitoring Program
COD	Chemical Oxygen Demand
CRM	Certified Reference Materials
CTR	California Toxics Rule
CWA	Clean Water Act
CWC	California Water Code
DDT	Dichlorodiphenyltrichloroethane
DEET	N,N-Diethyl-meta-Toluamide
E2	17 β -estradiol
EDC	Endocrine Disrupting Chemical
EE2	17 α -ethinyl estradiol
EEQ	Estradiol Equivalent
ELISA	Enzyme-linked Immunosorbent Assay
ER	Estrogen Receptor
FCG	Fish Consumption Goal
F/M Ratio	Food-Microorganism Ratio
GC-MS	Gas Chromatography-Mass Spectrometry
GCxGC/TOF-MS	Two Dimensional Gas Chromatography-Time of Flight Mass Spectrometry
GR	Glucocorticoid Receptor
GSI	Gonadosomatic Index
hER	Human Estrogen Receptor
HRT	Hydraulic Residence Time
HTP	High Throughput
K_{ow}	Octanol-Water Partition Coefficient

LAET	Low Apparent Effects Threshold
LC-MS	Liquid Chromatography-Mass Spectrometry
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
MARINe	The Multi-Agency Rocky Intertidal Network
MEC	Measured Environmental Concentration
MIC	Minimum Inhibitory Concentration
MOA	Mode of Action
MPA	Marine Protected Area
MRP	Municipal Regional Permit
M&RP	Monitoring and Reporting Program
MS	Mass Spectrometry
MS4	Municipal Separate Storm Sewer System
MTL	Monitoring Trigger Level
MTQ	Monitoring Trigger Quotient
NIHES/NTP	National Institute of Environmental Health Sciences/National Toxicology Program
NIST	National Institute of Standards and Technology
NOATEL	No Observed Adverse Transcription Effect Level
NOEC	No Observed Effect Concentration
NPDES	National Pollutant Discharge Elimination System
NRC	National Research Council
OEHHA	Office of Environmental Health Hazard Assessment
PAH	Polycyclic Aromatic Hydrocarbon
PBDE	Polybrominated Diphenyl Ether
PCB	Polychlorinated Biphenyl
PEC	Predicted Environmental Concentration
PFC	Perfluorinated Compound
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctane Sulfonate
PNEC	Predicted No Effect Concentration
POTW	Publicly Owned Treatment Works
PPCP	Pharmaceuticals and Personal Care Product
PR	Progesterone Receptor
PSD	Passive Sampling Device
QA/QC	Quality Assurance/Quality Control

QAPP	Quality Assurance Project Plan
QSAR	Quantitative Structure Activity Relationship
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
RfD	Reference Dose
RMC	Regional Monitoring Coalition
RMP	Regional Monitoring Program
RPA	Reasonable Potential Analyses
RW	Receiving Water
RWQCB	Regional Water Quality Control Board
SAB	Science Advisory Board
SCCWRP	Southern California Coastal Water Research Project
SDWA	Safe Drinking Water Act
SETAC	Society of Environmental Toxicology and Chemistry
SFB	San Francisco Bay Estuary
SFEI	San Francisco Estuary Institute
SIP	State Implementation Plan
SLWMBM	Screening Level Water Mass Balance Model
SMC	Stormwater Monitoring Coalition
SOT	Society of Toxicology
SRM	Standard Reference Materials
SRT	Solids Retention Time
SS	Suspended Solids
SWAMP	California Surface Water Ambient Monitoring Program
SMC	Stormwater Monitoring Coalition
SWPPP	Storm Water Pollution Prevention Plan
SWRCB	State Water Resources Control Board
TEQ	Toxic Equivalent
TIE	Toxicity Identification Evaluation
TMDL	Total Maximum Daily Load
TOC	Total Organic Carbon
TSS	Total Suspended Solids
UF	Uncertainty Factor
US	United States
USEPA	United States Environmental Protection Agency
VTG	Vitellogenin

WDR	Waste Discharge Requirement
WERF	Water Environment Research Federation
WET	Whole Effluent Testing
WHO	World Health Organization
WWTP	Wastewater Treatment Plant
YAS	Yeast Androgen Screening
YES	Yeast Estrogen Screening

1.0 INTRODUCTION

A panel of seven experts was tasked to present the current state of knowledge on the sources, fate and potential effects associated with chemicals of emerging concern (CECs) in aquatic systems in California that receive discharge from municipal wastewater treatment plants and stormwater. Based on this knowledge, the Panel was asked to develop a monitoring strategy to allow managers to make informed policy decisions on CECs. In response, the Panel developed a conceptual approach that focused the universe of possible CECs, considered their likely sources and fates, and adopted a risk-based screening framework to identify CECs that posed the greatest risk to the State's ecological resources and inhabitants. Using existing data on multimedia occurrence and toxicity to sensitive species, the Panel then applied this framework to three representative receiving water scenarios to create an adaptive monitoring strategy for CECs in receiving waters statewide.

1.1 Background

Modern life relies on availability and utilization of natural and synthetic chemicals which may enter ground and surface waters through runoff, industrial and municipal waste discharges, atmospheric deposition, or through releases from septic systems (Figure 1.1). While new chemicals are constantly introduced and others phased out, the concept of humans altering their exposure to chemicals through manipulation of the natural system is as long and rich as human history. Soot analyzed from the ceilings of pre-historic cave dwellings provides evidence of early exposure to potentially hazardous chemicals due to inadequate ventilation of open fires (Spengler and Sexton 1983). Smelting activities during Roman and medieval times caused wide-spread copper and lead pollution, which is detectable today in ice cores from Greenland (Hong et al. 1996). However, the link between water pollution and human illness was not clear until the mid-1800's when Dr. John Snow linked the spread of cholera to contaminated water (Newsom 2006). During this time, raw sewage from London was being conveyed by a primitive sewer system into the Thames River, causing the "Great Stench of London" in the summer of 1858 which threatened to move parliament because of the atrocious odor (Thompson 1991). However, what was more difficult in the 1800's was pinpointing the bacteria and/or chemicals which were responsible for illness and/or odor. Indeed, the ability to detect the presence of a particular chemical in the environment is a function of the analytical or bioanalytical method sensitivity and selectivity, and the concentration of that particular chemical/microbe in the environment. Today, nearly any imaginable chemical can be detected in water given ample sample volume and availability of purified standard material for instrument calibration.

Chemicals of Emerging Concern (CECs) encompass a vast number of chemicals that are generally unregulated in the U.S. or have limited regulation in environmental media (e.g., air, water, sediment and biota) around the world. CECs may include a wide variety of substances ranging from pharmaceuticals to flame retardants to newly registered contemporary use pesticides to newly developed commercial products such as nanomaterials. Generally, with the notable exception of new industrial or pharmaceutical compounds, many of these chemicals have likely been present in water bodies, sediments and tissues but at concentrations that were not detectable by commonly used analytical methods. However, recent advances in qualitative and quantitative analytical chemistry have now allowed detection in various environmental media and have led to initiatives to estimate the potential hazard of CECs. A multitude of chemicals that may be qualitatively identified cannot be quantified due to lack of standards or robust methods of measurement. Thus, regulators in the State of California have been trying to narrow the focus of chemical screening to compounds that have the greatest potential to pose a risk to human and ecological health.

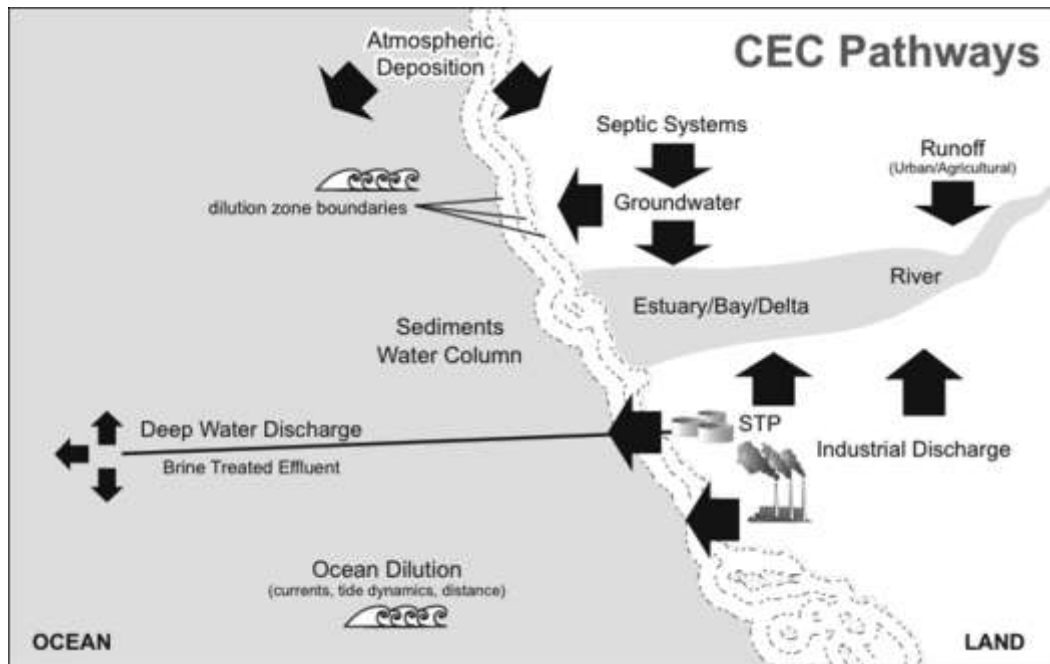


Figure 1.1. Potential sources and pathways for CEC introduction into the aquatic environment.

1.2 The Science Advisory Panel

Recognizing that consideration of CEC effects on aquatic life and human health is a rapidly evolving field and that regulatory requirements need to be based on best available science, the State Water Resources Control Board (SWRCB) established a Science Advisory Panel (“CEC Ecosystems Panel”) to provide guidance in developing monitoring programs that assess the potential ecological impacts and potential threats to human health of CECs in freshwater, estuarine and oceanic water bodies of California. Nominated and vetted through a stakeholder advisory committee represented by the discharger, non-governmental organization (NGO), regulator, and resource communities, the Panel was established in October of 2009 and included seven national experts in the fields of chemistry, biochemistry, toxicology, epidemiology, coastal and marine science, risk assessment, and engineering:

- Dr. Paul Anderson, ARCADIS and Boston University
- Dr. Nancy Denslow, University of Florida
- Dr. Jörg Drewes, Colorado School of Mines
- Dr. Adam Olivieri, EOA, Inc.
- Dr. Daniel Schlenk, University of California-Riverside (*Chair*)
- Dr. Geoffrey Scott, NOAA
- Dr. Shane Snyder, University of Arizona

A brief biography of each panel member and stakeholder advisor is provided in Appendix A. The Panel held six in-person meetings and numerous conference calls. The meetings included the opportunity for stakeholder input in clarifying their charge, exchange of information, dialog with the Panel and

consideration of public comments on the draft report. This report provides the results from the Panel's deliberations.

1.3 Charge to the Panel

The Panel was provided with six specific charge questions, but was generally asked to review the occurrence, relevance, and quantification of CECs in freshwater, estuarine and oceanic water bodies of California with the goal to provide recommendations for development of a monitoring program of CECs in freshwater, estuarine and oceanic water bodies of California. Reference is provided where in the report these charge questions are being discussed.

1. What are the relative contributions of CECs discharged into inland freshwater and coastal aquatic systems¹ from wastewater (including brines and septic tank effluents), stormwater, and atmospheric deposition? **(Section 3)**
2. What specific CECs, if any, are most appropriate for monitoring in discharges to inland freshwater and coastal aquatic systems and what are the applicable monitoring and detection methods and relevant detection limits? **(Sections 6 and 8)**
3. How are these priority constituents affected by the chemistry, biology and physics of treatment in wastewater systems, by discharge into and transport by streams, rivers lakes and estuaries, and as a result of mixing and dilution with fresh, brackish and oceanic receiving waters? **(Section 3)**
 - a. Revised question:
 - i. Which CECs are being removed by treatment?
 - ii. What happens to CECs after discharge into receiving waters?
4. What approaches should be used to assess biological effects of CECs to sentinel species in inland freshwater and coastal aquatic systems? **(Sections 7 and 8)**
5. What is the appropriate design (e.g., media, frequency, locations) for a CEC monitoring and biological effects assessment program given the current state of the art for monitoring methods, and what level of effects will be detectable with such a monitoring program? How does the sensitivity of the monitoring and assessment program vary with investment? **(Section 8)**
6. What concentrations of CECs or levels of biological effects should trigger further actions/assessments and what options should be considered for further actions? **(Sections 6 and 8)**

1.4 Organization of the Report

This report contains 9 sections and 6 appendices. The remainder of this section describes the Panel's conceptual approach to develop monitoring recommendations. Section 2 describes the regulatory framework for CECs in California and analytical issues associated with CEC monitoring. Section 3

¹ *Inland freshwater systems refer to surface waters including streams, rivers, lakes and reservoirs. Coastal aquatic systems are defined as the territorial marine waters as defined by California law, i.e., those extending out to three miles and including releases outside three miles that impact state waters and all ground and surface waters of fresh, brackish or saline water bodies within state boundaries that are hydraulically connected to the coastal ocean.*

addresses the sources and fate of CECs in California's receiving waters and introduces three exposure scenarios developed to test the conceptual approach. Section 4 provides toxicological relevance of CECs and Section 5 summarizes CEC occurrence information. Section 6 describes the risk-based screening framework the Panel developed to identify CECs that pose the greatest risk to aquatic life and human health. Section 7 discusses current and promising future biological approaches to assess exposure and impacts from unknown CECs. Section 8 illustrates the Panel's proposed monitoring program. Section 9 proposes future research and development to improve CEC monitoring and assessment efforts.

1.5 Conceptual Approach

Several reports have recently been published by state and non-governmental agencies to address the potential risks of CECs to ecological and human health (Snyder et al. 2010, Anderson et al. 2010). Many of these reports have utilized a risk-based framework to screen chemicals for monitoring and further study. Risk assessment strategies typically compare environmental concentrations of a chemical of interest with a biological threshold of adverse effect. Quantification of concentrations with quality assurance/quality control (QA/QC) as well as extensive biological characterization under the same conditions is required to reduce uncertainty in these assessments. When evaluating a large number of CECs, the availability of high quality occurrence and effects data is typically limited; thus, substantial uncertainty is often associated with most screening evaluations. While this approach has its limitations, it is currently the most efficient method for identifying CECs that have the greatest potential to pose a risk and require further study until the necessary information for reducing uncertainty can be obtained. The approach adopted by the Panel is illustrated in Figure 1.2 and is described in the following sections.

1.5.1 The Universe of Chemicals

Given the paucity of occurrence and toxicity information for many CECs, it is not possible to currently evaluate the risks of all chemicals detected in waterways. For similar reasons, it is also not possible to evaluate the potential risks of chemicals not yet detected in waterways. Thus, the Panel has categorized the universe of chemicals, including CECs, as follows:

Known knowns - Chemicals for which analytical methods are available for reliable measurement, that have been previously identified in surface waters, and for which measured concentrations are available in California surface waters, sediments, or biological tissues.

Unknown knowns - Chemicals that are known to occur in environmental media, but the concentrations at which they occur have not yet been quantified. Predicted environmental concentrations (PECs) could be developed for such compounds if use and other information are available. Bioassays can also be used to identify the potential presence and effects of such chemicals using toxicity identification evaluation (TIE) procedures.

Unknown unknowns - Chemicals that may be unknowingly released into the environment, or transformed within the environment, and for which there are currently no known identification and quantification methods. Bioassays could also be used to identify the potential presence and effects of such chemicals using TIE.

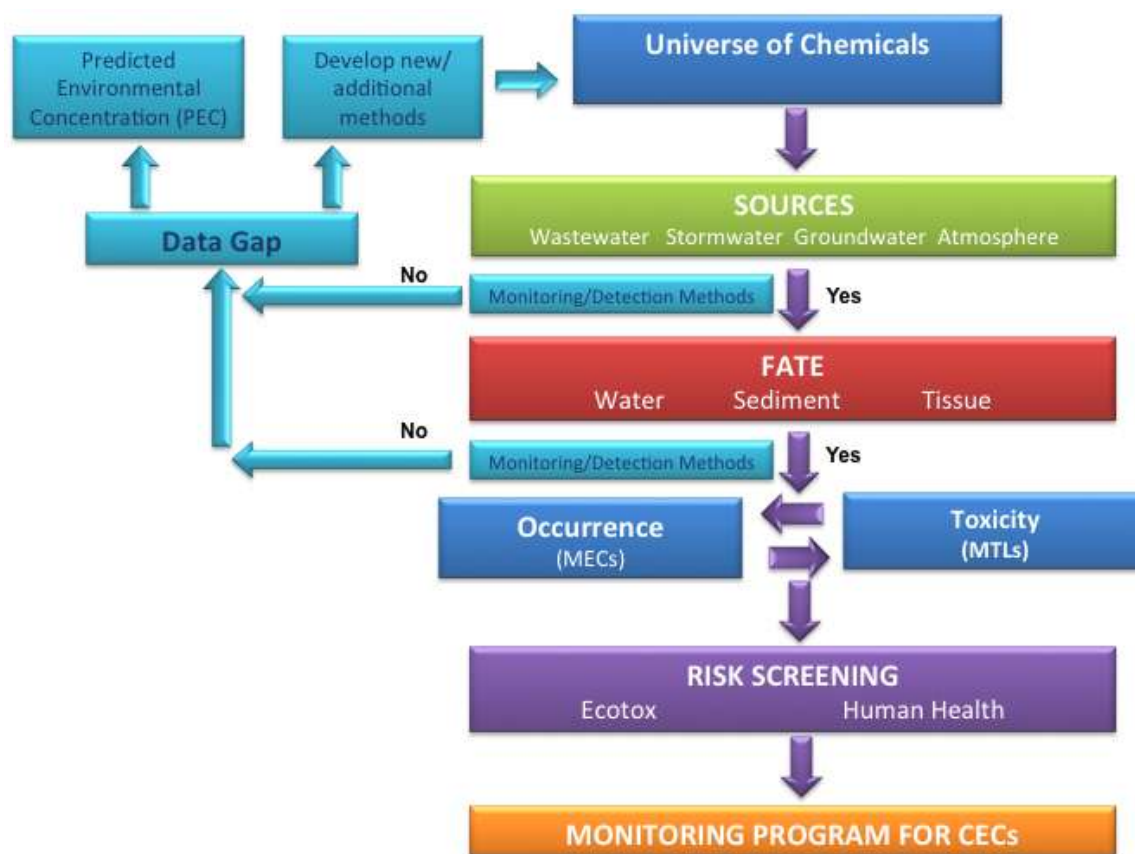


Figure 1.2. Conceptual approach for identifying CECs for risk assessment and monitoring considering both aquatic life and human health.

The universe of known chemicals considered by the Panel was derived from several databases, reports and studies. Compounds that were previously screened through the Science Advisory Panel for determining CECs in recycled water (“CEC Recycled Water Panel”) were initially used to make up the universe. Briefly, these CECs were derived from USEPA’s Candidate Contaminant List 3 (CCL3) (<http://water.epa.gov/scitech/drinkingwater/dws/ccl/ccl3.cfm>) and occurrence data specifically for California wastewater effluent qualities (Anderson et al. 2010). Given that the previous Panel focused specifically on potable reuse and landscape irrigation scenarios, data were only used from secondary or tertiary treated effluents. Chemicals provided by an advisory panel of the Water Environment Research Foundation (Diamond et al. 2011), which evaluated freshwater impacts were also screened as were high production volume chemicals evaluated from recent studies using persistence and bioaccumulation potential (Drewes et al. 2009; Kumar and Xagorarakis 2010; Howard and Muir 2010, 2011). Chemicals that had occurrence values of appropriate quality or methods for measurement were included in the universe as were CECs that were measured in tissues or sediments particularly in California. Review articles that evaluated the risk of CECs in various media from the peer-reviewed literature were also used to identify potential compounds for assessment. Lastly, some chemicals without occurrence data were included for assessment if review of toxicological studies revealed a no observed effect concentration (NOEC) of less than 0.1 mg/L. A preliminary screening was then conducted to develop a focused universe of chemicals to evaluate for monitoring.

The Panel also concluded that methods of detection must be established in at least one of three environmental matrices (water, sediment or biological tissue) (Section 2). Surface water measurements would include samples from freshwater, estuary, and/or oceanic sources. Given the propensity of hydrophobic CECs to partition into sediment organic matter, which can enhance exposure through the food web, sediment measurements would also be needed. Lastly, recent studies have reported the occurrence of CECs within aquatic biota, potentially leading to exposure of birds or mammals, including humans. Given the potential of exposure through the diet, CECs may also need to be measured in biological tissues.

Given the uncertainties associated with *unknown* CECs, the Panel concluded that providing an adaptive framework (i.e., one that can be modified through periodic re-evaluation as additional data or methodologies come forward) is the best approach to develop guidance for assessing the environmental risk of CECs at this time. For example, a recent report discussing current toxicological evaluation of chemicals for human health in the 21st Century has indicated that high through-put *in vitro* biological methods are necessary. Such methods are currently in development to allow regulators to focus on chemicals that elicit specific biological responses associated with “adverse outcomes” (USEPA 2009). While this approach is currently being implemented for human health, its application to ecological risk has not received the same attention.

1.5.2 Risk-based Screening Framework

The approach proposed by the Panel uses a chemical-by-chemical risk-based framework for screening of individual CECs (Figure 1.2). The Panel recognizes that biological methods will likely be used in future assessments for screening and identification of CECs that require specific monitoring. This framework was developed by the CEC Recycled Water Panel, also sponsored by the SWRCB to identify CECs and develop a monitoring strategy for recycled water used for urban landscape irrigation and indirect potable reuse projects throughout California (Anderson et al. 2010). The current Panel built upon this previous work to identify potential sources and CECs of interest (Charge Questions 1 and 2) for subsequent exposure assessment.

Both microbial and non-microbial effects of CECs were evaluated. For non-microbial effects, NOECs for reproduction, chronic growth/survival were adjusted using safety factors to derive monitoring trigger levels (MTLs) (Charge Question 6). The safety factors accounted for: 1) extrapolation of freshwater effects data to saltwater species; 2) CECs having specific modes of action (MOA) on developmental, neuroendocrine or immunological targets in eukaryotic organisms in the literature; and 3) CECs with an unknown MOA. If NOECs were not available, acute LC_{50s} were utilized. The potential for antibiotic resistance (ABR) was evaluated for indicator bacteria or pathogens as a basis for determining adverse effects within microbial communities or increased public health risks associated with recreational water use. The lowest observed concentration causing inhibition of bacterial growth (minimum inhibitory concentration of MIC) was used as the basis for establishing MTLs for antibiotic CECs, incorporating safety factors to account for the range in published MICs and the relative abundance of such published information for various antibiotics.

CECs concentrations for risk-based screening were determined based on measured or predicted environmental concentrations (MECs or PECs) in water, sediment or tissue. The maximum MEC was used as a conservative representation of potential exposure. PECs were calculated using dilution factors for estuary and oceanic sources from WWTP effluent and stormwater model parameters. The Panel

believes developing a process that allows for estimating the possible concentration of CECs in surface water is key to determining whether CECs for which MECs are not available or for which available analytical detection limits are well above toxicological thresholds. In concept, a process to develop screening level predicted concentrations of CECs in waters receiving discharge of WWTP effluent is simple. One needs to know how much of the compound is used each year in a household or per capita, make an assumption about how much water a person or household uses every day, estimate the amount entering a treatment plant, decide how many possible loss mechanisms occur during the use, transport and treatment process, and then predict a concentration in effluent or receiving water. Hannah et al. (2009) describe such a process to develop PECs for ethinylestradiol in US surface waters.

In order to screen the focused universe of CECs to identify those with the greatest potential to pose a risk to either ecological receptors or human health, a risk-based screening framework was developed (Charge Question 4). For each CEC the framework compares the MEC (or PEC) to the MTL to derive a monitoring trigger quotient ($MTQ = MEC \text{ [or PEC]}/MTL$). When the MTQ is less than 1.0, (i.e., $MEC < MTL$) the potential risk associated with a CEC based on currently available information is assumed to not be great enough to require monitoring. When the MTQ is greater than 1.0 (i.e., $MEC > MTL$), a CEC is assumed to have the potential to pose a risk and monitoring is recommended (Charge Question 5). This framework was applied to each of the three scenarios described in Section 1.5.3.

The Panel wishes to stress that an MTQ of greater than 1.0 does not indicate a risk exists. Several conservative assumptions were used to establish the MEC (or PEC) and the MTL. The Panel felt use of such conservative assumptions was appropriate for determining whether a CEC should be included in a monitoring program. Such conservative assumptions need to be refined prior to determining whether a potential risk may actually be present. Thus, a CEC with an MTQ greater than 1.0 can only be viewed as having the “potential to pose a risk;” it does not indicate the CEC poses a risk.

1.5.3 CEC Fate and Exposure Scenarios

A simple water balance model was used to guide the development of three representative exposure scenarios to test the conceptual approach and to provide examples of transport and potential exposure of select CECs to receptors of interest (Charge Question 3). The general fate of CECs in the environment was divided into particulate (bound) or aqueous (dissolved) phases. Subsequently, exposure routes to predict the likelihood of exposure through direct (aqueous) or indirect (dietary) routes were identified for each exposure scenario. The exposure scenarios, introduced below, were selected based upon the most common and relevant discharge scenarios across the State (Charge Question 1) and are further discussed in Section 3.

Scenario 1 - Effluent Dominated Inland Waterway: A highly modified and/or channelized waterway (freshwater) was selected to represent this scenario given the availability of data associated with wastewater and stormwater discharge. Exposures were conservatively assumed to be equal to the concentration of CECs in secondary/tertiary effluents from municipal wastewater treatment plants (WWTPs) or measured values from the literature. This scenario focuses on potential aqueous exposures given that Scenario 2 below uses a model to estimate indirect exposure from particulate-bound CECs generating an outcome that is also applicable to this scenario.

Scenario 2 - Coastal Embayment (“Estuary”): The San Francisco Bay estuary (SFB) was used as an example for this scenario because concentration data and a water flow model were available to the Panel. Aqueous exposures were based on occurrence data for SFB. Indirect exposure was modeled for

2,2',4,4'-tetrabromodiphenyl ether (PBDE 47), a representative hydrophobic CEC. Sediment concentrations derived from wastewater and stormwater were estimated using a 1-dimensional box model and the model output was compared to measured values for general corroboration at a screening level. Tissue concentrations were calculated from bioaccumulation factors derived from residues obtained from biota in SFB. The Panel believes that models of this type can be used to predict sediment and tissue concentrations of similar CECs.

Scenario 3 - Ocean Discharge of Treated Wastewater Effluent: CEC concentrations in off-shore discharges from select WWTPs in Southern California were used to represent conditions for this scenario. Aqueous concentrations of CECs were available for direct exposure assessment, and sediment/biota concentrations were used for indirect exposure assessments.

2.0 CURRENT REGULATORY AND MONITORING PARADIGM

State and federal regulations exist to protect the beneficial uses of California's water resources, ensuring that all fresh, brackish and ocean waters within the State are safe for human contact, harvested foodstuffs are safe to eat, and that aquatic life is not compromised. Monitoring of water quality parameters including chemical constituents is performed at local and regional scales on discharges of treated wastewater effluent and in waters that receive stormwater runoff to ensure compliance with receiving water objectives and effluent limits, water quality guidance, and to help evaluate potential controls (e.g., using conceptual models) (see Box 2.1). The trace measurement of CECs represent a challenge to existing analytical technology and methodologies, and thus requires careful attention to quality assurance/quality control (QA/QC) measures, as well as appropriate designs and planning that adequately address the goals of the monitoring program.

Box 2.1. Key Observations on Current Monitoring Efforts

There is a wide range of monitoring requirements in wastewater and stormwater NPDES permits and in receiving water monitoring requirements and programs in California (see Appendix B). While it is not possible to list and review all monitoring that is required across the State (e.g., operational monitoring of WWTPs and stormwater control monitoring), the following is a summary of key observations based on a review of some of the more significant monitoring efforts underway.

- Substantial resources are expended on the monitoring of regulated wastewater and stormwater discharges and receiving waters.
- Monitoring efforts occur at all levels of government (i.e., local, state, regional and federal levels).
- Monitoring/testing is performed on several media (i.e., water, sediments, tissue, organisms).
- Monitoring efforts address permit compliance with numeric limits for wastewater discharges and, in most cases, receiving water characterization and compliance with water quality objectives for stormwater.
- The specific question(s) to be addressed by monitoring programs are not always clearly evident and/or articulated.
- The use of consistent sampling and analytical testing protocols, and reporting formats is improving through statewide monitoring and data sharing efforts.
- Coordination and collaboration of the various efforts is evident but is largely ad hoc in nature.
- Efforts to link monitoring results with management decisions are being made and need to continue.
- Some monitoring has been initiated on CECs. Such efforts are investigative and are being performed to establish the scientific basis for setting thresholds and/or discontinuing monitoring for constituents with ill-defined occurrence/impacts.

2.1 Regulation of Wastewater and Stormwater in California

Water Quality regulation in California involves the melding of state and federal processes for activities such as setting water quality standards, issuing discharge permits and operating grants programs. Regulation and administration of stormwater (includes municipal, industrial, and construction), industrial and municipal wastewater treatment and disposal, and monitoring is carried out by the State Water Resources Control Board (SWRCB) and nine Regional Water Quality Control Boards (RWQCBs)(see Box 2.2). The SWRCB has overall responsibility for setting statewide policy on the administration of water rights and water quality control. Each RWQCB is responsible for adoption and implementation of

water quality control plans (“Basin Plans”), issuance of waste discharge requirements (WDRs), and performing other functions concerning water quality monitoring and control within their respective regions, subject to SWRCB review or approval.

Box 2.2. Nine Regional Water Quality Control Boards (RWQCBs) in California

In recognition of the regional differences in water quality and quantity, the state is divided into nine regions for the purposes of regional administration of California’s water quality control program. The boundaries of the Regional Boards are based on watersheds, also known as hydrologic areas. (1) North Coast, (2) San Francisco Bay, (3) Central Coast, (4) Los Angeles, (5) Central Valley, (6) Lahontan, (7) Colorado River Basin, (8) Santa Ana, and (9) San Diego.

2.1.1 Clean Water Act

The Clean Water Act (CWA), officially known as the Federal Water Pollution Control Act, was enacted by Congress in 1972. Ten major bills have subsequently revised the 1972 statute. The objective of the CWA is to “restore and maintain the chemical, physical, and biological integrity of the nation’s waters to make all surface waters “fishable” and “swimmable.” The USEPA has delegated authority to California to implement provisions of the CWA. One provision of the CWA prohibits discharge of pollutants into federal waters unless a permit is issued that complies with the CWA. Under federal law, a discharge permit is officially known as a National Pollutant Discharge Elimination System (NPDES) permit. The State and Regional Water Boards issue WDRs that serve as NPDES permits in California.

2.1.2 Porter Cologne Water Quality Control Act (California Water Code – CWC)

The Porter Cologne Act legislation (aka the California Water Code or CWC) was enacted by the California Legislature in 1970. Portions of it became the model for the 1972 CWA amendments. In many respects, the CWC surpasses the federal act, allowing the water boards to comprehensively regulate both surface and ground waters. It also allows the water boards to establish requirements for nearly any source of waste discharge, including nonpoint sources and certain other sources exempted from the federal act’s permitting requirements. It further provides for the adoption of Basin Plans and the implementation of these plans by adopting WDRs for individual dischargers or classes of dischargers.

2.2 Monitoring Regulated Discharges

This section provides a brief summary of monitoring of regulated discharges and receiving waters at the local, regional and state level. Various sections of the CWA and the CWC authorize the State and Regional Water Boards to require technical and monitoring reports. These monitoring requirements are most typically contained in the State discharge permits issued by the Regional Water Boards. A more detailed discussion on State monitoring and reporting requirements is provided in Appendix B.1, which includes several case examples to illustrate the variety, breadth and variability of monitoring efforts.

2.2.1 Wastewater Discharges

The NPDES permit Monitoring and Reporting Program (M&RP) for municipal and industrial wastewater discharges establishes monitoring and reporting requirements to implement federal and State requirements. The monitoring program typically contains definitions of terms, and sets out requirements for reporting of routine monitoring data in accordance with NPDES regulations, the CWC, and RWQCB policies. The M&RP also defines the sampling stations and frequency, the pollutants to be

monitored, and additional reporting requirements. Pollutants to be monitored include all parameters for which effluent limitations are specified. Monitoring for additional constituents, for which no effluent limitations are established, is also required to provide data for future completion of reasonable potential analyses (RPAs).

2.2.2 Stormwater

Regulating and monitoring stormwater is generally addressed as part of State permits and requirements in three main categories described below. Examples of stormwater monitoring are covered in more detail in Appendix B.1.

Municipal Stormwater (MS4s). In 1987, the CWA was amended to specify the requirements for NPDES permits for stormwater discharges. California municipalities are required to comply with CWC² and federal requirements to control the discharge of pollutants in stormwater runoff from their municipal separate storm sewer systems (MS4s). MS4s are regulated by NPDES permits issued by the RWQCBs that contain monitoring, commercial and industrial requirements, inspections and TMDL requirements³. In addition to largest municipal discharger (Caltrans), there are currently 21 Phase I municipal permits and 125 permittees enrolled in the Phase II municipal permit. For example, the San Francisco Bay RWQCB issued a Municipal Regional Permit (MRP) covering 76 local agencies, including cities, counties, and flood management districts, that contains requirements for the following pollutants of concern: Pesticides, Trash, Mercury, PCBs, Copper, PBDEs, Legacy Pesticides, and Selenium.

General Industrial Permit. This is an NPDES permit issued by the SWRCB that regulates discharges associated with 10 categories of industrial activities. There are approximately 10,000 active permittees in this program area. Monitoring requirements are tailored to capture the overall (and not peak) impact of stormwater discharge on receiving waters. The minimum required monitoring is for four indicators (pH, TSS, oil & grease, and specific conductance), and additional constituents can be required based on the industrial category and activity (e.g., ammonia, Mg, COD, As, CN, Pb, Hg, Se, Ag, Fe, Al, Zn).

Construction General Permit. Dischargers whose projects disturb ≥ 1 acres of soil or disturb < 1 acre but are part of a larger common development plan that in total disturbs ≥ 1 acre, are required to obtain coverage under this category. Construction activity subject to this permit includes clearing, grading and disturbances to the ground such as stockpiling, or excavation, but does not include regular maintenance activities performed to restore the original line, grade, or capacity of the facility. This General Permit requires the development and implementation of a Storm Water Pollution Prevention Plan (SWPPP), consisting of a visual monitoring program; a chemical monitoring program to be implemented if there is a failure of best management practices (BMPs); and a sediment monitoring plan if the site discharges directly to a water body listed on the 303(d) list for sediment. The permit requires effluent monitoring and reporting for pH and turbidity in stormwater discharges and suspended sediment concentration

² The California Toxics Rule (CTR) promulgated by USEPA added numeric water quality criteria for a number of constituents (i.e., 30 volatile substances, 58 semi-volatile substances, 15 inorganics, 25 pesticides, and polychlorinated biphenyls (PCBs)) to Water Quality Controls Plans. Subsequently, the State Water Board adopted a State Implementation Plan (SIP) that includes the CTR which states "This Policy does not apply to regulation of stormwater discharges."

³ A total maximum daily load (TMDL) is a plan that is targeted to reduce a specific pollutant in order to meet water quality standards in a 303(d) listed water body. Once a TMDL is developed, the stormwater NPDES permits must be adopted that are consistent with the TMDL.

under certain conditions. In addition, the permit calls for receiving water monitoring (e.g., bioassessments) under high-risk situations.

2.3 Regional, State and Federal Receiving Water Monitoring Efforts

There are several regional, statewide and federal water quality monitoring programs for surface waters within California (see Box 2.3). These programs differ in the geographical extent and specificity, but address many of the same questions regarding the severity, extent and temporal trends associated with chemical contaminants and water/habitat quality, such as:

- Are chemical concentrations cause for concern, and are associated impacts likely?
- What are the sources, pathways, loadings, and processes leading to contaminant-related impacts in receiving waters?
- What future sources, concentrations and potential impacts of contaminants should we be concerned about?

Box 2.3. Regional, State and Federal Water Quality Monitoring Programs

San Francisco Estuary Regional Monitoring Program (RMP) - a collaborative effort among regulators and dischargers to collect data annually on spatial and temporal trends in contamination (including CECs) in water, sediment and biota, evaluate toxic effects on sensitive organisms, and communicate water quality information (<http://www.sfei.org/>).

Regional Monitoring Coalition (RMC) – assesses water quality and the condition of beneficial uses in urban creeks and rivers; investigates stormwater treatment control effectiveness; and geomorphic projects to assess creek restoration and protection (San Francisco Bay Area Municipal Regional Permit Co-permittees).

Southern California Bight Regional Monitoring Program (“Bight”) - a collaborative of several regional programs, focusing on the quality of coastal watersheds, wetlands and the marine environment of the southern California Bight. This multi-component effort has been conducted every 5 years since 1994 (<http://www.sccwrp.org/ResearchAreas/RegionalMonitoring/>).

Stormwater Monitoring Coalition (SMC) Regional Watershed Monitoring Program - a regional watershed monitoring program for stormwater quality in southern California to facilitate greater data collection and provide a regional context to address site- and watershed-specific questions.

California Surface Water Ambient Monitoring Program (SWAMP) - a program to coordinate all water quality monitoring conducted by the State and Regional Water Boards. SWAMP’s mission is to provide resource managers, decision makers, and the public with timely, high-quality information to evaluate the condition of all waters in the State.

Marine Protected Areas (MPAs) and Areas of Special Biological Significance (ASBS) – a statewide collaborative program of more than 30 regulated agencies to define "natural" water-quality conditions in coastal lands and waters subject to restricted uses and discharges.

MARINE and Bivalve Monitoring Programs [The Multi-Agency Rocky Intertidal Network \(MARINE\)](#) partnership assesses habitat quality, species abundance, invertebrate counts, and other surveys in California’s rocky intertidal environments. The National Oceanic and Atmospheric Administration (NOAA) recently re-focused their Mussel Watch Program on CECs, with the initial pilot conducted in collaboration with multiple California entities.

National Coastal Assessments - Regional agencies and statewide programs working in collaboration with the USEPA and other federal entities to determine how the condition of California’s resources compare to the rest of the nation.

Sacramento Coordinated Monitoring Program (CMP) – CMP partnering agencies support many area programs committed to safeguarding river water quality in the Sacramento and American rivers (<http://www.srcsd.com/cmp.php>)

2.4 Analytical Methods to Monitor CECs

A critical component in the ability to assess environmental health is the identification and quantification of CEC in environmental media. The validity of applied methodologies is critical because erroneous data can impact risk calculations, ultimately leading to questionable environmental management decisions. State-of-the-art analytical techniques can now precisely identify and quantify trace concentrations of CECs in the environment. However, the measurement of CECs in environmental matrices often requires methodologies that are not “standardized” and rarely rely on consistent QA/QC protocols. Moreover, the majority of CECs occur in water at levels of less than one $\mu\text{g/L}$, with some relevant to aquatic health at levels of less than one ng/L (Caldwell et al. 2008). A detailed discussion of analytical method considerations for measuring CECs in water is provided in the CEC Recycled Water Panel report related primarily to monitoring for human health considerations through drinking water (Anderson et al. 2010). In addition, numerous reviews have been published regarding the breadth and diversity of available analytical methods (Lee 1999; De Alda and Barcelo 2001; Snyder et al. 2003; Gros et al. 2006; Richardson 2006, 2007). Given that this effort focuses on ecological health, some important differences arise. For instance, many CECs induce adverse effects in aquatic organisms at water concentrations far lower than those that would be expected to cause human health impacts (see Section 4). This is due in part to the greater duration and magnitude of exposure for certain aquatic organisms as opposed to humans who are exposed primary through periodic ingestion of water.

2.4.1 Quality Assurance/Quality Control

Quality control (QC) is the ability to determine and minimize systematic and random errors. A systematic error (or “bias”) is one in which reported values are consistently different from the true value. The ability to reproducibly determine the same value from a given sample is called the precision of the measurement. The ability to determine the true value in an environmental sample is known as accuracy. Random errors are more difficult to track and can affect both the accuracy and precision of an analytical method. Detection of an analyte when it is actually absent is a Type I error (“false positive”), while an error that results in non-detect when the analyte actually is present is a Type II error (“false negative”). Quality assurance (QA) is the step mandated in a particular protocol and/or laboratory to produce accurate and precise analytical data, thus minimizing Type I and Type II errors. Generally, a quality assurance project plan (QAPP) is established before actual environmental testing begins. The QAPP will specify QA/QC procedures that are to be followed and documented at each step of the particular protocol. In environmental monitoring, QAPPs address seven key considerations: problem definition, sample program design, field sampling, sample preparation, chemical analysis, data analysis, and reporting (Batley 1999). The components are discussed in detail in Appendix B.2.

Most aspects of QA/QC for environmental monitoring are well understood and readily attainable by well-regarded scientific research and commercial laboratories. Ultra-trace analysis (sub- ng/L) is inherently more difficult in terms of potential for Type I and Type II error. However, modern analytical techniques such as isotope dilution and automated on-line solid-phase extraction offer tremendous promise for continually improving analytical data. A detailed QAPP is critical in addressing the question(s) for which the particular study was initiated. Ultimately, through proper planning, QA/QC, and ensuring the samples selected and collected are relevant for addressing monitoring goals, accurate and precise analytical data are possible which allow environmental managers to make the best possible decisions.

2.4.2 Unique Analytical Aspects of Tissue and Sediment Analyses

Although the majority of data concerning CECs in the environment are from aqueous samples, the advancement of analytical protocols has allowed for detection of some CECs (e.g., PBDEs and pyrethroids) in sediment and biological tissues (Maruya et al. 1997, Snyder et al. 2001a, Schlenk et al. 2005). The analysis of CECs in these matrices requires additional analytical considerations, e.g., the need to homogenize sediment and tissue samples, which are described in detail in Appendix B.2. Another challenge with tissues and sediments is the degree and complexity of matrix interferences that are co-extracted with the target CECs. Cleanup and/or fractionation steps are typically warranted to isolate the target CECs from matrix interferences as well as co-occurring chemicals. In order to gauge efficiency and method accuracy, parallel analysis of certified and/or standard reference materials (CRMs/SRMs), if available, is highly recommended. Since the availability of such materials is scarce at best, the Panel recommends that the State engage in a dialogue with agencies such as the National Institute of Standards and Technology (NIST) to facilitate the creation of CRMs/SRMs for priority CECs in sediment and tissue matrices.

2.4.3 Non-targeted Analysis for Unidentified or Unknown CECs

Routine monitoring of chemicals in environmental samples relies on *a priori* knowledge of the chemical of interest (so called “targeted” analysis). Instrumental analysis of known CECs (e.g., pharmaceuticals, household and high volume production commercial chemicals) using GC-MS and/or LC-MS/MS requires a purified standard to represent each CEC of interest. While targeted analytical methods allow for reliable quantitation, they are not designed to periodically screen for new or unexpected chemicals (e.g., *unknown* CECs). Howard and Muir (2010) reviewed Canadian and U.S. chemical databases representing ~25,000 substances for chemicals with the potential for persistence and bioaccumulation based on theoretical calculations. They concluded that among the approximately 600 potentially persistent and bioaccumulative compounds, roughly 500 are neglected by targeted monitoring surveys.

Modern analytical tools are available for non-targeted chemical identification and are largely mass spectrometric based (Snyder et al. 2003, Ibanez et al. 2004). For instance, analytical methods and data reporting systems designed to identify and document unknown and/or previously unidentified CECs in controlled reference materials (Hoh et al. 2009) are now coming on-line for environmental samples (Hoh et al. submitted). For relatively non-polar and volatile to semi-volatile organics, sample extracts can be analyzed using a comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC/TOF-MS) system. The GCxGC analytical component allows for enhanced resolution of individual compounds in a complex mixture, with resolved peaks identified by first searching a reference mass spectral database, followed by manual interpretation of spectra not found in existing catalogs. Analysis of a single dolphin blubber sample resulted in identification of more than 270 individual organic compounds representing 20 or more structural homologs or “classes” (Figure 2.1). Approximately 50% of those identified in this example are not routinely targeted for monitoring, and the majority of these can be traced to anthropogenic sources (e.g., polychlorinated styrenes and polybrominated biphenyls). Many water soluble CECs, including most transformation products, pharmaceuticals and personal care products are generally not suitable for GC analyses. Snyder et al. (2001b) utilized liquid chromatography (LC) coupled to high resolution MS to identify novel compounds in the waters of Lake Mead, NV. More recently, the advent of LC coupled with a “QTOF” (a hybrid quadrupole – time of flight) detector can be used in a similar fashion (Vanderford et al. 2008, Perez-Parada et al. 2011). These techniques are especially powerful when combined with statistical software that can differentiate the hundreds of potential compounds identified during these types of analyses (Vaclavik et al. 2011). While such non-targeted methods are useful in creating an inventory of, e.g.,

persistent and bioaccumulative compounds in sediment and tissue samples, they are particularly attractive as a periodic screening (and not a routine monitoring) tool for directing targeted chemical or bioanalytical analysis (i.e., TIEs) as the composition of CECs in WWTP effluent and/or waters receiving stormwater discharge changes in the future.

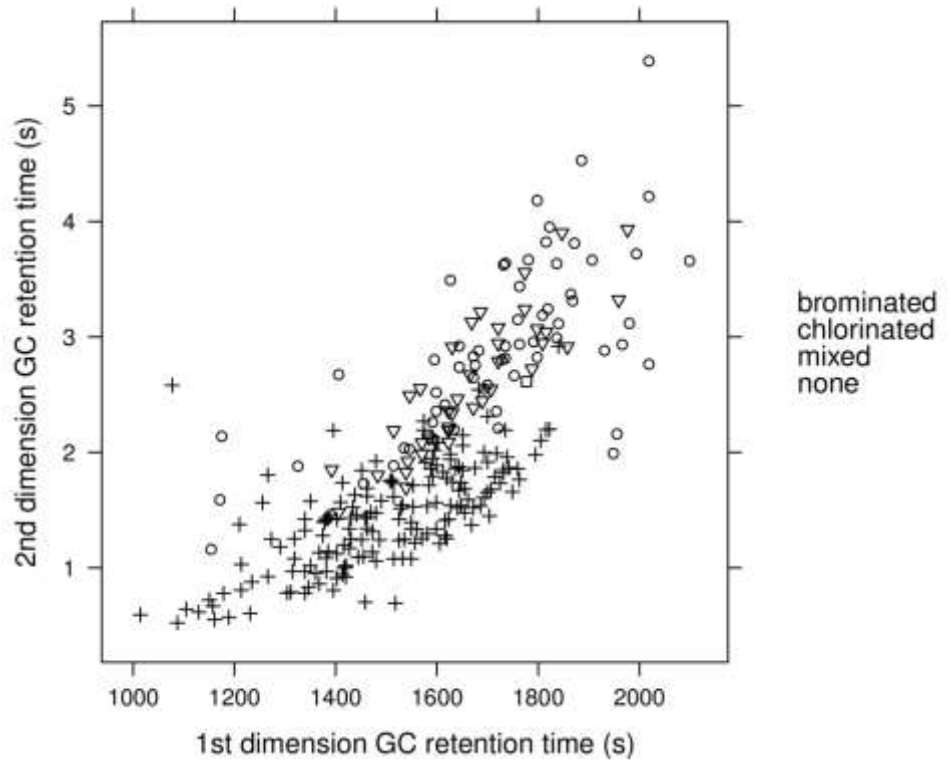


Figure 2.1. Non-targeted analysis using two-dimensional gas chromatography coupled to time of flight mass spectrometry (GCxGC-TOF) identified more than 270 individual compounds in a complex environmental matrix. Approximately 50% of the identified compounds are not routinely analyzed using targeted methods in receiving water monitoring programs. (Hoh et al. submitted).

3.0 CEC SOURCES, FATE AND EXPOSURE SCENARIOS

Treated wastewater effluent and stormwater runoff represent major sources of CECs to California's inland waterways and coastal aquatic systems. Once discharged, CECs can remain dissolved in the water column or can associate strongly with sediment and are subject to transformation and uptake by wildlife. The Panel developed a screening level water mass balance model to address dilution of potential CEC contributions among sources and to generate three scenarios that represent CEC exposure across receiving waters of the State. Within these scenarios (inland, coastal embayment and open ocean), additional models were employed to address CEC exposure to receptors at multiple trophic levels via direct aqueous uptake and through indirect mechanisms (i.e., bioaccumulation and trophic transfer).

The State of California, one of the largest in the USA with more than 155,000 square miles of land populated by more than 37,000,000 people⁴, is home to a wide array of businesses and industries and represents one of the top-10 largest economies in the world⁵ and largest state economy in the USA⁶. California contains 1,700 km of shoreline⁷ along with countless rivers, lakes, and streams. Some areas of Northern California receive abundant rainfall (e.g., nearly 67"/annum⁸) whereas southern California is arid, averaging 10" of rainfall per annum⁹. With its population and economic base comes a tremendous amount of waste potentially containing CECs that, upon discharge, may affect receiving waters throughout the State.

The initial charge to the Panel was to provide recommendations regarding appropriate monitoring strategies for CECs in coastal and marine waters of the State. The Panel considered oceanic waters but also bays and estuaries with brackish water. Subsequent to the initial charge, the SWRCB expanded the Panel's charge to include inland freshwaters. Potential sources of CECs to inland waterbodies have been characterized in the past and are relatively well understood (e.g., direct discharge from wastewater treatment plants (WWTPs)). However, the Panel had limited success in finding CEC occurrence information for stormwater runoff, groundwater and atmospheric contributions, and was unable to find readily available information on the relative magnitude of the other potential sources (e.g., septic systems) of CECs to coastal environments. In response, the Panel created a screening level water-mass balance model to estimate the degree of dilution of CEC sources at and beyond the land-sea interface. The results of this model were used to develop scenarios to represent exposure of CECs in the State's coastal and marine ecosystems. Previously developed models to estimate the fate and effects of CECs were linked and applied to these scenarios to generate MECs for use in the risk-based screening framework (Section 6).

3.1 Sources

Figure 1.1 illustrates the interaction of various sources of CECs with coastal receiving waters. Among the several sources, treated municipal wastewater ("WWTP effluent") and stormwater discharged to inland (ponds, streams, rivers, lakes and reservoirs) and/or coastal receiving waters (bays, estuaries, ocean) are

⁴ <http://quickfacts.census.gov/qfd/states/06000.html>

⁵ http://www.msnbc.msn.com/id/16600877/ns/business-us_business/#.Tv4WadX2LI8

⁶ http://www.usatoday.com/money/economy/2011-06-20-state-gdp-growth_n.htm

⁷ http://resources.ca.gov/ocean/html/chapt_5c.html

⁸ <http://cdo.ncdc.noaa.gov/climatenormals/clim20/ca/042147.pdf>

⁹ <http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?ca7740>

widely regulated and subject to routine monitoring (see Section 2) and are thus of primary interest to the Panel.

3.1.1 Wastewater Treatment Plant (WWTP) Effluent

Effluent discharged from municipal wastewater treatment plants is a major source of CECs to the receiving water environment. Although most CECs occur in trace concentrations ($\mu\text{g/L}$ or lower) in WWTP effluent, the large volume (e.g., close to 1 billion gallons per day into the southern California Bight alone) discharged to receiving waters in California throughout the year can result in total mass loadings that are comparable to regulated environmental contaminants (e.g., heavy metals). CEC concentrations in WWTP effluent vary depending on the strength of wastewater and the level of treatment (Ort et al. 2010). In general terms, the higher the level of treatment (progressing from primary (lowest degree of treatment) to secondary to advanced treatment including reverse osmosis (the highest degree of treatment)), the lower the concentration of CECs (see Box 3.1).

3.1.2 Stormwater Runoff

Stormwater runoff is another source of CECs to California's receiving waters. The total volume of stormwater discharged to receiving waters annually is roughly equivalent to WWTP effluent discharge in the southern California Bight (Lyon and Stein 2009) yet CEC concentration data are scarce. In populated areas of central and northern California, stormwater runoff can be expected to comprise a higher proportion of discharge relative to WWTP effluent due to higher annual precipitation rates (see also San Francisco Bay example for Scenario 2). Unlike WWTP effluent, the vast majority of annual stormwater runoff and discharge occurs during the 6-month wet season (Dec – May) in all but the most arid regions of the State, resulting in greater than 90% of annual CEC loading associated with runoff occurring during this period (Lyon and Stein 2009). The intensity and duration of major precipitation events and their individual contribution to annual CEC loading, however, can vary widely. In contrast to WWTP effluent, discharged stormwater is subject to minimal treatment prior to entering the State's waterbodies. As a result, the level of total suspended solids (TSS) in stormwater typically far exceeds that of WWTP effluent. Moreover, attenuation of CECs present in stormwater occurs primarily *in situ*, i.e., under ambient conditions.

3.1.3 Other Sources

Discharge from septic systems, concentrated brine disposal, dry weather runoff, industrial discharges, groundwater, and atmospheric fallout and exchange (i.e., wet and dry atmospheric deposition) are additional potential sources of CECs to the State's receiving waters. CEC occurrence data are currently very limited for these sources. Evaluation of discharge (controlled and/or incidental) from agricultural operations was not considered by the Panel. The panel acknowledges that agricultural sources may contain CECs such as antibiotics often used in livestock production; however agricultural sources were outside of the scope of this panel and were thus not considered but could be evaluated in the future by this or other panels.

Box 3.1. Effects of conventional wastewater treatment on CEC concentrations in effluent

Removal of CECs in WWTPs depends on their biodegradability and physicochemical properties, such as water solubility, hydrophobicity (as measured by K_{ow}) and volatility. These properties influence whether a CEC will remain in the aqueous phase (like many pharmaceuticals) or sorb to particles that end up as sludge (e.g., estrogens or certain antibiotics) (Stevens-Garmon et al. 2011). Multiple studies have demonstrated that sorption, aerobic and anaerobic biotransformation, abiotic degradation via hydrolysis, and volatilization are the primary attenuation mechanisms for CECs in WWTPs.

Biological unit processes (“Secondary” treatment)

Biotransformation of CECs during secondary treatment consisting of aerobic (trickling filters, activated sludge treatment) and anaerobic (sludge digestion) processes occurs for most CECs. Although degradation of bulk organic matter is well understood (Tchobanoglous et al. 2003), the effects of such treatment processes on ultra-trace level CECs (ppt or ng/L) level have received relatively little study. Several operational factors can influence removal of CECs in activated sludge systems, including biochemical oxygen demand (BOD_5), suspended solids (SS) loading, hydraulic residence time (HRT), solids retention time (SRT), food-microorganism ratio (F/M ratio), mixed liquor suspended solids (MLSS), pH and temperature (Drewes 2007). These operational details, however, are usually lacking in studies reported in the literature. Moreover, determination of CEC biotransformation rates can be extremely difficult due to the large number of unknown products formed (Ternes et al. 2004). No systematic and comprehensive work has described the dimensions of CEC issues in wastewater treatment, including origins, distributions, fate and transport.

Tertiary treatment processes

Tertiary treatment processes are largely ineffective in attenuating CECs. Chang et al. (2007) showed that coagulation was ineffective in removing steroid hormones from secondary effluent over a range of ferric chloride dosages and pH, a finding corroborated by a bench-scale drinking water study on ethinyl estradiol (Westerhoff et al. 2005). Three full-scale WWTPs in Sweden employing only chemical precipitation had no significant reduction in estrogenic activity (Svenson et al. 2002). A fourth plant with only lime softening at pH >11 was more effective, removing 73% of estrogenic compounds. Golet et al. (2003) reported minimal removal of ciprofloxacin ($4 \pm 1\%$) and norfloxacin ($3 \pm 2\%$) using flocculation/filtration, likely due to sorption of fluoroquinolones to remaining particles and precipitates.

Wastewater disinfection processes

Chlorine doses of 10-20 mg/L are commonly applied with contact times >10 minutes for disinfection of wastewater (Tchobanoglous et al. 2003). A common structural characteristic of estrogenic chemicals is the presence of a phenolic ring that is susceptible to transformation upon chlorination. Drewes et al. (2006) collected composite samples before and after chlorination of tertiary effluent (chlorine dose = 3.5 mg/L; 45 minutes contact time). Estrogens present in this effluent were removed below detection limit (<0.4 ng/L). Lee et al. (2004) explored the removal of 17 β -estradiol (E2) during oxidation with free chlorine at 1-7 mg/L. Whereas low chlorine levels required >36 hours for complete E2 removal, a 10 minutes contact time at the highest dose (7.5 mg/L) achieved complete removal. Westerhoff et al. (2005) demonstrated complete removal of steroid hormones in surface water with a contact time of 24 hours using chlorine dosages between 3.5 and 3.8 mg/L. Other CECs (acetaminophen, diclofenac, naproxen, oxybenzone, sulfamethoxazole, and triclosan) also exhibited a high degree of reactivity with chlorine resulting in concentrations below the limit of detection in this study.

To circumvent information gaps, various approaches have been proposed to predict CEC concentrations in WWTP effluent, including those focused on closed systems (Kümmerer et al. 1997), prescription rate and per-capita wastewater volume (Stuer-Lauridsen et al. 2000, Huang et al. 2001) or mass balance approaches (Ternes et al. 2004, Khan and Ongerth 2004). These predictions can only be considered as qualitative due to the lack of and uncertainties associated with input data, and model limitations. However, these studies can assist in highlighting priorities for further research into the fate and transport of CECs during wastewater treatment.

3.2 Fate

Most CECs are attenuated via physicochemical or biological processes in conventional WWTPs, resulting in effluent concentrations that are lower than in raw wastewater (see Box 3.1). Stormwater, on the other hand, is usually subject to minimal (if any) engineered treatment resulting in little (if any) attenuation prior to discharge. Once discharged into receiving waters, CECs are subject to physical, chemical and biological processes that may result in attenuation (lower concentrations), enrichment or magnification (higher concentrations) in a given environmental compartment or media (Figure 3.1).

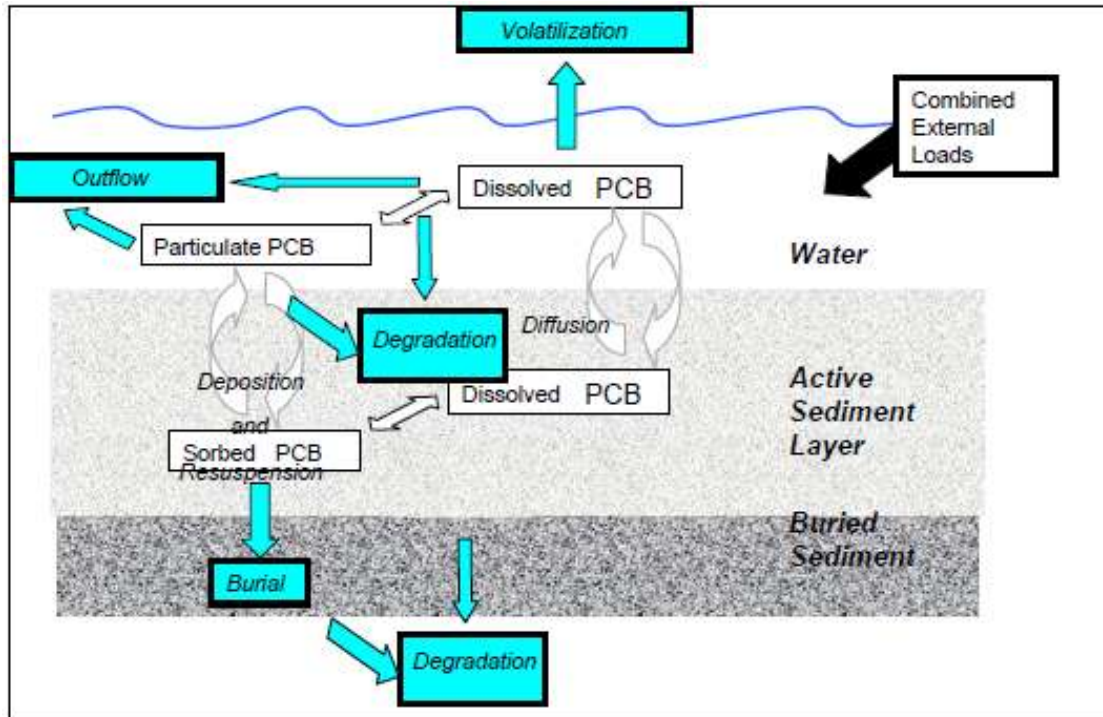


Figure 3.1. Environmental processes that affect the fate of CECs in aquatic systems (Davis 2003).

3.2.1 Aqueous vs. Particle Association of CECs

CECs may be generally classified as water soluble (hydrophilic) or insoluble (hydrophobic) based on the value of their octanol-water partition coefficient (K_{ow}). Water soluble CECs ($\log K_{ow} < 3$) are preferentially found in the aqueous (dissolved) phase and can be transported with water masses (advection), transformed via abiotic and biological pathways, and can impact aquatic organisms via direct uptake and subsequent toxicological activity. In contrast, un-ionized hydrophobic compounds (those with $\log K_{ow} > 3$) tend to be associated with suspended or bedded sediment particles in aquatic systems, with the fraction sorbed increasing with increasing K_{ow} . CECs may also leave the aquatic environment through volatilization, a property measured by the Henry's Law constant (K_H) or vapor pressure. The physicochemical characteristics of the receiving water can also influence the fate and concentrations of CECs, particularly temperature, salinity and pH. In general, CECs become less soluble with decreasing

water temperature and increasing salinity. The solubility and speciation of CECs with ionizable structural moieties (e.g., acid and basic groups) may be affected by pH; however, the relatively narrow pH range in most receiving waters (6-8) minimizes this impact for many CECs.

3.2.2 Transformation and Persistence

CECs can be transformed by abiotic and biological processes. Examples of abiotic processes acting primarily on water soluble CECs include photodegradation and hydrolysis. Biodegradation by microorganisms is the primary biotransformation process acting on CECs in both the water column and in bedded sediments. In the latter medium, both abiotic and biological processes are possible; however, in most cases attenuation of light at the sediment-water interface limits photo-induced transformation. Biotransformation can occur under both oxidizing and reducing conditions, however, transformation rates are typically lower under reducing conditions. CECs that are resistant to transformation are referred to as persistent. In water, persistent compounds can be transported over large distances via currents and other mass water movement, whereas in sediment persistent CECs become buried in deeper compacted layers in depositional environments. In contrast, compounds that undergo transformation in engineered or natural systems but whose source/input levels are high and constant enough to counterbalance transformation rates are called “pseudo-persistent”.

3.2.3 Wildlife Exposure

Wildlife living in receiving waters can be exposed to CECs via direct uptake of aqueous phase (dissolved) CECs (i.e., via uptake across gill membranes and skin) and through ingestion of prey containing CECs (i.e., indirect or food chain exposure). In trophic transfer and biomagnification, un-ionized, hydrophobic, recalcitrant CECs can accumulate in lower trophic level organisms and “transfer” their CEC body burden to higher trophic level organisms that consume them as food. Ingestion of particles (e.g., sediment ingestion) may also contribute to overall exposure. For un-ionized CECs, the relative contribution of indirect (i.e., via the diet) versus direct (i.e., via gills and skin) exposure to overall exposure increases with increasing K_{ow} . Benthic organisms may be exposed to hydrophobic CECs via direct uptake of dissolved CECs in sediment interstitial water.

3.3 Exposure Scenarios

Based on the charge to the Panel in the Fall of 2009 of applying current knowledge to coastal and marine receiving waters, the Panel formulated two scenarios that address discharge of WWTP effluent and stormwater into coastal embayments (Scenario 2) and ocean discharge of WWTP effluent (Scenario 3). Upon expansion of the charge in early 2011 to include all receiving waters within California, the Panel developed a single scenario to cover freshwater systems that are highly affected by WWTP effluent discharge (Scenario 1). The following sections describe each of these scenarios (see Box 3.2) in more detail, as well as the assumptions and tools used to further develop and define these scenarios.

Box 3.2. Exposure Scenarios Representing California's Receiving Waters.**Effluent dominated freshwater systems in California (Scenario 1)**

With its Mediterranean climate, California is home to streams, rivers, ponds and lakes where WWTP effluent constitutes the majority of base flow/volume during the 6-month dry season (June-Nov). The source water in these systems is municipal wastewater subjected to tertiary (or better) treatment prior to discharge ("WWTP effluent" see also Section 3.1). Waterbodies that fit this description include the Los Angeles, San Diego, San Gabriel and Santa Ana Rivers. Numerous impoundment, ponds and small (recreational park) lakes across the State, particularly those in urban centers adjacent to WWTPs, also fall within this category. *The primary concern in this scenario is wildlife exposure to the aqueous phase CECs discharged in WWTP effluent.* However, CECs derived from stormwater runoff as well as indirect dietary uptake of CECs from particulate bound materials considered in Scenario 2 (Coastal embayment) are also relevant to this scenario.

Coastal embayment – San Francisco Bay estuary (Scenario 2)

California is home to dozens of coastal embayments and estuaries that receive discharge from both WWTP effluent and stormwater runoff. None is larger than San Francisco Bay, a vibrant aquatic ecosystem covering up to 4,160 km² and home to ~8 million residents, and fed in large part by the Sacramento-San Joaquin River delta. CECs are continually discharged via WWTP effluent at a number of locations within the Bay, whereas stormwater from local watersheds contribute largely during the wet season (Dec – May). The quality of the Bay's receiving waters is closely monitored through a collaborative managed by the San Francisco Estuary Institute (SFEI) known as the Regional Monitoring Program (RMP). A conceptual mass-balance model that treats the entire Bay as a single "box" was employed by the Panel to estimate CEC concentrations in water and sediment based on inputs from WWTP effluent, the delta and stormwater. The output of this "1-box model" is coupled to biota-sediment accumulation factors (BSAFs) to predict CEC concentrations in prey items of ecological receptors at the top of the Bay food web, for subsequent comparison with tissue-based MTLs in the risk-based framework (see Section 6).

Ocean discharge of WWTP effluent to the southern California Bight (Scenario 3)

The five coastal counties of southern California are home to more than 20 million residents, making this coastline among the most densely populated in the U.S. WWTP effluent is discharged into the Bight at mid-Shelf depths (50 to 100 m) from facilities that are collectively capable of discharging ~ 1 billion gallons of effluent per day. The quality of the Bight's receiving waters and the health of its ecosystem are monitored every 5 years through a collaborative managed by the Southern California Coastal Water Research Project (SCCWRP) known as the Bight Program. The screening level water mass balance model (SLWMBM) developed by the Panel (see Section 3.3.2.1) was used to estimate dilution factors in 3 regions of increasing distance from the coastline that were then applied to determine MECs for aqueous exposure in the Panel's risk assessment (see Section 6). As in Scenario 2, BSAFs were used to predict CEC concentrations in ecological receptors at the top of the marine food web, for subsequent comparison with tissue-based thresholds.

3.3.1 Scenario 1 - Effluent-dominated Inland Waterway

Scenario 1 represents an inland freshwater waterway during summer low-flow conditions where WWTP effluent is the dominant source of CECs (see Box 3.2 for examples). Because stormwater and other possible CEC sources (i.e., groundwater or atmosphere) are assumed to have little to no influence on the loading and concentrations of CECs under these conditions, concentrations derived from WWTP effluent are used to determine MECs and/or PECs. This represents a conservative assumption given the likely possibilities of dilution by non-effluent inputs and *in situ* transformation of CECs resulting from photo-oxidation and/or biotransformation in the receiving freshwater body. The Panel notes, however, that transformation does not always lead to compounds of lesser toxicity and this report does not address metabolites specifically. Dry weather run-off (e.g., incidental urban runoff) that may also contain detectable concentrations of CECs is assumed to be a negligible source of CECs relative to WWTP effluent in this scenario, based on a flow contribution as low as 2% of the total water discharge (Stein and Ackerman 2007). Although this scenario is limited to low-flow conditions, the Panel acknowledges

that stormwater input during the wet season can result in CEC exposure to ecological receptors in inland freshwater systems, a situation that is addressed in Scenario 2 (see Section 3.3.2.2).

3.3.2 Coastal and Marine Scenarios

3.3.2.1 Modeling CEC Source Contributions

To better understand the relative importance of the major CEC sources to California's coastal and marine environments, the Panel created a screening level water mass balance model (SLWMBM) based on inputs to the Southern California Bight (SCB). Details and model assumptions are described in detail in Appendix C.1. Briefly, the model divided the SCB into three regions based on distance from shore and estimated the amount of water entering each region from multiple CEC sources including (1) WWTP effluent; (2) stormwater discharge; (3) precipitation; (4) coastal groundwater discharging into the ocean; and (5) ocean currents causing advection of seawater into and out of each region. The Panel acknowledges that other sources of CECs could also be contributing to CEC loads in these constructs. For example, certain areas of the SCB (as well as other parts of the coast of California) have historical sediments that contain compounds that are herein considered CECs and that may contribute CECs to the environment (e.g., phthalates).

The relative importance of modeled CEC sources was estimated by calculating the dilution factor for each source and coastal region. Within each region, dilution factors were estimated for four different ocean current scenarios as represented by exchange volume in Table 3.1. The Panel notes that several observations become apparent from a comparison of dilution factors, keeping in mind that these dilution factors assume complete and instantaneous mixing within each of the three modeled regions.

- **Mid- and off-shore regions.** Dilution factors for source inputs range from 27 for the mid-shore region to 36,000 for the off-shore region. A recent study on CECs in treated effluent and receiving seawater from large WWTP outfalls in the SCB suggested outfall dilution¹⁰ factors of ~1000 or more in near-bottom water (Vidal-Dorsch et al. 2011). These dilution factors are large enough to suggest that investigating effects associated with offshore discharges is not a priority compared with effluent-dominated freshwater systems (Scenario 1) and near-shore coastal releases (Scenario 2). If potential effects are predicted or demonstrated for the latter scenarios, then further assessment of effects associated with offshore discharges may be warranted.
- **Near-shore.** Dilution factors were lowest for combined WWTP and stormwater (5 to 71) and highest for rainfall (200 to 2,600). (In the SLWMBM "rainfall" refers only to precipitation that falls directly on the portions of the ocean being evaluated. "Stormwater" refers to the precipitation that fell on land and runs off to a receiving water.) The next lowest dilution is predicted for WWTP effluent (9 to 120), followed by groundwater (27 to 360). Note that the potential for near-field effects at coastal discharge locations is not ruled out by SLWMBM results and that relatively low dilution of WWTP discharges in effluent dominated coastal waterways would occur under dry season, low-flow conditions.

¹⁰ When pollutants are introduced into receiving waters, they are subject to physical processes which result in their dilution, one of the main processes that reduces their concentration after discharge. Dilution is more important for reducing the concentration of conservative (e.g., metals) vs. non-conservative substances (e.g., some organics). Dilution capacity can be defined as the effective volume of receiving water available for dilution. The effective volume can vary according to tidal cycles and transient physical phenomena such as stratification and rainfall. The process of dilution can be separated into initial dilution and secondary mixing. For microbial agents a third attenuation process is die-off. In general, discharges occur through pipes and diffusers located below the air-water interface, and wastewater or stormwater qualities (sometimes blended with brines) contain a mixture of pollutants. Initial dilution occurs as the buoyant discharge rises towards the surface because of the density differential between saline receiving waters and freshwater effluents. Secondary dilution occurs as part of the vertical and horizontal dispersal of the discharge plume until the density and thus pollutant concentration differential becomes inconsequential.

- Precipitation. Dilution factors ranging from 200 to 26,000 were estimated for rainfall in all coastal regions. Unless a CEC is found to be present at substantially higher concentrations in rainfall than in WWTP effluent, stormwater or groundwater, precipitation is not likely to represent an important source of CECs to inland freshwater systems or coastal oceanic waters.

Table 3.1. Dilution Factors for CEC sources in three coastal regions using a screening level water mass balance model (SLWMBM).

Dilution Factors for Different Coastal Regions					
Ocean Current (km/day)	Rainfall	WWTP Effluent	Stormwater	WWTP and Stormwater	Groundwater
Near-Shore Coastal Region (0-1 km)					
0	200	9	14	5	27
1	440	20	30	12	60
5	1400	63	97	38	190
10	2600	120	180	71	360
Mid-Shore Coastal Region (0-5 km)					
0	200	44	68	27	140
1	440	98	150	59	300
5	1400	310	480	190	970
10	2600	580	900	350	1800
Off-Shore Coastal Region (0-10 km)					
0	2000	880	1400	540	2700
1	4400	2000	3000	1200	6000
5	14000	6300	9700	3800	19000
10	26000	12000	18000	7000	36000

A cursory review of the modeled dilution factors suggests that the greatest potential for CEC exposure is associated with stormwater and WWTP effluent sources in the near-coastal zone. However, WWTP effluent is not generally discharged immediately adjacent to the shoreline. In the SCB, the large WWTP outfalls are well beyond the 1 km distance that defines the near-shore coastal region. Both stormwater and groundwater are discharged in the immediate vicinity of the shoreline. Second, WWTP effluent and groundwater are discharged continuously to the coastal system and thus, assuming complete mixing, the dilution factors in Table 3.1 may represent the relative long-term impacts of these two sources. In contrast, stormwater does not represent a continuous discharge, with the vast majority of the annual input occurring during the 6-month wet season, often over just a few days with heavy rainfall. During storm events, substantially lower dilution of stormwater may be occurring in the near-shore coastal region than suggested in Table 3.1. The Panel recognizes that this scenario may only be present for the few days during and immediately following a major storm event. However, the Panel believes these relatively short-term, potentially high CEC concentration events should be evaluated closer to determine whether they may pose a risk to aquatic receptors. This evaluation would also be applicable to the potential effects of CECs in stormwater on inland freshwaters (see Section 3.3.1).

Beyond providing insight about the relative importance of different sources of water to the SCB, the SLWMBM could also be used to combine CEC occurrence data for the various sources released to the SCB. With that information, a mass balance for key CECs could be developed to better understand the relative contributions of the primary input sources to the SCB. For inland waters, measured concentrations of CECs in WWTP effluents and runoff could be used directly to understand the relative importance of those two sources (assuming minimal dilution in an effluent dominated river during low flow conditions).

The observed differences in modeled dilution across the three coastal regions along with the temporal discontinuities of source input led the Panel to create two distinct exposure scenarios for coastal and marine receiving waters, one to represent CEC input at the land-ocean interface and into a coastal embayment (Scenario 2) and the second to address ocean discharge of WWTP effluent to the off-shore marine environment (Scenario 3). In combination with the effluent dominated inland waterway (Scenario 1), the Panel believes the three exposure scenarios represent the broad range of settings where potential effects from CECs may be of concern to California regulatory agencies and the citizens of the State.

3.3.2.2 Scenario 2 – Coastal embayment (“estuary”)

Scenario 2 represents the coastal embayments and estuaries along the California coast that receive either direct or indirect (i.e., upstream) discharge of WWTP effluent and stormwater runoff. In this scenario, the Panel assumes a higher degree of dilution of municipal WWTP effluent as compared to Scenario 1. In addition, stormwater runoff is included as a potential source of CECs. Direct aqueous exposure to CECs present in diluted WWTP effluent and undiluted stormwater are both considered. Because stormwater also transports particle-reactive compounds via suspended sediment that eventually discharge to coastal embayments, indirect exposure to CECs becomes an important route of exposure to wildlife and humans and is considered in this scenario. Because of its unique geographic and demographic characteristics and relative wealth of available CEC data and modeling resources, the Panel selected the San Francisco Bay estuary (SFB) to evaluate potential CEC exposure in this scenario (see Box 3.2).

To estimate CEC source contributions, a mass balance “one-box” model previously developed for PCBs (Davis 2003) was adapted and used to predict concentrations of a model compound 2,2',4,4'-tetrabromodiphenyl ether (PBDE 47) in SFB water and sediments. A full description of this approach is included in Appendix C.2. Selection of PBDE 47 is appropriate for particle-bound CECs based on its hydrophobicity ($\log k_{ow} = 6.81$), low volatility ($k_H = 0.56 \text{ Pa}\cdot\text{m}^3/\text{mol}$), persistence, bioaccumulation, trophic transfer and biomagnification (Shaw and Kannan 2009). The model estimates a roughly equal mass loading contribution of PBDE 47 from WWTP effluent and stormwater discharge into San Francisco Bay (see Box 3.3).

To estimate potential exposure of PBDE 47, biota to sediment accumulation factors (BSAFs) derived from monitoring data were used to estimate tissue concentrations in receptors of interest (i.e., birds, mammals). To validate the estimates, predicted concentrations were compared to measured values obtained from monitoring studies (Shaw and Kannan 2009, Meng et al. 2009). Measured or predicted tissue concentrations were then compared to tissue-based MTLs for PBDEs (i.e., bird eggs) to determine whether monitoring might be required (see Section 6). In addition, an estimate of the range of initial dilution for source inputs throughout SFB is on the order of 10:1 to 100:1, based on modeling assumptions. Based on these results, the CEC screening framework utilizes a conservative initial dilution estimate of 10:1¹¹.

¹¹ Use of the conservative initial dilution of 10:1 for San Francisco Bay is consistent with the conservative assumptions contained in the San Francisco Bay Water Quality Control Plan. This assumption was only made for the purposes of screening CECs as part of the report framework. Initial and secondary mixing dilution in the Bay has been documented at levels equal to or greater than 100:1.

Box 3.3. Estimating contributions of PBDE 47 from treated municipal wastewater effluent and stormwater discharged into San Francisco Bay.

The mass loading of CECs discharged into receiving waters is estimated as

$$\text{Loading } (\mu\text{g/d}) = \text{Discharge (L/d)} * \text{Concentration } (\mu\text{g/L})$$

Using the 1-box mass balance model for San Francisco Bay*,

Discharge from WWTPs: 3.33×10^9 L/d

Avg. WWTP Effluent Concentration: 5.2 ng/L

Discharge from Stormwater: 4.55×10^9 L/d

Avg. Stormwater Concentration: 5.6 ng/L

Loading from WWTPs: 17.3 g/d (~40% of total WWTP, stormwater)

Loading from Stormwater: 25.5 g/d (~60% of total WWTP, stormwater)

In this example, estimated loading contributions of PBDE 47 from WWTP effluent and stormwater are roughly equal (17 vs. 26 g/d). The proportions of WWTP effluent and stormwater discharge into the southern California Bight are reversed, i.e., 40% (stormwater) and 60% (WWTP effluent) (Lyon and Stein 2009). Assuming the average PBDE 47 concentrations above are representative of sources feeding the Bight, the relative loading contribution would be reversed (i.e., ~60% from effluent; ~40% from stormwater). This example illustrates the value of source and receiving water data (i.e., stream flow and discharge rates, and concentrations) and environmental fate models. Please note that the above model estimates show reasonable agreement with a limited validation dataset, and thus will tend to drive the discussion on potential management options.

* from Tables C.3 and C.5 (see Appendix C.1)

3.3.2.3 Scenario 3 – Ocean Discharge of Municipal WWTP Effluent

Scenario 3 represents discharge of CECs associated with WWTP effluent into the coastal ocean. As in Scenario 1, concentrations derived from WWTP effluent are used to determine MECs and/or PECs in this scenario. In this scenario, WWTP effluent is discharged to near bottom waters at mid-Continental Shelf depths (50 to 100 m) where relatively rapid dilution by ambient ocean water can be expected to occur. Marine organisms that inhabit the near bottom and benthic habitat near these outfalls are subject to CEC exposure via direct (aqueous) and indirect mechanisms (near outfall sediment). Like Scenario 2, a number of recent studies on the occurrence of CECs and biological responses of near outfall sentinel organisms have been recently performed at marine outfalls of the SCB (see Box 3.2), and were used to provide the most relevant occurrence data in effluent, water, sediments and fish tissues, which in turn were used to predict food web trophic transfer and biomagnification using the approach described in Scenario 2.

4.0 EFFECTS ASSESSMENT

For non-microbial effects, no observed effects concentrations (NOECs) for survival, growth & reproduction in sensitive aquatic species were compiled from published studies. The Panel targeted compounds with NOECs <0.1 mg/L (100,000 ng/L) for aqueous exposure. The rationale for evaluating only CECs with NOECs <0.1 mg/L was based on the assumption that most compounds occur in concentrations within the ng-µg/L range. If a worst case safety factor of 1000 was applied, then compounds with (NOEC/1000) in the ng/L range may exceed one and pose a potential risk. Sediment NOECs were obtained only for CECs with known occurrence data. The NOECs for compounds linked to antibiotic resistance were estimated based on the most sensitive minimum inhibitory concentrations (MICs).

4.1 Assessing Non-Microbial Toxicity Endpoints

Substantial information exists about toxicity of CECs to environmental species including fish and other aquatic species in the literature. The Panel reviewed the literature to find the most sensitive species and relied heavily on studies that examined survival, growth and reproduction. The literature review revealed that bacterial communities, birds, invertebrates, fish, and mammals/humans can be affected by CECs. A current listing of no observed effects concentrations (NOECs) for CECs was compiled (see Appendix D, Tables D-1 and D-2) for use in deriving monitoring trigger levels (MTLs) for the risk-based framework. As described above, the Panel restricted the focused universe of chemicals to those with NOECs <0.1 mg/L (Table 4.1).

To determine the most sensitive species and associated NOECs, we made use of two main databases: the EPA EcoTox web site (URL: <http://cfpub.epa.gov/ecotox/>) and the MistraWikiPharma database (URL: <http://www.wikipharma.org/welcome.asp>) (Molander et al. 2009). Both are updated frequently. In addition, we checked *Pubmed*, *SciFinder Scholar* and *Web of Science* for journal articles that provided information about toxicity. References within these databases were then individually checked for accuracy for CECs with calculated MTQ >1 in any of the exposure scenarios (see Section 3). A review of the manuscripts that describe toxicities for these substances appears in Appendix D.

The sentinel species considered included fish, algae, and invertebrates. Microbes were also considered for antibiotic resistance (ABR) and are discussed separately below. Human health was also evaluated because of concerns about potential exposures associated with consumption of fish. It is likely that different species will be the most sensitive in different scenarios, depending on their life-cycles, their exposure and assimilation of CECs and whether or not they live in the water column or sediments. The majority of the studies were for freshwater species. Few studies of salt water species were identified. We added a 10-fold safety factor for sensitivities in salt water if only fresh water data was available. In some cases saltwater may reduce the toxicities of some chemicals, but to be conservative, the above safety factor was incorporated. Studies in salmonids and other euryhaline fish have indicated enhanced toxicity in hypersaline conditions (El-Alfy and Schlenk 1998, Wang et al. 2001, El-Alfy et al. 2001, Bawardi et al. 2007, Lavado et al. 2009a). If a predicted no effect concentration (PNEC) that incorporated responses from saltwater species was provided, no safety factor was administered. Most of the toxicity studies surveyed included chronic exposures and measured survival, reproduction, and growth, the most important endpoints for use in environmental risk assessment. However, for some of the chemicals, only acute exposures have been investigated. In some cases, the only toxicity information that exists is changes in physiology (e.g., heart rate), tissue morphology (e.g., histopathology) or biochemical function (e.g., enzymatic action). These are described in the appendix for compounds that

had MTQs >1. We did not use manuscripts with new molecular data that are based on gene expression changes, as these have not yet been vetted for estimation of potential risk.

In each case, we attempted to find a NOEC for CECs. However, NOECs could not be estimated from every study. Some of the studies measured the actual concentrations used in their experiments, but yet others relied on nominal concentrations. In cases where nominal data were available we used the lowest observed effects concentration (LOEC) for the endpoint to be conservative in our estimation of threshold. In a few cases, the lowest value reported was the LC₅₀, thus care should be taken in the final evaluation of the NOECs. The panel recognized the discrepancies associated with using deterministic metrics of toxicity (i.e., NOECs) and recommends that MTLs be regularly revisited as additional data becomes available so that studies that use a more probabilistic assessment of toxicity (i.e., Species Sensitivity Distribution) may be used. We have grouped the compounds by route of exposure, treating aqueous and sediment exposure generically (i.e., across all scenarios) (see Section 6).

The panel did not conduct specific toxicity assessments for stormwater since only one CEC in the focused universe (Table 4.1; bisphenol A) was detected in a special study to obtain data for this informational gap (Table 5.3). The panel recognized that risk assessments of stormwater CECs should likely use acute toxicity thresholds given the shorter duration of exposure. Since chronic NOEC or PNEC values are used in this assessment, an additional level of conservatism is included, because chronic threshold values tend to be less than acute threshold values.

There are also new compounds that have been recently discovered to have robust toxicologic effects in aquatic species, but for which there may be very scant occurrence data. It is critical to start collecting occurrence data for these to make sure they do not pose a risk in California receiving waters. In particular, progestogens and glucocorticoids have come to the attention of Europeans and new work is currently being pursued on both the effects and occurrence side on these chemicals. Detailed descriptions of the toxicological properties of all compounds are in Appendix D.

4.2 Human Health

The Panel also considered the need to develop monitoring trigger levels (MTLs) based on the potential effects of CECs released to receiving waters on human health. For most CECs considered, the potential for human health exposure occurs if receiving water is used as a potable water supply and people are exposed by drinking this supply. The Panel assumed such potential exposures are limited to freshwater settings (i.e., Scenario 1). Because the focus of the CEC Recycled Water Panel was identification of CECs for monitoring in reused water (i.e., potable water supplies), this Panel did not evaluate potential drinking water exposures again as part of Scenario 1. This Panel also judged potential direct contact exposures to CECs in receiving waters (e.g., while swimming or wading) to be small enough to not warrant quantitative evaluation. Such exposures are anticipated to be small because frequency of contact is low for most people and dilution is expected to be high in coastal waters (see Section 3.3.2.1).

The other potential human health exposure pathway the Panel considered was exposure to CECs via the consumption of aquatic organisms. While most CECs are not expected to bioaccumulate in aquatic biota (i.e., finfish and shellfish), CECs with a log K_{ow} greater than 3, that remain largely un-ionized in receiving waters and are not rapidly metabolized by aquatic organisms, have the potential to bioaccumulate (see occurrence chapter 5). While this Panel did not have the resources to conduct an exhaustive review of the bioaccumulation potential of all the CECs evaluated in this report, the Panel selected PBDEs as a model bioaccumulative CEC to demonstrate how such a compound might be evaluated for inclusion in a

monitoring program. For PDBEs the establishment of an allowable concentration in fish consumed by humans is based on the Fish Consumption Goal (FCG) of 310 µg/kg recently derived by the State of California (<http://oehha.ca.gov/fish/gtlsx/pdf/PBDEs052311.pdf>). FCGs are based solely on public health considerations relating to exposure to each individual contaminant, without regard to economic considerations, technical feasibility, or the counterbalancing benefits of fish consumption are based solely on public health considerations relating to exposure to each individual contaminant, without regard to economic considerations, technical feasibility, or the counterbalancing benefits of fish consumption.” (<http://oehha.ca.gov/fish/gtlsx/pdf/PBDEs052311.pdf>). Concentrations of PBDEs predicted to be in fish (based on a biota sediment accumulation factor (BSAF) derived from turbot liver data in the SCB, see Appendix C) were not found to exceed this threshold. The Panel believes this approach can be used to derive FCGs for other CECs, as long as CEC-specific reference doses (RfD) are available.

Table 4.1. CECs with toxicity NOECs less than 0.1 mg/L in fish and non-fish species.

Fish	Non-Fish	Non-fish (cont.)
p-nonylphenol*	AHTN	Ibuprofen*
octylphenol	p-nonylphenol**	Miconazole
AHTN (tonalide)	octylphenol	Nonylphenol monoethoxylate (NP1EO)
Atrazine	Atenolol	Octocrylene
Bisphenol A (BPA)	Atorvastatin	PBDE-47, PBDE-99**
Chlorpyrifos	Atrazine	Permethrin*,**
Cis-androstenedione*	Azithromycin [#]	PFDA
Diclofenac*	Bifenthrin***	PFOS
Droperinone	Bis (2-ethylhexyl) phthalate**	Progesterone
17-beta estradiol (E2)*	Butylbenzyl phthalate**	Sulfamethoxazole [#]
Estrone*	Carbamazepine	Testosterone
Galaxolide	Chlorpyrifos*	Triclosan [#]
Ibuprofen*	Ciprofloxacin [#]	Trimethoprim [#]
Levonorgestrel	Desulfinyl fipronil	Ziprasidone
Miconazole	di-n-butylphthalate**	
Nonylphenol monoethoxylate (NP1EO)	Erythromycin [#]	
PBDE-47	Fenofibrate	
PBDE-99	Fipronil*	
Permethrin	Fluorouracil	
Propranolol	Fluoxetine	
Setraline	Galaxolide*	
Triclosan	Gemfibrozil	

[#]Antibiotic resistance

*CECs with monitoring trigger quotient (MTQ) >1 in at least one of the scenarios (see Section 6).

**CECs with calculated MTQ >1 in sediments.

4.3 Assessing Microbial and Antibiotic Resistance Hazards of CECs

In this section we describe the process used to identify NOECs for antibiotic mortality and resistance for subsequent risk assessment (Section 6). Antibiotics may adversely affect bacteria resulting in death at high clinical, therapeutic doses whereas at lower doses bacteria may survive and adapt to exposure by mutations which may result in development of antibiotic resistance (ABR) (Spellberg et al. 2011, Uyaguari et al. 2011). Minimum Inhibitory Concentrations (MICs) are generally the antibiotic concentrations that will cause bacterial death. MICs are routinely reported for every antibiotic. Published MIC data for *E. coli* or other gram negative bacteria (including some pathogens) commonly used in water quality or environmental monitoring or research were used to determine toxicity thresholds (see Appendix D; Table D-3) for antibiotics that have been measured in monitoring studies conducted throughout the US (Parveen et al. 1997, Van Dolah et al. 2000, Huang et al. 2001, Koplin et al. 2002, USGS 2002, Webster et al. 2004, NOAA 2011) or in monitoring studies in California (Klosterhaus 2010, Maruya et al. 2011).

Standardized metrics for reporting ABR have been developed for medical/clinical settings, such as most resistant MIC values which determine what levels of bacteria have adapted to antibiotic exposure. Most environmental reporting of ABR has focused on levels in WWTP plant effluents or surface waters and use methods modified from medical applications. Standardized ABR methods have not been developed for many aspects of environmental settings, and in particular have not been applied to sediments and tissue. Exposures to antibiotics in the environment are generally lower than therapeutic doses that kill bacteria (Pomati et al. 2006, 2008). Consequently, there has been concern that ABR may occur in these instances. In addition, it has been suggested that ABR may be conferred not only from chemical exposure to antibiotics but from gene mutations associated with plasmids (packets of external DNA) exchanged between naïve and antibiotic resistant bacteria [e.g., bacterial conjugative plasmids, transposons, and integrons (Bennett 2008, Garriss et al. 2009)]. This panel focused on aqueous exposures for Scenarios 1 and 2 because only aqueous MIC and NOECs were available (see Section 6). The Panel could not locate sediment- or tissue-based MICs and NOECs and therefore did not evaluate the potential hazards of antibiotics and antibacterial levels in sediments and tissues.

5.0 OCCURRENCE OF CECs

Multimedia occurrence data for CECs were compiled using a tiered relevance framework with preference given to data generated within California. To impart conservatism to subsequent risk analyses, maximum concentrations of CECs in WWTP effluent, receiving waters receiving stormwater runoff, other CEC sources, sediment and biological tissues were considered. For aqueous exposure scenarios, the compilation effort was further focused by considering only those CECs for which NOECs <0.1 mg/L (= 100,000 ng/L) (described in Section 4) have been reported. Peer-reviewed literature values for other geographical regions were considered when no data from California were identified for a specific CEC.

5.1 Introduction

The State of California has a rich database of environmental monitoring data (Section 2). Beyond what has been mandated, water agencies, universities, government agencies, environmental groups, and others have conducted numerous studies for a diversity of environmental chemicals in a breadth of matrices. It is plausible that California has accumulated more environmental monitoring data than any other State in the USA. However, new chemicals continue to be developed and subsequently introduced to the environment. Additionally, analytical methodologies continue to improve in sensitivity and selectivity, constantly unveiling new discoveries of chemicals in the environment. The CEC Recycled Water Panel described monitoring programs undertaken through water recycling programs, and aggregated monitoring data for CECs in WWTP effluent from 2007 through 2009 provided by water agencies within the State (Section 5; Anderson et al. 2010). Because such data result from targeted analysis, the list of chemicals amassed by the previous Panel do not represent all possible or plausible chemicals within State waters, but a small snapshot of those for which analytical methods were developed or available. More importantly, analytical detection limits often are more a function of practical analytical capability as opposed to ecosystem or health bioeffects relevance.

As part of the current Panel charge, we investigated the occurrence of those organic chemicals which have documented, or propensity, to induce adverse biological effects to ecological receptors in multiple environmental matrices, including WWTP effluent, receiving waters, sediment and biological tissue (Figure 5.1). This required the panel to consider not only wastewater treatment monitoring data, but also those monitoring data available for California rivers, streams, atmosphere, estuaries, and coastlines. The Panel also did not interpret their charge to include biological vectors (i.e., bacteria, viruses, and prions) nor inorganics (i.e., arsenic, chromium, perchlorate). From our investigation, 82 organic chemicals were selected for initial screening as a “focused universe of CECs” based on the prioritization of pharmaceuticals and personal care products, endocrine disrupting chemicals, and persistent and bioaccumulative organic chemicals in commercial use and in water (Kumar and Xagorarakis 2010; Howard and Muir 2010, 2011; Diamond et al. 2011), availability of toxicological information (e.g., NOECs <0.1 mg/L) needed to compute MTQs and maximum occurrence criteria consistent with the NOEC criteria (Table 5.1). In addition, five commercial laboratories were contacted to determine which of the 82 CECs selected were available for analysis. Only 17 of the 82 CECs (21%) were not analyzed by one of the four responding laboratories; however, it is possible that other commercial laboratories do offer analytical services for the 17 CECs (Table 5.1). It is important to note that compounds not listed in Table 5.1 were not considered for further consideration, including those reported in various studies throughout the State.

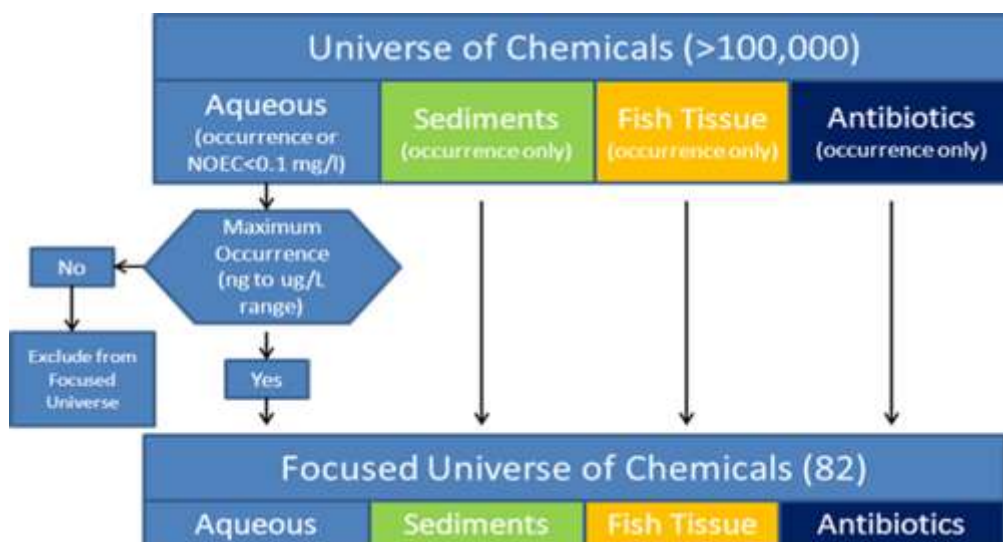


Figure 5.1. Chemicals and environmental media considered in the CECs selection process.

5.2 CECs in Source and Receiving Water

5.2.1 Effluent-dominated Freshwater System (Scenario 1)

Table 5.2 summarizes the maximum concentrations of CECs representing the focused universe of chemicals used to represent exposure to undiluted WWTP effluent that is discharged to freshwater ecosystems, and in some cases the actual receiving surface water (Scenario 1). Because there were far more available occurrence data for water in this scenario for most CECs, as compared with the other scenarios, the panel decided to adopt the following hierarchical tiered approach to evaluate and include occurrence data for CECs:

1. (highest relevance) CEC Recycled Water Panel for WWTP effluents;
2. from within the State of California;
3. from within the United States;
4. (lowest relevance) from foreign countries; and
5. no occurrence data could be located.

Using this ranking system, greatest reliance is upon those data which were compiled by the CEC Recycled Water Panel. This is justified because the current (“Ecosystems”) Panel had general confidence in the QA/QC applied and in the locations from which samples originated. Tier 2 data come from both agency reports and peer-reviewed published literature for samples originating in California. Tier 3 data originate primarily from peer-reviewed literature and government documents demonstrating occurrence within the United States. When multiple references were located for Tier 3 data, deference was given to peer-reviewed published literature if all other considerations were equal. Tier 4 (lowest relevance) data come from peer-reviewed literature from foreign countries. Because of differences in regulations and environmental management, the panel believed that data originating from samples collected in foreign lands maybe less representative of US conditions. Tier 5 is for those compounds for which no environmental occurrence data could be located.

Table 5.1. Individual compounds for which occurrence data were included in this study, their primary use, and commercially available laboratory services (“L”).

Compound	Primary Use(s)	L	Compound	Primary Use(s)	L
17-alpha estradiol	Steroid hormone	Y	Hydrocodone	Pain medication/narcotic	Y
17-beta estradiol	Steroid hormone	Y	Ibuprofen	OTC Pain reliever	Y
Acetaminophen	OTC Pain reliever	Y	Iopromide	X-ray contrast media	Y
AHTN (tonalide)	Synthetic fragrance	N	Levonorgestrel	Pharmaceutical/synthetic progestin hormone	Y
Amphetamine	Pharmaceutical/Illicit Drug	Y	Meprobamate	Pharmaceutical/anti-anxiety, tranquilizer	Y
Atenolol	Heart medication/beta-blocker	Y	Metformin	Pharmaceutical/diabetes	Y
Atorvastatin	Cholesterol medication/statin	Y	Miconazole	Pharmaceutical/anti-fungal	Y
Atrazine	Herbicide	Y	Naproxen	OTC Pain reliever	Y
Azithromycin	Pharmaceutical/antibiotic	Y	NP1EO	Alkylphenol surfactant (one ethoxylate)	Y
Beclomethasone	Pharmaceutical glucocorticoid, asthma treatment	N	NP2EO	Alkylphenol surfactant (two ethoxylates)	Y
Benzophenone	Sunscreen ingredient	N	Octocrylene	Sunscreen ingredient	N
Bifenthrin	Pyrethroid insecticide	Y	Octylphenol	Alkylphenol surfactant degradant	Y
Bisphenol A	Monomer of epoxy/polycarbonate	Y	o-Hydroxy atorvastatin	Pharmaceutical degradant	N
Butylated hydroxyanisole	Food additive/anti-oxidant	N	Oxybenzone (benzophenone-3)	Sunscreen ingredient	Y
Butylated hydroxytoluene	Food additive/anti-oxidant	N	PBDE -47	Brominated flame retardant	Y
Butylbenzyl phthalate	Plasticizer for PVC	Y	PBDE -99	Brominated flame retardant	Y
Carbamazepine	Pharmaceutical/anti-seizure	Y	Permethrin	Pyrethroid insecticide	Y
Chlorpyrifos	Organophosphate insecticide	Y	PFBA	Perfluorinated organic chemical	Y
Ciprofloxacin	Pharmaceutical/antibiotic	Y	PFDA	Perfluorinated organic chemical	Y
Cis-androstenedione	Steroid hormone	Y	PFDoA	Perfluorinated organic chemical	Y
Clarithromycin	Macrolide antibiotic	Y	PFHxS	Perfluorinated organic chemical	Y
DEET (N,N-diethyl-meta-toluamide)	Insect repellent	Y	PFNA	Perfluorinated organic chemical	Y
Diazepam	Pharmaceutical/anti-seizure, anti-anxiety	Y	PFOA	Perfluorinated organic chemical	Y

Table 5.1 (cont.)

Compound	Primary Use(s)	L	Compound	Primary Use(s)	L
Diazinon	Organophosphate insecticide	Y	PFOS	Perfluorinated organic chemical	Y
Di-n-butylphthalate	Plasticizer	Y	PFOSA	Perfluorinated organic chemical	Y
Diclofenac	Non-steroidal anti-inflammatory drug	Y	PFUdA	Perfluorinated organic chemical	Y
Dilantin	Pharmaceutical/anti-convulsant	Y	p-Hydroxy atorvastatin	Pharmaceutical degradant	N
Bis(2-ethylhexyl) phthalate (BEHP)	Plasticizer for PVC	Y	p-nonylphenol	Alkylphenol surfactant degradant	Y
Drospirenone	Pharmaceutical/synthetic progestin hormone	N	Prednisolone	Pharmaceutical anti-inflammatory hormones	Y
Erythromycin	Pharmaceutical/antibiotic	Y	Progesterone	Steroid hormone	Y
Estrone	Steroid hormone	Y	Propranolol	Pharmaceutical/anti-anxiety, beta-blocker	Y
Fenofibrate	Cholesterol medication/fibrate	N	Sertraline	Pharmaceutical/anti-depression	Y
Fipronil	Phenylpyrazole insecticide	N	Sulfamethoxazole	Pharmaceutical/antibiotic	Y
Fipronil desulfinyl	Degradant of phenylpyrazole insecticide	N	TCEP	Chlorophosphate flame retardant	Y
Fipronil sulfide	Degradant of phenylpyrazole insecticide	N	TCPP	Chlorophosphate flame retardant	Y
Fipronil sulfone	Degradant of phenylpyrazole insecticide	N	Testosterone	Steroid hormone	Y
Fluorouracil	Pharmaceutical/cancer treatment	N	Triamterene	Pharmaceutical/diuretic	N
Fluoxetine (Prozac)	Pharmaceutical/anti-depression	Y	Triclocarban	Antimicrobial compound	Y
Furosemide	Pharmaceutical/diuretic	Y	Triclosan	Antimicrobial compound	Y
Galaxolide (HHCB)	Synthetic fragrance	Y	Trimethoprim	Pharmaceutical/antibiotic	Y
Gemfibrozil	Cholesterol medication	Y	Ziprasidone	Pharmaceutical/anti-psychotic	N

Table 5.2. Maximum aqueous concentration of CECs (ng/L) with a monitoring trigger quotient >1 (Scenarios 1 and 2; see Section 6, Tables 6.1 and 6.2).

Compound	CEC Ecosystems Panel			WERF CEC5R08a		
	Data Tier	Matrix	ng/L	ng/L	Data Tier	Matrix
Bifenthrin	2	Surface	85	NA	5	No Data
Bisphenol A	1	Effluent	520	12000	3	Stream
Chlorpyrifos	2	Effluent	190	310	3	Stream
Diclofenac	1	Effluent	230	2500	4	Stream
17-beta estradiol	1	Effluent	8.4	74	3	Stream
Estrone	1	Effluent	73	112	3	Stream
Galaxolide (HHCB)	2	Effluent	2780	970	3	Reservoir
Ibuprofen	1	Effluent	1000	27256	4	Effluent
Permethrin	2	Surface	45.8	0.27	3	River

The primary sources for occurrence data within each Tier are 1) results from the Recycled Water Panel investigation, 2) studies provided and/or executed by regional entities including SCCWRP and SFEI (including special studies), 3) the Water Environment Research Foundation (WERF) trace organic chemical database published in 2010 (see below), and 4) published literature identified via Thomas Reuters Web of Knowledge™ (<http://wokinfo.com/>) search engine. The literature search was performed using “topic search” with initial keywords of the CEC name followed by “California”. If no relevant data were found with the above search criteria, the additional search of CEC name followed by keywords “wastewater OR river” was performed. When numerous results were obtained, the five most recently published manuscripts were reviewed and ranked in accordance with the tiered system described previously. All values were vetted through the Panel, at times resulting in the review and incorporation of additional data. Table 5.2 provides the aqueous occurrence values used by the panel for CECs with a monitoring trigger quotient greater than 1 (see Section 6). The occurrence data for all 82 CECs evaluated is provided in Appendix E.

In addition to peer-reviewed literature and published reports, the Panel considered relevant data from certain completed and on-going studies from within the State of California. For instance, the Los Angeles RWQB, SCCWRP and collaborators are nearing completion of a CEC occurrence study on surface water samples collected in 2011 from two effluent dominated freshwater river systems in southern California. Maximum surface water concentrations for selected CECs obtained from this study are provided in Table E.1. Maximum concentrations reported from this study were generally similar to those reported in the Recycled Water Panel report (Tier 1 data element).

The aforementioned WERF study in 2010 entitled, “Diagnostic Tools to Evaluate Impacts of Trace Organics” (Project CEC5R08a) served as a supplemental occurrence database on a national scale (Table 5.2 and Table E.2). A summary of the findings of this study have recently been published in peer-reviewed literature (Diamond et al. 2011). For the Panel’s compounds of interest, the occurrence metric used by the WERF team for risk valuation was considered and the data source located (data sources provided in Table E.2). Using the tiered structure described, selected CEC concentrations from WERF CEC5R08a were utilized. When considering Tier 3 data (US values other than California), the Panel chose to rely on peer-reviewed and published studies in open literature preferentially to government documents and agency reports.

5.2.2 Storm, Rain, and Embayment Water (Scenario 2)

Occurrence data for the focused universe of CECs in storm, rain, and embayment water were relatively sparse. The tiered hierarchical approach described previously also was utilized for the occurrence metrics for storm and embayment water. A literature review using Web of Knowledge™ was conducted as described previously, only substituting the keywords “storm*”, “rain”, and “bay” in place of rivers and streams. Occurrence data obtained via a study that was performed in support of the current panel was designed to determine storm and rain water contribution of CEC concentrations. Surface grab samples were collected in March 2010, and February and May of 2011 from urban streams in southern California and the San Francisco Bay margins during storm events. A single rainwater sample was collected using a stainless steel funnel and bucket from the roof of the SCCWRP building in Costa Mesa during the March 2010 storm event. Different analytical laboratories were used for each sampling event, therefore the targeted compounds vary. Between the two methods, 35 of the panel’s selected CECs were evaluated. The entire occurrence dataset for rain and stormwater is provided in Table E.3, while occurrence values for CECs with a MTQ greater than 1 (see Section 6; Table 6.2) are provided in Table 5.3.

5.2.3 WWTP Effluent Discharged to the Coastal Ocean (Scenario 3)

SCCWRP and collaborators investigated the occurrence of certain CECs at four ocean outfalls from large wastewater treatment plants in southern California (Vidal-Dorsch et al. 2011). Samples were collected quarterly for one year. Effluent samples were collected from the final effluent of the wastewater treatment plant before ocean discharge, whereas seawater samples were collected near the outfall close to the seafloor. The results from this monitoring can be found in Table E.4. A literature review was conducted as described previously to locate CEC occurrence studies relevant to ocean water. It should be noted that many of the highest levels in effluent assumed to be discharged to the ocean were from samples taken from a WWTP operating at an advanced primary level of treatment. The CEC concentrations measured in primary effluent were not considered in the evaluation of aqueous occurrence provided in Table 5.2, as this particular effluent represents a unique discharge to the marine environment and is not representative of WWTP discharges to inland or nearshore waters.

Table 5.3. Maximum concentration of CECs (ng/L) in stormwater and rainwater with monitoring trigger quotients >1 (Scenario 2; see Section 6, Table 6.2).

Compound	SCCWRP Stormwater Max (ng/L)	SCCWRP Rainwater Max (ng/L)	Data Tier	Literature Max (ng/L)	Matrix
Bifenthrin			2	29.8	Urban Runoff
Bisphenol A	14357	500	3	158	Urban Runoff
Chlorpyrifos			2	220	Urban Runoff
17-beta estradiol			2	3*	Ag Runoff
Estrone			4	52*	Ag Runoff
Galaxolide (HHCB)			5	NA	
Permethrin			1100*	2	Ag Runoff

*= estimated value; Ag = agricultural
NA = no data available

5.3 CECs in Sediment and Biological Tissue

5.3.1 Sediment

Maximum concentrations of CECs in ocean and embayment sediments are provided in Table 5.4. Data were obtained from the aforementioned southern California ocean outfall and San Francisco Bay studies, as well as from selected California studies including surveys of pyrethroid insecticides and PBDEs as part of the 2008 Southern California Bight Program.

5.3.2 Tissue

Maximum concentrations of CECs in biological tissue of various freshwater, estuarine, and marine species are summarized in Table 5.5. Data were obtained from various sources and include monitoring programs for fish, pinnipeds, and bird eggs. Many of these data were provided by the San Francisco Estuary Institute (SFEI) and are available electronically (<http://www.sfei.org/rmp/wqt>). The majority of data in Table 5.5 are from samples collected within California; however, freshwater fish tissue data from a US study of wastewater dominated surface waters also were utilized (Ramirez et al. 2009).

Table 5.4. Maximum concentration of CECs (ng/g dry weight) representing a focused universe of chemicals in California ocean and estuary sediments. NM = not measured

Compounds	Ocean ng/g	Estuary ng/g	References
Acetaminophen	NM	ND	(Klosterhaus 2010)
Bifenthrin	NM	80	(Maruya et al. 2011)
Butylbenzyl phthalate	100	NM	(Maruya et al. 2011)
Carbamazepine	0.12	NM	(Maruya et al. 2011)
DEET	NM	3	(Klosterhaus 2010)
Diazepam	0.07	NM	(Maruya et al. 2011)
Di-n-butylphthalate	44	NM	(Maruya et al. 2011)
Bis(2-ethylhexyl) phthalate (BEHP)	490	NM	(Maruya et al. 2011)
Erythromycin	NM	3	(Klosterhaus 2010)
NP1EO	NM	40	(Klosterhaus 2010)
NP2EO	NM	19	(Maruya et al. 2011)
PBDE -47 + 99	NM	171	(Oros et al. 2005)
Permethrin	NM	190	(Lao et al. 2010)
p-nonylphenol	420	86	(Klosterhaus 2010, Maruya et al. 2011)
Sulfamethoxazole	NM	1	(Klosterhaus 2010)
Triamterene	NM	11	(Klosterhaus 2010)
Triclocarban	NM	33	(Klosterhaus 2010)
Triclosan	8.6	40	(Klosterhaus 2010, Maruya et al. 2011)
Trimethoprim	NM	18	(Klosterhaus 2010)

Table 5.5. Maximum concentration of CECs (ng/g wet weight) representing a focused universe of chemicals in biological tissues.

Compound	Ocean Fish Liver	Estuary Fish	Freshwater Fish (non-CA data)	Pinnipeds	Mussels	Bird Eggs	References
AHTN (tonalide)			290				(Ramirez et al. 2009)
Amphetamine					4		(Klosterhaus 2010)
Atenolol					0.3		(Klosterhaus 2010)
Carbamazepine			3.1		5		(Ramirez et al. 2009)
DEET					14		(Klosterhaus 2010)
Diazepam	110						(Maruya et al. 2011)
Erythromycin					0.1		(Klosterhaus 2010)
Galaxolide (HHCB)			2100				(Ramirez et al. 2009)
NP1EO					41		(Klosterhaus 2010)
NP2EO					192		(Klosterhaus 2010)
Octylphenol					ND		(Klosterhaus 2010)
PBDE - SUM 47+99	480			33700*	10.4	14596^	(Oros et al. 2005, She et al. 2008, Meng et al. 2009, Ramirez et al. 2009, Maruya et al. 2011)
PFDA						28.3	(Sedlak and Greig 2012)
PFDoA		3.4				19.5	(Sedlak and Greig 2012)
PFHxS						40.1	(Sedlak and Greig 2012)
PFNA						39.5	(Sedlak and Greig 2012)
PFOA						28.7	(Sedlak and Greig 2012)
PFOS		43.4			0.2	1760	(Klosterhaus 2010, Sedlak and Greig 2012)
PFUdA						10.7	(Sedlak and Greig 2012)
p-nonylphenol	360				95		(Klosterhaus 2010, Maruya et al. 2011)
Sertraline			19		1		(Ramirez et al. 2009, Klosterhaus 2010)
Triamterene					0.6		(Klosterhaus 2010)
Triclocarban					2		(Klosterhaus 2010)
Triclosan			5.2		ND		(Ramirez et al. 2009, Klosterhaus 2010)

* sum of 14 PBDE congeners reported

^ calculated wet weight value based on lipid weight value from She et al. (2008)

6.0 RISK-BASED SCREENING FRAMEWORK

CEC-specific risk screening was performed by estimating monitoring trigger quotients (MTQs) defined as the ratio of the monitoring trigger level (MTL) derived from NOECs obtained in Section 4 to the measured or predicted environmental concentration (MEC or PEC) identified in Section 5. This approach was applied to aqueous, sediment and tissue matrices as appropriate for the three exposure scenarios described in Section 3. CECs with MTQs that exceeded unity were considered for monitoring as described in Section 8.

6.1 Background

The Panel used a risk-based framework to identify those CECs with the greatest potential to pose a risk to California receiving waters and, therefore, which should be considered for monitoring. The risk-based approach simply divided the measured environmental concentration (MEC) or the predicted environmental concentration (PEC) by the MTL to derive a monitoring trigger quotient (MTQ). If the MTQ exceeded 1.0, the Panel assumed the CEC posed a sufficiently large potential to pose a risk to be considered for monitoring. When the MTQ was equal to or less than 1.0, the Panel assumed the potential risk associated with the CEC did not currently warrant consideration for monitoring.

The Panel wishes to stress that an MTQ of greater than 1.0 does not indicate a risk exists. Several conservative assumptions were used to establish the MEC (or PEC) and the MTL. The Panel felt use of such conservative assumptions was appropriate for determining whether a CEC should be included in a monitoring program. Such conservative assumptions need to be refined prior to determining whether a potential risk may actually be present. Thus, a CEC with an MTQ greater than 1.0 can only be viewed as having the “potential to pose a risk;” it does not indicate the CEC poses a risk.

MTLs were derived by dividing toxicity benchmarks (e.g., NOECs, LOECs, predicted no effects concentration (PNEC), etc.) by appropriate safety factors. To be conservative each non-ABR safety factor was assigned a value of 10. Safety factors were applied to a: CEC with an unknown mode of action (MOA); to CECs where a potential endocrine disrupting mode of action was not incorporated into either the PNEC or NOEC; to extrapolate from freshwater to saltwater; and to extrapolate from an acute to chronic NOEC. A safety factor of 100-1000 was used to derive ABR MTLs from ABR NOECs. For some CECs and exposure scenarios, MTLs were derived without the use of a single safety factor, for other CECs, multiple safety factors were used, as appropriate.

The Panel adopted a tiered risk-based screening approach that focused on the most sensitive receptor of interest for each of the three exposure scenarios (see Section 3). Aqueous concentrations and NOECs were used in every scenario, with PECs developed for Scenarios 2 and 3 by applying dilution factors of 10 and 1000, respectively, to secondary WWTP effluent when MECs were not available. Indirect exposure using sediment and tissue values were determined for Scenario 2. When available, sediment and tissue based thresholds of effect were used for MTQ determination. If NOEC values were not available through the literature, EPA’s ECOSAR were used to estimate effects, and the lowest NOEC was utilized. Non-bacterial MTQs will be addressed in Sections 6.2 and 6.3 with bacterial endpoints (ABR) addressed in Section 6.4.

6.2. CEC Monitoring trigger quotients

6.2.1 Aqueous Exposure for Effluent-dominated Inland Waterway (Scenario 1)

Nine compounds exceeded thresholds for aqueous exposure to CECs in scenario 1 (Table 6.1). Current use pesticides had the highest MTQs with pyrethroids in the 50-200 range. While only two pyrethroids were evaluated in the current screening, the Panel notes that permethrin and bifenthrin were used as models, and it is likely that other pyrethroids of similar occurrence and potency would also present MTQ values exceeding unity. This also applies to diazinon, (like chlorpyrifos) an organophosphate insecticide. The steroid hormone 17-beta estradiol, the hormone degradate/metabolite estrone was also above unity as were the pharmaceuticals ibuprofen and diclofenac. The MTQs for the fragrance galaxolide (HHCB) and industrial plasticizer bisphenol A were also above one.

Table 6.1. CECs with Monitoring trigger quotients >1 for aqueous exposures in effluent dominated inland waterways (Scenario 1).

Compound	MEC (ng/L)	NOEC or PNEC (ng/L)	Safety Factor	Freshwater MTL (ng/L)	MTQ
Bifenthrin	85	4	10 ^a	0.4	210
Permethrin	46	10	10 ^a	1	46
Chlorpyrifos	190	50	10 ^a	5	38
Estrone	73	6	1	6	12
Ibuprofen	1000	1000	10 ^b	100	10
Bisphenol A	520	60	1	60	8.7
17-beta estradiol	8.4	2	1	2	4.2
Galaxolide (HHCB)	2780	7000	10 ^b	700	4.0
Diclofenac	230	1000	10 ^b	100	2.3

^aEDC mode of action not incorporated into PNEC or NOEC

^bUnknown mode of action

6.2.2 Coastal Embayment (Scenario 2)

To estimate exposure, PECs were derived from MECs obtained in Scenario 1 with a 10-fold dilution to simulate embayment dilution. Table E.4 shows the relationships between measured values in San Francisco Bay and the PECs derived from dilution. The panel felt that since aqueous values from Scenario 1 were well characterized, it would be more consistent to use the diluted Scenario 1 values rather than measured values for a relatively few number of compounds in SF Bay receiving waters.

6.2.2.1 Aqueous Exposure

Seven compounds had MTQs greater than 1.0 for Scenario 2 (Table 6.2). Most of these also exceeded unity for Scenario 1 (Table 6.1) indicating a high priority for potential monitoring.

6.2.2.2 Sediment exposure

Data from a limited number of studies and for a handful of CECs were available for estuarine and marine sediments (see Table 5.4). Permethrin, bifenthrin and PBDE 47/99 were detected in estuarine sediments

allowing for comparison to MTLs. All four compounds had an MTQ greater than 1.0 (Table 6.3). The NOECs used for MTQ calculation are not normalized for organic carbon and thus are considered by the Panel to be quite conservative given the uncertainty. An additional safety factor of 10 was included since threshold values were derived from acute toxicity rather than chronic NOEC endpoints of reproduction, growth or survival (see Section 4 and Appendix D). The occurrence of bifenthrin and permethrin in sediments and in aqueous Scenarios 1 and 2 provides additional evidence of enhanced prioritization for pyrethroid monitoring.

Table 6.2. Monitoring trigger quotients >1 for aqueous exposure for coastal embayments.

Compound	MEC (ng/L)	PEC (ng/L)	NOEC or PNEC (ng/L)	Safety Factor	Estuarine MTL (ng/L)	MTQ
Bisphenol A	14400**	ND	60	10 ^a	6	2400
Bifenthrin	30**	ND	4	100 ^{a,b}	0.04	750
Permethrin	46*	4.6	10	100 ^{a,b}	0.1	46
Chlorpyrifos	220**	ND	10	10 ^b	1	220
Estrone	73*	7.3	6	10 ^a	0.6	12
17-beta estradiol	8.4*	0.84	2	10 ^a	0.2	4.2
HHCB –Galaxolide	2780*	278	7000	100 ^{a,b}	70	4.0

^aFreshwater to saltwater

^bEDC mode of action

*Values are from freshwater

**Stormwater

PEC = estimated concentration assuming an initial dilution of 10:1

ND = no dilution

Table 6.3. CECs with Monitoring trigger quotients >1 for sediment exposure in coastal embayments.

Compound	MEC (ng/g)	NOEC (ng/g)	Safety Factor	Estuarine Sediment MTL (ng/g)	MTQ
Bifenthrin	80	5.2	1000 ^{a,b,c}	0.052	1500
PBDE-47; -99	171	3	100 ^{a,b}	0.03	5700
Permethrin	190	73	1000 ^{a,b,c}	0.073	2600

^aFreshwater to saltwater

^bEDC mode of action

^cAcute to Chronic NOEC

6.2.3 Ocean Discharge of Municipal WWTP Effluent

6.2.3.1 Aqueous Exposure

No CECs had an MTQ of greater than 1.0, primarily due to the assumed nominal 1000-fold dilution that is observed at these near bottom marine outfalls located in 50-100 m on the mid-Shelf (see also Section 3). The Panel appreciates that dilution for some ocean outfalls may be less than the 1000-fold assumed above. For such outfalls the framework created by the Panel has the flexibility to incorporate a lower

dilution factor and derive a monitoring list of CECs specific to that outfall. It bears pointing out that even if the 1000-fold dilution factor assumed by the Panel is decreased to 100-fold, the proposed list of CEC's for monitoring at ocean outfalls would change very little; it would consist of a single compound, bifenthrin (with an MTQ equal to approximately 2). This suggests that unless dilution is less than approximately 200-fold, MTQs at ocean outfalls will be less than or equal to 1.0 and monitoring of surface water would not be required. Readers should also appreciate that the Screening Level Water Mass Balance Model developed by the Panel considers only dilution (see also Section 3.3.2.1). Other factors (e.g., degradation) would also serve to reduce the concentration of CECs in surface water in the vicinity of ocean outfalls. Therefore, actual MTQs associated with ocean outfalls are likely to be lower than those based strictly on the results of the SLWMBM.

6.2.3.2 Sediment exposure

Five CECs associated with ocean outfall sediments had MTQs greater than 1.0 (Table 6.4). The two highest MTQs were for butyl benzyl phthalate and the sum of PBDE 47 and 99. These PBDEs were also identified in sediment exposures for the coastal embayment (Scenario 2) (Table 6.3) and suggest high prioritization for monitoring.

Table 6.4. CECs with Monitoring trigger quotients >1 for sediment exposure in the ocean discharge of municipal WWTP effluent.

Compound	MEC (ng/g)	NOEC (ng/g)	Safety Factor	Marine Sediment MTL (ng/g)	MTQ
Bis(2-ethylhexyl phthalate (BEHP)	490	1300	10 ^b	130	3.8
p-nonylphenol	420	1400	100 ^{a,b}	14	30
PBDE-47; -99	4.4	3	10 ^b	0.30	15
Butylbenzyl phthalate (BBP)	100	63	10 ^b	6.3	16

^aFreshwater to saltwater

^bEDC mode of action

6.3 Tissue-based MTQ Calculations

As described in Section 3, CECs that are considered hydrophobic ($\log K_{ow} > 3$), remain un-ionized in either freshwater or saltwater environments and that are persistent have the potential to bioaccumulate in aquatic biota. The resulting risk can be posed directly to the organism in which a CEC accumulates if its concentration exceeds a critical body burden. The potential risk associated with bioaccumulated CECs can also be indirect, i.e., by trophic transfer and biomagnification in higher trophic level receptors (e.g., birds, marine and terrestrial mammals). Moreover, an organism with a sub-critical CEC body burden that comprises the majority of the diet of a higher level trophic receptor may pose an unacceptable risk to the predator organism should biomagnification result in a CEC concentration that exceeds the critical body residue for the predator.

Comparison to critical body burden. While several of the CECs considered by the Panel have the potential to bioaccumulate, only two (PBDE 47 and PFOS) had NOECs from which body burden-based MTLs could be derived. For this assessment, PBDE 47 and PFOS were measured in bird eggs (in units of ng/ml yolk). In order to convert from a volume to mass based MEC, a density of 1 was used. Both PBDE

47 and PFOS had MTQs greater than 1.0 (Table 6.5). In piscivorous birds, PBDE concentrations in eggs ranging from 5 to 369 ng/g have been detected with highest concentrations observed in CA in San Francisco Bay (2,160-9,420 ng/g) and Canada (486-5,359 ng/g) with dominant congeners of 47, 99 and 100 (Shaw and Kannan 2009). The Panel recognizes an MTQ of 850 for PBDE-47 and -99 (Table 6.5) is extremely high, which is the result of the maximum concentration reported in bird eggs from San Francisco Bay (see Table 5.5). Note however that PBDE concentrations of similar magnitude were reported in blubber of pinnipeds stranded off the southern California coast. A ten-fold safety factor was included since PBDE target the endocrine system (thyroid gland).

Table 6.5. CECs with Monitoring trigger quotients >1 in tissues.

Compound	MEC (ng/g)	NOEC (ng/g)	Safety Factor	Tissue MTL (ng/g)	MTQ
PBDE-47, -99	24465	289	10 ^a	28.9	850
PFOS	1760	1000	1	1000	1.8

^aEDC mode of action

Evaluation of dietary intake. Measured or predicted tissue concentrations of CECs for aquatic biota that comprise the diet of higher trophic level receptors can be compared to allowable dietary concentrations to determine if the higher trophic receptors are at a potential risk. An example is the State of California's Fish Contaminant Goals (FCGs) for PBDEs, which the Panel compared to PECs for PBDEs in San Francisco Bay fish based on a screening level one-box model combined with BSAFs derived from paired sediment and fish concentration data (see Section 3). The predicted fish tissue concentrations of PBDE 47 (11 ng/g) and total PBDE (33 ng/g) were all less than the FCG (310 ng/g) indicating that potential risks were not high enough to warrant monitoring of PBDEs in fish tissue for protection of human health. The Panel was not able to identify an allowable dietary fish concentration of PBDE for marine mammals and, therefore, did not evaluate PBDE in fish tissues for protection of marine mammals (see Box 6.1). The Panel believes the process used to evaluate PBDEs is applicable to other CECs, assuming allowable dietary concentrations and either PECs or MECs are available.

Box 6.1 Marine Mammals

The Panel was not able to identify allowable concentrations of PBDEs in fish for protection of marine mammals that could serve as MTLs. The Panel believes such marine mammal-based MTLs could be derived using the same general approach as used to derive FCGs for protection of human health. The key differences would be in the selection of an aquatic biota consumption rate and an allowable daily intake (ADI) of a CEC for marine mammals. Both would likely be higher for marine mammals than for humans. Although the Panel has not attempted to derive an ADI for marine mammals, it expects that a smaller safety factor would be used to establish such an ADI for marine mammals. If an uncertainty factor of 30 (instead 3000) were used, the human and marine mammal-based MTLs would be identical. If a smaller safety factor was used for marine mammals, the human-based MTL was less than the marine mammal-based MTL. If the State believes that MTLs based on marine mammals are important to develop, this Panel recommends that a subsequent panel be convened to develop recommendations about the assumptions to use to derive marine mammal-based MTLs.

6.4 Antibiotics

Assessment of potential bacterial effects was based on the range of MICs reported for each antibiotic. MICs are identified to develop dosing regimens for antibiotics. Ranges of MICs are often reported for individual antibiotics because some studies are conducted with naïve strains (no resistant genes = most sensitive strains) while others use bacterial strains that have developed specific gene mutation based resistance (Most Resistant MIC). A highly resistant strain will have a higher MIC than a naïve strain. Antibiotics with at least 3 independent MICs (Most Resistant MIC, Most Sensitive MIC and an Intermediate MIC Values) were judged to have a complete data set and a safety factor of 100 was applied to the most sensitive MIC (NOEC from Section 4) to derive the MTL. This safety factor of 100 was applied to account for uncertainty associated with the use of indicator bacteria that are not aquatic/marine (safety factor = 10) and for the uncertainty of development of ABR from plasmids or other molecular constituents (safety factor = 10). For antibiotics with less than 3 independent MICs a Safety factor of 1000 (the safety factor of 100 mentioned above plus an additional safety factor of 10 to account for incomplete acute toxicity (MIC) data) was used to derive the MTLs discussed in Appendix D. NOECs were derived from most sensitive MICs (lowest concentration causing toxicity) and were set at concentrations less than the most sensitive MIC value reported (see Section 4.3).

6.4.1 Aqueous Exposure for Effluent-dominated Inland Waterway (Scenario 1)

Monitoring trigger quotients for antibiotics/antibacterial agents in the effluent dominated inland waterway (Scenario 1) are listed in Table 6.6. One compound, triclosan, an antimicrobial agent, had an MTQ >1.

Table 6.6. Monitoring trigger quotient estimates for antibiotics/antibacterial agents in the effluent dominated inland waterway (Scenario 1).

Antibiotic	MEC (ng/L)	NOEC (ng/L)	Safety Factor	MTL (ng/L)	MTQ
Triclosan	510	25,000	100	250	2.0

6.4.2 Aqueous Exposure for Coastal Embayment (Scenario 2)

No antibiotics/antibacterial agents had an MTQ of greater than 1.0 in the coastal embayment likely due to the 10-fold dilution within the embayment Scenario 2.

6.4.3 Aqueous Exposure for Ocean Discharge of WWTP Effluent (Scenario 3)

No antibiotics/antibacterial agents had MTQs >1 for aqueous exposure in Scenario 3. In all cases, MTQs for antibiotics/antibacterial agents were reduced by an order of magnitude (factors of 19-90) or more (factors of 917-1000) at ocean outfalls when compared to Scenarios 1 and 2. This suggests that the risks for developing ABR is much lower in waters around marine outfalls than in effluent dominated inland and coastal embayment waters, due to the greater dilution of CEC sources in oceanic waters. These findings are consistent with results that illustrate the dilution effects of tidal range on the rate of antibiotic resistance as measured in other regions of the U.S. (Table 6.7).

Table 6.7. Rates of Antibiotic Resistance (ABR = % of *E. coli* bacteria that had antibiotic resistance). Parentheses indicate adjusted ABR value for a common tidal range (SC = 7 ft.) at each site.

Watershed	Site ABR Urban	Site ABR Rural	% Difference Urban vs. Rural	Reference
Florida (Apalachicola Bay)	25 (3.5)	13 (1.9)	47	Parveen et al. 1997
Maryland (Anacostia River, Annapolis Harbor, and Baltimore Harbor vs. Chester River, Miles River, Wye River and Love Point)	9 (4.5)	2.8 (1.4)	69	Kaspar et al. 1990
South Carolina (Broad Creek vs. Okatee River)	3	1	67	Van Dolah et al. 2000

The importance of ABR in coastal waters may also be a cause for concern in marine bacteria, particularly transfer of ABR to marine pathogens. In marine bacteria such as *Vibrio*, extremely high levels of ABR have been found in *V. parahaemolyticus* (Baker-Austin et al. 2008) and in *V. vulnificus* (Baker-Austin et al. 2009) in both highly urbanized areas with WWTP discharges and urban runoff (e.g., Charleston Harbor, SC and Brunswick Harbor, GA) as well as in more pristine National Estuarine Research Reserve sites (ACE Basin NERRS in SC), suggesting that anthropogenic factors such as antibiotics and genetic elements that may confer ABR from WWTPs as well as natural factors such as naturally occurring trace metals and possibly climate change/sea level rise may affect ABR in the marine environment.

7.0 SCREENING FOR CECs USING BIOLOGICAL METHODS

Bioanalytical techniques (“bioassays”) that integrate the exposure of CECs acting with a common mode of action and produce a response that can be linked to higher order impacts (e.g., survival, growth and reproduction) are being developed to complement current chemical-specific analytical methods. In vitro high-throughput bioassays that target endocrine disrupting chemicals have been validated for chemical screening programs and show promise for use in water quality monitoring, particularly as a cost-effective screening step. Remaining challenges include adaptation and validation of bioassays that target other relevant endpoints in ecological receptors (e.g., genotoxicity, immunotoxicity, antibiotic resistance), as well as the establishment of linkages between screening level bioassay results and in vivo, whole-organism and population level impacts.

7.1 Background

Biological monitoring methods (or “bioassays”) have been developed to quantify CECs that may be unknowingly released into the environment and for which there are currently no known chemical analytical methods for their quantification. These methods may offer additional safeguards for human and ecological health in the three exposure scenarios described in this report. The main advantage of bioassays is that they are able to detect the presence of chemicals based on their bioactivity rather than on their detection by analytical chemistry. Moreover, bioassays are one of the primary ways to evaluate the occurrence of unknown CECs. However, adaptation and implementation of these methods require the development of robust, reproducible and high-throughput *in vitro* assays. In addition, it is imperative to specify the endpoint of concern in this process. While the main focus by USEPA has been on compounds that interfere with estrogen, androgen and thyroid hormone responses, there are over 22 other nuclear hormone pathways that also can lead to adverse outcomes, and these pathways should also be explored. Other potential candidate endpoints of concern include genotoxicity and steroidogenesis. Genotoxicity refers to degradation of DNA within cells, which can occur when chemicals covalently bind to nucleotides in DNA in a way that precludes the cells' capacity to repair the DNA. This degradation can lead to cancer if it occurs within sensitive genes. Steroidogenesis is the biochemical process by which sex steroid hormones are synthesized within the gonad or brain. If this process is disrupted, insufficient sex hormones will be produced, which may adversely effect reproduction. An in-depth discussion of bioanalytical tools that are available for safeguarding human health was included in the previous report of the California’s Science Advisory Panel for Chemicals of Emerging Concern (CECs) in Recycled Water (Anderson et al. 2010). In this report we primarily concentrate on bioanalytical assays as they pertain to endpoints relevant to receiving waters.

Based on recommendations in the previous report (Anderson et al. 2010), the SWRCB initiated an ongoing study to determine the usefulness of bioanalytical assays for monitoring recycled waters. The objective of the study was to test commercially available bioanalytical assays for endpoints that are expected to be altered by contaminants not removed by secondary treatment and to compare the results of these assays with traditional chemical-specific analysis. A multi-investigator team has been assembled and work is in progress to evaluate a few of the most promising high throughput *in vitro* bioassays that are relevant to human health. These assays are listed in Table 7.1, along with their relevant endpoints. Chronic and high activation of these receptors have been linked to disease outcomes in some human populations. However, low-level activation of the receptors, as may occur from constituents in the water that bind and activate the receptors in *in vitro* assays, does not predict that these diseases will occur, and may only indicate that the respective classes of chemicals are present in the water. As part of this study, the bioassay results will be translated into toxicity equivalent units

(TEQs) for measured bioactivity; these TEQs will then be compared to human health thresholds and to chemical-specific concentrations. The plan is to determine whether the bioanalytical tests are useful for a multi-tiered monitoring program for recycled water applications.

Table 7.1. Bioanalytical assays for endpoints of concern to human health.

Assay	Acronym	Mechanism	Potential Health Implications
Estrogen receptor activity	ER	Estrogen signaling	Reproduction, cancer
Androgen receptor activity	AR	Maintenance of male sexual phenotype	Androgen insensitivity syndrome
Progesterone receptor activity	PR	Embryonic development, cell differentiation, homeostasis	Cancer, diabetes, hormone resistance syndromes
Peroxisome proliferator-activated receptor gamma	PPAR γ	Fatty acid storage and glucose metabolism	Obesity, diabetes, atherosclerosis, and cancer
Glucocorticoid receptor	GR	cortisol, glucocorticoids	Development, metabolism, immune response, neuroendocrine integration
Genotoxicity		DNA mutations	Cancer
Cytotoxicity		General toxicity	Tissue integrity

Several commercial companies offer high throughput assays for soluble hormone receptors that are stably transfected cells, but most depend on transient transfection. BDS has developed the CALUX assays for several soluble hormone receptors including ER and AR, among others; these receptors are stably transfected into a human osteoblastic osteosarcoma cell line which is devoid of any soluble hormone receptors. The assays depend on the full receptor for activity. Invitrogen (a Division of Life Technologies, Inc.) offers stably transfected assays for 22 different soluble hormone receptors; these are chimeric assays that depend on the Gal4 DNA domain for transactivation of transcription. The assays are straightforward and should be relatively easy to implement in current testing laboratories. Promega has cassette vectors that also use the Gal4 DNA domain that can be manipulated to insert any ligand binding domain of interest. However, these cassette vectors require further development for use in water quality monitoring. Other companies (e.g., SwitchGear Genomics in Menlo Park, CA) perform transient transfection assays as a service. AttaGene provides a multiplex method capable of evaluating 50 transcription activities at one time in a proprietary assay that also requires transient transfection. The ToxCast program recently tested 309 chemicals using a battery of receptor assays and AttaGene methodology (Martin et al. 2010). Most commercial companies provide research support; however, for monitoring and regulatory purposes, such assays must be evaluated in round-robin experiments at multiple locations to test for robustness. These high throughput assays offer considerable potential for water quality testing. Several groups of researchers are currently evaluating the use of these assays for water quality testing in round-robin type trials to establish validity of this application and to develop standard operating procedures. Results from these trials should be in the reviewed literature within 2 years. Lastly, it should be emphasized that bioassays can be used to measure synergistic, additive, and antagonistic interactions among compounds that may be present as a mixture, in highly complex effluents. This is important as toxicity evaluations based on single-chemical analyses will generally miss the synergistic, additive, or antagonistic potential found in mixtures, thus providing an inaccurate assessment of potential risk.

7.2 Bioanalytical Screening Tools for Ecotoxicology

Over the past 20 years, several bioassays have been developed through academic laboratories to assess the potential for environmental contaminants to affect wildlife. Most of these assays have not been fully validated, and thus are not currently used for regulatory purposes. However, several of these assays merit attention for further research, as they appear to bridge the gap between molecular biomarkers and higher order effects in survival, reproduction, development and susceptibility to disease. Current bioanalytical assays can be used to assess exposure, but more work is required before they can be used to assess adverse effects. Some of the more common assays are described below.

1) Yeast Estrogen Screen/Yeast Androgen Screen (YES/YAS) Assays

Yeast estrogen screen (YES) and yeast androgen screen (YAS) assays were developed almost 15 years ago and have been useful to derive total estrogenicity (or androgenicity) values for effluents. The YES assay first described by Routledge and Sumpter (1996) depends on a transgenic yeast strain into which the human estrogen receptor (hER) and a *lac-Z* gene (encoding the enzyme β -galactosidase) reporter construct were stably cloned. The YAS assay works by similar principles for the androgen receptor, and it was used for ecotoxicology by Sohoni and Sumpter (1998). The assays are quite sensitive and have a broad dynamic range, with the YES assay able to measure estrogenic compounds in the 1.5 to 3,000 ng/L range and the YAS assay in approximately the same range. Both assays have been used extensively to measure activities of estrogen, androgen and their antagonists in effluents (Routledge and Sumpter 1996, Sohoni and Sumpter 1998, Thomas et al. 2002). The most attractive feature of these assays is that they can be used to calculate net estrogenic potency (estrogen equivalency, EEQ) or net androgenic potency (androgen equivalency, AEQ) of environmental samples, as described in work by Bulloch et al. (2010) and Caldwell et al. (In press). In a recent test with 106 chemicals, both assays were used in a careful analysis, and although the results corresponded well with known activities, even pure compounds (estrogen, androgen and respective antagonists) exhibited a 12 to 30% rate of false negatives and a 3 to 13% rate of false positives (Kolle et al. 2010).

There are also detractions in using these assays. First, yeast do not have the full metabolism potential of vertebrate livers; thus, chemicals that are routinely metabolized to generate active components are missed. Second, there appears to be a high degree of variability for the assay that is due to interferences from other substances in the tested media. For example, cytotoxic microconstituents present in the media could reduce growth of the yeast and interfere with the assay. Still, some procedures can be used to get around cytotoxicity, but these require extraction and separation of components in the tested samples (Teske et al. 2007, Colosi and Kney 2011, Citulski and Farahbakhsh 2012, among others). In a study by Leusch et al. (2010), the YES assay was not as sensitive as other *in vitro* assays tested.

2) Zebrafish Early Life-stage Assay

Due to Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) legislation in Europe, there has been a push to go towards cell-based and fish embryo assays, as fish embryos are not considered live animals until the swim-up stage. A very successful and easy to perform assay was developed Padilla et al. (2012). This assay uses zebrafish embryos from 6 to 8 hours post fertilization to 5 days post fertilization. The endpoints measured are death and developmental deformities. The advantages of this assay are that it includes a whole-organism test for a sensitive window of exposure – early life development -- and can be performed in a high throughput manner. However, the MOAs for the endpoints are not specific and can be achieved through many multiple pathways. Thus, this test

would be much more non-specific for MOA than either the YES/YAS assays and would be more difficult to align with specific groups of offending chemicals. In addition, it is likely that endocrine related changes in adults are not captured by the embryo assay, creating potential for some chemicals to escape detection.

3) Biomarkers of Estrogen and Androgen Exposure: Vitellogenin/Spiggin

In the environmental arena, two biomarkers have surfaced as being very specific for endocrine disruption: vitellogenin (Vtg) which is secreted from the liver in response to estrogens in male fish (Heppell et al. 1995, Sumpter and Jobling 1995, Folmar et al. 2001) and spiggin which is secreted from the kidney of the three-spined stickleback females in response to androgens (Katsadiaki et al. 2006). Both of these assays have been validated with environmental ranges of estrogens and androgens, and they both have large dynamic ranges: as much as 6 orders of magnitude for Vtg and up to 4 orders of magnitude for spiggin. Spiggin seems to be produced only in the three-spined stickleback and thus this approach would not be relevant for other fish. Commercially available ELISAs have been validated for both biomarkers and antibodies (Nilsen et al. 2004, Eidem et al. 2006, Sanchez et al. 2008, Berg et al. 2009); however, neither assay has been linked to population level effects.

More interesting are biomarker assays that have been linked to population level effects, including decreased Vtg in females and decreased plasma steroid hormones in males and females (Miller et al. 2007, Ankley et al. 2008), suggesting that these biomarkers could be used in the regulatory framework. Empirical proof for the population level effects of constant exposure to a low concentration of a strong estrogen came from a whole-lake experiment with 5 ng/L ethinylestradiol that was carried out in Canada (Kidd et al. 2007). The population of fathead minnows in a lake was decimated during long-term exposure to high levels of estrogen. An emerging challenge in the environmental arena is to understand the effects of long-term exposure to progestogens, as these too are found in the environment (Paulos et al. 2010)(see also Appendix D).

4) *In situ* Bioassays

A National Research Council panel recommended flow-through biomonitoring systems as a potential tool for certain water quality conditions (NRC 1998). To implement such a system, fish were utilized by the Orange County Water District as an investigative model to develop a standard test platform, and evaluate the water quality of shallow ground water originating from the Santa Ana River (Deng et al. 2008). The endpoints focused on chronic exposure (3 months) and included histopathology (i.e., cancer), endocrine and reproduction metrics. A more developed system has been employed in Singapore primarily for acute impacts of water quality (<http://www.pub.gov.sg/mpublications/Pages/PressReleases.aspx?ItemId=178>). Disadvantages of these systems include the questionable ability to differentiate non-chemical and chemical stressors and the difficulty in establishing appropriate controls for assessing potential adverse effects *in situ*.

5) Fish Short-term Reproductive Assay

The fish short-term reproductive assay uses small fish, such as the fathead minnow, zebrafish or medaka, in a 21-day reproduction test (Ankley et al. 2001). The test is carried out with reproductively active females and males, the reproductive capacity of which are determined for at least 10 days before the start of the trial. The test is carried out for 21 days, and cumulative egg production, number of eyed eggs (fertility) and number of hatched eggs are recorded. At the end of the exposure, the fish are sacrificed, and endpoints, including: ovary and testis weight, gonadal-somatic index (GSI), plasma Vtg, plasma sex hormone concentrations and changes in secondary sex characteristics are measured.

Appearance of Vtg in male plasma is an indication that the test chemicals have estrogenic effects. A decrease in female Vtg is an indication that pathways important for egg quality have been disrupted, and these values can go into population models as described above.

6) Amphibian Metamorphosis Assay

Thyroid hormone axis disruption is one of the activities that are of high priority to the USEPA. A sensitive assay for this mode of action is the frog metamorphosis assay, as it tests for metamorphosis changes in *Xenopus laevis* tadpoles during their development into frogs (Furlow and Neff 2006). The assay works by treating tadpoles at developmental stage 51, just before they start metamorphosis, with the test chemicals. The endpoints examined are mortality, developmental stage (advanced or delayed), hind limb length, snout-vent length, wet weight and thyroid histopathology. This assay is especially valuable when coupled with histopathology of the thyroid gland to evaluate tissue structure differences. Some of the endpoints measured may also be altered by mechanisms other than thyroid disruption.

A variation of this assay, called the C-fin Assay, has been recently developed (Hinther et al. 2010). The C-fin assay depends on culturing tail fin biopsies of *Rana catesbeiana* tadpoles in a 96-well plate format and evaluating responses at the gene expression level of known thyroid hormone responsive genes, such as thyroid hormone receptor beta and rana larval keratin type 1. The assay can be performed within 48 hours and has been used effectively to study the effects of environmental levels of chemicals such as triclosan and triclocarban (Hinther et al. 2011).

7) Other Nuclear Receptor Cell-based Assays for Ecological Species

As with human nuclear hormone receptor assays, a number of different academic groups have cloned out receptors from lower vertebrates and invertebrates and developed *in vitro* transfection assays (Ikeuchi et al. 1999, Ackermann et al. 2002, Menuet et al. 2004, Sabo-Attwood et al. 2007, Blum et al. 2008, Katsu et al. 2008, Gaertner et al. 2012, among others). For the most part, these assays depend on transient transfection, i.e., each time the assay is performed the cells must be transfected with two constructs: one for the nuclear hormone receptor and one for a reporter. These assays have shown that there are important differences between nuclear hormone receptors in environmental species and in mammalian species, suggesting that the environmental assays should be used when environmental organisms are of concern (Matthews et al. 2000, Shyu et al. 2011). One of the major findings from these projects was the discovery that teleosts has a least 3 (and in rainbow trout 4, Nagler et al. 2007) functional estrogen receptors, rather than two as found in mammalian organisms. For teleosts, the receptors are expressed differentially in tissues and appear to have specific functions. Thus, to fully understand the effects of CECs on environmental organisms, these assays should be developed further and commercialized. At this point, none of the assays for non-mammalian systems are commercially available.

8) Microarray Analyses

With new advances in DNA sequencing technology that have been credited with making significant contributions to human-health approaches, it is now feasible to use these techniques for under-represented species. Gene microarrays are now available for a number of different non-model environmental species and commercially available for several species including zebrafish and fathead minnow, among others. The arrays have been used in both laboratory and field exposure studies with great success. Fish can be exposed to surface waters, effluents or other matrices in the laboratory or in the field for short periods of time to determine tissue-specific gene expression changes resulting from the exposure (Garcia-Reyero et al. 2009, 2011; Sellin-Jeffries et al. 2012; Weil et al. 2012). Once

calibrated for a specific mode of action, these assays should provide an indication of the “type” of compounds present within the matrix. With subsequent studies using refined *in vitro* assays in a TIE approach, specific compounds can eventually be identified. Even more importantly these assays have the potential to determine the “no observed adverse transcription effect level (NOATEL)”. To be considered for regulatory use, these assays must be vetted in round-robin tests and commercial laboratories will have to be trained in their proper use.

9) Transgenic Fish Models

There are a number of transgenic zebrafish and transgenic medaka that have been developed to quickly assess endocrine disruption for different molecular pathways, including: estrogen signaling (Chen et al. 2010, Hano et al. 2011, among others), aryl hydrocarbon receptor function (Mattingly et al. 2001), thyroid hormone function (Terrien et al. 2011), and neurotoxicity screening (Fan et al. 2011), among others. Using live animals, usually embryos, these transgenic constructs have the ability to quickly identify the presence of CECs for environmental monitoring. These assays have potential for HTP formats, with one embryo per well in a 96-well plate. However, not all possible hormone receptors are represented at this time, and validation of the sensitivities and specificities of these assays has not yet been performed.

10) Antibiotics and Antibiotic Resistance

As described in Section 4.3, there is a concern that low concentrations of antibiotics in aquatic environments could promote antibiotic resistance (ABR) in bacteria. This has been demonstrated in the laboratory with naïve strains of bacteria exposed to levels of antibiotics below the minimum inhibitory concentration (MIC)(NOAA 2011), thus creating concerns that similar resistance may occur in the environment. At concentrations of antibiotics within the range of bacterial sensitivity, some bacteria will be sensitive and succumb, while others that harbor genes for ABR will flourish. The removal of sensitive bacteria from the environment gives those that harbor the resistance gene a distinct advantage, and they eventually take over the population. Potentially more important is the release of ABR genes (via plasmids) into receiving waters. These released plasmids can then be transformed back into new bacteria, a process that is aided by the high ionic concentrations of bivalent metals present in the discharge. Notably, there are numerous studies that indicate that sediments from rivers that are contaminated with antibiotics are rich in these types of plasmids (Reinthal et al. 2003, Kristiansson et al. 2011, Uyaguari et al. 2011).

NOAA (2011) has developed an effective assay to screen for antibiotic resistance. In its current format, the assay tests 26 different antibiotics using 3 concentrations of each that are related to the MIC for *E. coli* (10% MIC, 100% MIC and 200% MIC). This combination of doses provides a determination of antibiotics for which there may be resistance and provides an overall quantitative assessment of the strength of the resistance for each. Also, because these panels are custom made, it may be possible to design panels specifically for antibiotics of concern based upon initial monitoring results. This type of panel has been effectively used with *E. coli* isolated from positive fecal coliform samples collected for compliance monitoring purposes; random colonies are picked from each plate and analyzed for growth in the presence of each antibiotic. Colonies growing at or >MIC values are considered to be ABR.

7.3 Strengths and Weaknesses of Bioassays

As discussed in Anderson et al. (2010), many bioanalytical assays are still under development and not available on a commercial basis. However, progress has been considerable in this regard, and assays are

undergoing inter-laboratory testing for robustness and predictability of endocrine disruption. For environmental species, it is clearly important to develop assays that link to higher order endpoints, including: survival, reproduction, development, and susceptibility to disease (see Box 7.1). Some assays described above are limited to specific endpoints, e.g., the fish reproduction assay. Others are more mechanistic and describe specific molecular pathways that could potentially lead to these higher endpoints. The linkage of the Vtg and plasma hormone levels to population effects for females (Miller et al. 2007, Ankley et al. 2008) clearly suggests that biomarkers will become more useful as they are linked to adverse outcome pathways. As these assays are used to measure unknown/unknowns, it will be critical to also consider an array of analytical tools to identify the chemical structures of the constituents. Along these lines, progress continues to be made in non-targeted analytical methods using sensitive instruments such as LC-MS/MS or GC-TOF/MS.

Transgenic zebrafish (or medaka) models also have the potential to be very useful in monitoring surface waters, as these tests are easy to perform and link to a visible biomarker that develops within a few hours. However, a potential drawback is that the assays have not been fully vetted for sensitivity and robustness and are not commercially available yet. As with other newly developed bioassays, comparison of test results to the current “gold standard” of analytical chemistry is needed to determine the utility of bioassays in monitoring applications.

Another important aspect of bioassays is that they can be used in MOA assessments of individual chemicals and in cell-based assays to help distinguish agonist from antagonist activities. Some cell types allow metabolism to occur during the test, thus allowing health assessment tests for metabolites that may be less or more toxic than the parent chemical. Several HTP *in vitro* bioassays have undergone round-robin testing for estrogenic activity, steroidogenic impacts and genotoxicity. The USEPA and the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP) are using these assays in screening tiers for chemical testing purposes. Predictive models are being developed to link *in vitro* assays with human disease (Sipes et al. 2011).

While strengths include exposure assessment for known and unknown CECs, the primary weakness of using bioassays is the uncertainty surrounding the potential for quantifying adverse effects in humans at the individual level and in ecological receptors at the population level. Few of these bioassays have been calibrated to these higher order effects. There is a possibility of false positives especially for low concentrations of chemicals (i.e., *in vitro* the chemicals signal activity but *in vivo* they fail to do so, or vice versa). The most likely explanation for these inconsistencies is metabolism and whole-organism integrated responses compared to specific bioassay response. In addition, extraction procedures have not been evaluated in round-robin intercalibration studies. For the most part, the *in vitro* assays rely on chemical extraction of contaminants from water, sediments or ecological receptor tissues without knowing if the extraction methods quantitatively and reliably obtain all relevant chemical contaminants. For example, perchlorate would have been missed by these assays. In addition, there is uncertainty as to the appropriate volumes of water, and masses of sediments or specific tissues to extract in order to get an *in vitro* response, and how these concentrations can be extrapolated to human and ecological health.

Box 7.1. Bioassays that Target Effects at the Population/Ecosystem Level

In ecological systems, effects at the population level are measured as changes in mortality, growth, reproduction, development and susceptibility to disease. Newer biochemical endpoints can also be entered into population predictions, as has been done for copepods (Chandler 2004, Chandler et al. 2004a) and for fish with vitellogenin production in females (Miller et al. 2007; Ankley et al. 2008). These two approaches are highlighted below.

The copepod (*Amphiascus tenuiremis*) bioassay developed for assessment of endocrine disrupting chemicals features a built-in Leslie Matrix population forecasting tool (Chandler et al. 2004b). *A. tenuiremes* is a tiny (<1 mm) sediment-dwelling copepod that grows rapidly, creating very large densities of animals in a short period of time. It serves as a predominant food for juvenile fishes and macroinvertebrates, thus forming a direct link among these different groups. Based on a complete life-cycle test and modeled using a life-stage classified Leslie matrix approach, this bioassay allows far-reaching population simulations to be predicted from multi-generational tests. Required endpoints for model input are survival, development rates of nauplii and reproductive effects over 3 generations.

This assay has produced findings that suggest that copepods may be impacted by exposure to CECs in the first two generations, but then appear to adapt and develop resistance in later generations. This has been demonstrated with organochlorines and organophosphate insecticides, as well as with some CECs (e.g., fipronil), providing a different perspective for risk assessment. For chemicals in which initial testing discerns effects, a tiered approach could be implemented to direct multi-generational testing to more effectively predict long-term chronic impacts. This assay has been used to examine resistance of copepods to organophosphate insecticides.

Another promising approach is the identification of a biomarker linked to adverse outcome pathways that can be linked to population effects (Miller et al. 2007, Ankley et al. 2008). These investigators utilized vitellogenin (Vtg), the egg yolk protein in oviparous animals, as a biomarker for endocrine disruption. Instead of focusing on Vtg in males as a biomarker that is up-regulated by exposure to environmental estrogens, the investigations monitor plasma levels of Vtg in females, as an indication of endocrine disruption that can originate from a number of different molecular mechanisms. Indeed, plasma Vtg can decrease as chemicals compete for binding to the estrogen receptors in the liver where this biomarker is synthesized, or which alter the steroidogenic synthesis of endogenous hormones (estradiol or testosterone) in the gonad, or even by chemicals that alter the release of gonadotropins (e.g., LH or FSH) from the pituitary. In a series of studies, investigators have shown how concentrations of plasma Vtg in females can be linked to populations using a Leslie Matrix for androgens (17 β -trenbolone and 17 α -trenbolone), aromatase inhibitors (fadrozole and prochloraz) and the antiestrogen fenarimol. In each case, the contaminants were dosed in 21-day reproductive assays resulting in dose-dependent suppression of fecundity in females, which was subsequently linked to population effects. The population model predicted profound effects on populations of fish from a 25% decrease in plasma Vtg levels in females.

These types of approaches need to be further developed in order to fully be able to utilize molecular biomarkers in risk assessment. As more adverse outcome pathways are identified and linkages between the alteration of the biomarker and population effects are established, the more likely it will be possible to consistently protect the environment without having to add safety factors to address uncertainties.

Few commercial testing companies currently have the equipment and trained staff to perform bioassays, creating a significant need for training. However, it is likely that suppliers of the biological test systems and kits (such as Invitrogen/Life Technologies Inc. and SwitchGear Genomics Inc.) will provide courses to personnel in commercial testing companies to teach them how to perform and implement the assays under GLP conditions. Alternatively, continuing education courses associated with the Society of Toxicology (SOT) or Society of Environmental Toxicology and Chemistry (SETAC) may provide this service.

Another short-term problem with bioassays is that many, particularly *in vivo* and microarray assays, still need to be vetted in round-robin studies to determine the limits of the methodology, the variability of response and the robustness and sensitivities of the assays. In addition, special emphasis should be placed on extraction procedures as most round-robin tests are carried out on a common extract. This level of QA/QC validation will require resources in parallel with other tests that are ongoing. While in the short run these additional resources will cost more than traditional chemicals-specific analyses, in the

long run the bioassays may help reduce the overall costs of monitoring surface waters. In this scenario, the bioassays could indicate which analytical methods should be employed to identify chemicals of the greatest concern (Leusch et al. 2010).

7.4 Use of Bioanalytical Tools in Risk Assessment

While significant challenges still lie ahead for the full implementation of bioanalytical methods into environmental risk assessment (see Section 9), this has been the topic of multiple international symposiums, especially with a focus on toxicogenomics methods (Ankley et al. 2006, 2009; Van Agellen et al. 2010). Many scientists are working to overcome the road blocks and aspire to the notion quoted from Ankley et al. (2006) that “the successful incorporation of toxicogenomics into regulatory frameworks may someday be regarded as the most important intellectual and practical contribution from this generation of ecotoxicologists.”

Traditionally there has been mistrust among practitioners on the use of biomarkers in ecotoxicology, mainly because the available markers related more to exposure than to effect and were not specifically linked into higher-order adverse effects on populations (i.e., survival, reproduction, development and susceptibility to disease). This perception is slowly changing, and a few biomarkers have now been linked to population level effects. For aquatic oviparous vertebrates, these biomarkers include the decreased plasma Vtg and decreased plasma sex steroid hormones in females, both of which have been linked through modeling to population declines. As such, molecular events that lead to uncovering adverse outcome pathways may be useful to risk assessments. Through toxicogenomic studies, it is now clear that many adverse outcome pathways are linked to the action of soluble nuclear receptors involved in gene activation. These receptors, including activation of estrogen and androgen receptors, have already been linked to human disease. Commercial assays, in the form of kits, are now being assembled for many of the receptors. However, research is still required to fully test these assays in comparison to the trusted gold standard of analytical chemistry and subsequently determine their utility in water quality monitoring and assessment (see also Section 9.1).

In the short term, bioanalytical assays may have a larger role in assessing the presence of unknown contaminants that activate specific hormone receptors than in assessing toxicity. To this end, the assays could be used in conjunction with chemical-specific analytical methods to identify unknown/unknowns. Conversely, bioanalytical assays may also be used to determine that additional chemical testing is not necessary in receiving waters where bioactivity is not measureable. Additional research is necessary to determine the suite of bioanalytical assays necessary to determine the overall safety of receiving waters.

To use bioanalytical assays for toxicity determination, more work is required to link their output to higher order effects in reproduction, survival, development and susceptibility to disease. As pointed out above with Vtg in females and plasma sex hormone concentrations, it is possible to make these types of linkages with population models. More work is required to determine contaminant concentrations associated with adverse outcomes at these levels.

8.0 MONITORING APPROACH

The Panel recommends a phased monitoring approach that develops a list of CECs from a risk-based framework, performs initial monitoring at appropriate spatial and temporal scales using validated analytical methods, analyzes and interprets initial monitoring data using the most current information and modeling tools, and implements control actions for CECs commensurate with risk. The Panel also recommends an adaptation to the findings of CEC monitoring, by revisiting the conceptual approach periodically (i.e., at least every 5 years) to respond in a timely fashion to future changes in the usage and state of knowledge concerning CECs. To maximize the resources committed to water quality monitoring across the State, the Panel recommends taking full advantage of existing monitoring programs in obtaining the necessary information and as testbeds for new, improved monitoring technologies.

8.1 Phased Monitoring Program

The Panel recommends an adaptive monitoring approach with four (sequential) phases that balances the potential risks identified for CECs, including uncertainty, against escalating actions (Figure 8.1). The first step (Phase 1) develops an initial list of CECs by applying the risk-based screening framework on the focused universe of CECs (Figure 1.2). This initial list is used to design and perform monitoring and special studies (Phase 2), develop and test alternative tools, analyze initial monitoring data and update the initial screening list as needed (Phase 3), and implement control actions as needed (Phase 4). The phases are based on aligning a presumed CEC exposure and toxicity and resultant potential risk with an appropriate monitoring level.

8.1.1 Phase 1 - Develop Initial CEC List(s) Based on Panel Screening Framework

Phase 1 has essentially been completed by the current Panel. An initial list of CECs (Table 8.1) was identified by comparing MECs/PECs to biological effects thresholds (MTLs) that incorporated appropriate safety factors for the media (aqueous, sediment and tissue) identified in Section 6. While analytical chemistry methods have been vetted by the Panel (Section 2 and Table 5.1) and are clearly available for these CECs, it is unclear whether they would be commercially available for monitoring each listed CEC in the specified media. For example, most of the CECs have well-characterized methods for chemical detection in aqueous media, but may not be wide-spread for sediments or biota. If methods are not feasible, then analytical methods would need to be developed or PECs estimated (e.g., using a conceptual source and fate model) before the CEC can be considered for Phase 2 monitoring.

In addition, the Panel also recognized the potential for the development of less expensive bioanalytical screening tools, which may also be utilized for detecting mixtures of “known” compounds, but also “unknown unknowns” and “known unknowns” (see Section 7). Investigators are currently evaluating a battery of commercially available HTP *in vitro* bioassays for application in recycled water quality monitoring in California, as well as in other parts of the world (e.g., Australia). As these and other chemical methods become available, periodic reassessment by subsequent advisory panels can be used to move CECs or newly validated methods to subsequent phases.

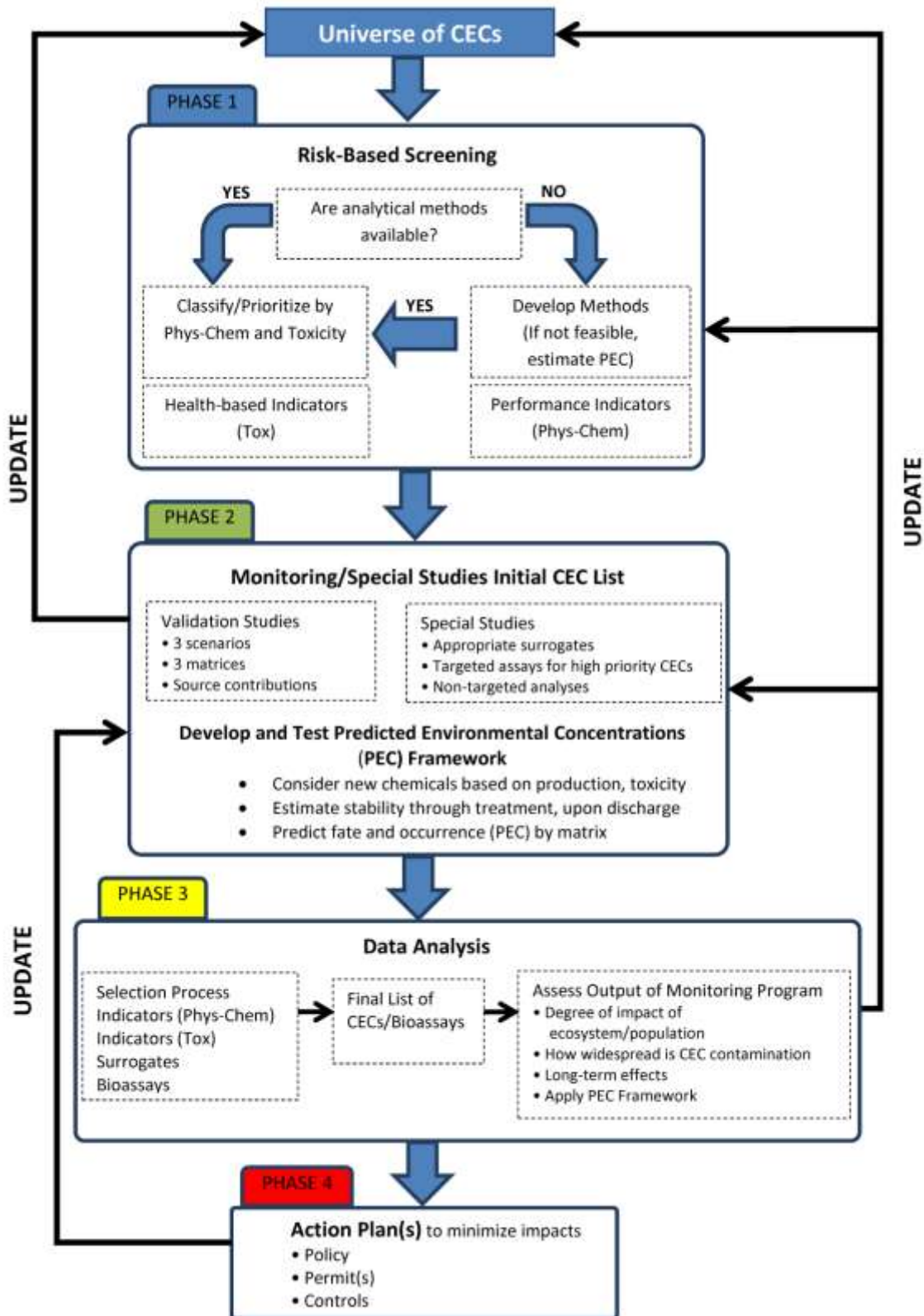


Figure 8.1. A phased monitoring strategy for CECs considers the compounds with the highest risk and available analytical methods. Periodic revisits are key to adapting to changes in sources, emerging toxicological data and (bio)analytical technology.

8.1.2 Phase 2 - Implement Monitoring of Phase 1 List of Initial CECs

Phase 2 involves implementation of monitoring for CECs that have MTQs >1 (Section 6, Tables 6.1-6.6; summarized in Table 8.1). The overall objectives of Phase 2 are to: 1) to verify the occurrence of targeted CECs in aqueous, sediment and tissue samples; 2) initiate compiling a data set as part of special studies that characterize their occurrence in sources and receiving waters (e.g., WWTP effluents and effluent dominated receiving waters, stormwater impacted freshwaters, marine waters, coastal embayment and estuarine waters, and background receiving water, and in the appropriate environmental matrices (water, sediment and tissue); 3) begin to evaluate potential improved/supplemental methods and surrogate measures including non-targeted analysis (see Section 2.4.3), passive sampling devices (see Appendix B, Box B.1) and bioassays for CECs and ABR (see Section 7); and 4) initiate development of conceptual models to aid with monitoring data assessments (Phase 3) and policy analysis.

Purposive¹² monitoring is proposed to characterize the presence of selected CECs in three main categories of receiving waters throughout the State.

- Freshwater creeks, streams and rivers – representative urban and non-urban freshwaters in southern and northern regions of the State
- Coastal embayments and estuaries – e.g., San Francisco Bay and San Diego/Newport Bay
- Marine waters – southern California Bight

8.1.2.1 CEC Monitoring Questions and General Approach

To date, various industrial and municipal dischargers as well as stormwater agencies have either directly implemented significant monitoring efforts or provided resources to the State (e.g., SWAMP) and/or regional programs (e.g., SCCWRP-Bight and SMC, SFEI-RMP and RMC) to conduct such efforts. These efforts have focused on site-specific water quality issues and treatment plant performance and compliance with effluent limits. The monitoring efforts, while significant, have not focused on CECs and what few CEC data are available are limited to a specific CEC, are research driven, and/or generally are not sufficient to characterize key CECs in the various categories of State receiving waters identified by the Panel (see Section 3). The monitoring effort described below provides broad guidance to the State to address the specific questions listed below, consistent with the recommended phased monitoring approach (Figure 8.1), and to cost-effectively integrate the proposed CEC monitoring program with ongoing state-wide and regional monitoring efforts.

1. Which CECs are detected in freshwaters and depositional stream sediments, and in which large California watersheds are they detected?
2. Which CECs are detected in marine waters and sediments adjacent to WWTP and significant stormwater outfalls and how quickly do they attenuate?
3. Which CECs are detected in coastal embayment/estuarine water and sediments?
4. What is the relative contribution of CECs in WWTP effluent vs. stormwater?

¹² As used in this report, purposive monitoring is monitoring conducted in a deliberative and non-random fashion to achieve a certain goal.

5. What is the extent and magnitude of PBDE and PFOS contamination in tissues of aquatic wildlife across the State? Does tissue occurrence correspond with sediment occurrence?
6. What is the direction and magnitude of change in CEC concentrations (in water, sediment and tissues) over a multi-year (3 to 5 year) time period?
7. How does the Panel's assumed relationships, based on the new CEC data (e.g., MEC or PEC, NOEC and MTL), change the estimated MTQs?
8. Does the new information (Question 7 above) modify the Panel's assumption regarding CEC potential risk and if so, does it trigger the need to evaluate CEC control efforts?
9. Do toxicity estimates from NPDES testing (i.e., acute WET; periodic chronic) provide adequate safety at the population level for CECs that have sublethal impacts on endocrine, immune or reproductive endpoints in aquatic organisms?
10. How do we effectively monitor for antibiotic resistance (ABR) and how do we link microbial assessment endpoints with analytical chemistry monitoring for antibiotics and other pharmaceuticals to better discern multiple pathways for development of ABR?

The cost-effective collection of relevant and reliable data that provide meaningful insight on the above questions requires collaboration at the local, regional and State levels. Thus, the Panel recommends integration of CEC monitoring efforts with ongoing monitoring efforts, e.g., as noted below:

- a) Freshwater locations - coordinate and integrate with local, regional and State monitoring programs (e.g., California's Surface Water Ambient Monitoring Program (SWAMP); the Stormwater Monitoring Coalition (SMC) and Regional Monitoring Coalition (RMC) municipal stormwater programs in southern and northern California.
- b) Coastal embayments/estuaries - coordinate and integrate with local, regional and State-wide monitoring programs (e.g., the SMC and the SFB RMP).
- c) Marine locations - coordinate and integrate with local, regional and State-wide monitoring programs (e.g., the Southern California Bight program, ASBS/MPA monitoring efforts).

8.1.2.2 Monitoring Program Design Guidance

The Panel recommends that detailed monitoring workplans be developed to define the effort as outlined in Table 8.2 and that the workplans be reviewed by the Panel prior to implementation. The workplans need to clearly identify sampling locations and frequencies to characterize the specific matrix for the various scenarios. The CEC monitoring workplans also need to consider sampling methods (see Appendix B.2) and toxicity drivers (e.g., acute vs chronic toxicity). The monitoring effort should be conducted as part of select special studies coordinated through the appropriate monitoring efforts (e.g., SWAMP, Bight and SMC, RMP and RMC) and regional permits (where necessary). Further, the monitoring plans need to be developed in coordination with the appropriate regional monitoring program(s) to ensure use of consistent sampling and analysis methods as well QA/QC and data reporting methods. It is anticipated that Phase 2 would occur over a five year period with development and Panel review of coordinated plans occurring during year one, monitoring occurring during years two through four, and independent review of results conducted by the CEC Panel during year five.

Table 8.1. CECs recommended for initial monitoring (Phase 2) by scenario and environmental matrix (i.e., aqueous, sediment, tissue). M = include in monitoring program (discharges to E = embayments; F = freshwater, O = ocean waters; NA = not applicable. Monitoring Trigger Quotient values from Section 6 appear in parentheses.

Compound	Scenario 1 - Inland Waterbody Aqueous (Tables 6.1 & 6.6)	Scenario 2 - Embayment Aqueous (Table 6.2)	WWTP Effluent	FW Stream - Stormwater (Aqueous and Sediment) ^a	Scenario 2- Embayment Sediment (Table 6.3)	Scenario 3 - Marine Sediment (Table 6.4)	Tissue (Table 6.5)
Bis(2-ethylhexyl) phthalate	NA	NA	M-O	NA	NA	M(3.8)	NA
Bisphenol A	M(8.7)	M(2400)	M-E/F	M	NA	NA	NA
Bifenthrin	M(210)	M(750)	M-E/F	M	M(1500)	NA	NA
Butylbenzyl phthalate	NA	NA	M-O	NA	NA	M(16)	NA
Permethrin	M(46)	M(46)	M-E/F	M	M(2600)	NA	NA
Chlorpyrifos	M(38)	M(220)	M-E/F	M	NA	NA	NA
Estrone	M(12)	M(12)	M-E/F	M	NA	NA	NA
Ibuprofen	M(10)	NA	M-F	M	NA	NA	NA
17-beta estradiol	M(4.2)	M(4.2)	M-E/F	M	NA	NA	NA
Galaxolide (HHCB)	M(4.0)	M(4.0)	M-E/F	M	NA	NA	NA
Diclofenac	M(2.3)	NA	M-F	M	NA	NA	NA
p-Nonylphenol	NA	NA	M-O	NA	NA	M(30)	NA
PBDE -47 and 99	NA	NA	M- E/F/O	M	M(5700)	M(15)	M(850)
PFOS	NA	NA	M- E/F/O	M	M ^b	M ^b	M(1.8)
Triclosan	M(2.0)	NA	M-F	M	NA	NA	NA

^a Addresses data gap on relative contributions of stormwater discharge and WWTP effluent (see Monitoring Question 4)

^b Addresses route of exposure and data gap for estimation of BSAFs for tissue CECs (see Monitoring Question 5)

Table 8.2. Guidance for developing targeted/pilot CEC monitoring workplans. FW = fresh water; M = include in monitoring programs; NA = not applicable; RW = receiving water

General Monitoring Design Parameters	Large POTW Discharging to Ocean ^a	Small POTW Discharging to Embayment ^b	Stormwater (MS4) Discharge -- Receiving Water Stations ^c	POTW Discharging to Effluent Dominated Waterway ^d
Parameter List	Table 8.1	Table 8.1	Table 8.1	Table 8.1
Spatial coverage – Receiving Water (RW)	2-D grid (up to 6 sites each location)	2-D gradient (up to 6 sites in estuary)	1-D gradient (up to 6 sites for each location)	1-D (up to 6 sites for each location)
Number of POTW and/or FW Locations	Two POTWs and corresponding RWs	Five POTWs in one estuary/embayment	Two large FW streams and the Delta	One POTW and RW
Frequency	Semi-annual over three years	Semi-annual over three years	Wet and Dry Season over three years	Wet and Dry Season over three years
Background	M	M	M	M
Aqueous (non-filtered)	NA	M	M	M
Sediment (top 5 cm)	M	M	M	M
Tissue ^e	M	M	M	M
Bioanalytical Screening Assays ^f	Pilot evaluation and validation studies	Pilot evaluation and validation studies	Pilot evaluation and validation studies	Pilot evaluation and validation studies
Toxicity ^g	Pilot screening study at one POTW	Pilot screening study at one POTW	NA	Pilot screening study at POTW
Antibiotic Resistance ^h	NA	Pilot investigation at one POTW	NA	Pilot investigation at one POTW
Passive Sampling Devices (PSDs) ⁱ	Pilot investigation at one POTW	NA	NA	Pilot investigation at one POTW

a – Daily discharge ≥ 100 mgd; potentially conduct pilot investigation in southern California (coordinate with Bight program).

b – Daily discharge < 100 mgd; potentially conduct pilot investigation in San Francisco Bay (coordinate with the Regional Monitoring Program).

c -- Potentially conduct pilot investigation for one stream in the San Francisco Bay Area (coordinate with BASMAA – RMC); one stream in Southern California (coordinate with the Stormwater Monitoring Coalition), and the Sacramento-San Joaquin Delta (coordinate with Regional Monitoring Program and the appropriate Delta organization(s)).

d – Potentially conduct pilot investigation in Southern California (coordinate with the Stormwater Monitoring Coalition).

e -- Identify appropriate species and tissues (e.g., bivalve and fish tissue for PBDEs; bird eggs for PFOS) in conjunction with local, regional and Statewide monitoring programs (e.g., SWAMP Bioaccumulation Workgroup; Bight, RMP and National Mussel Watch Programs)

f – Conduct evaluation and validation of bioanalytical screening methods that combine bioassays and subsequent non-targeted analyses to identify bioactive substances using a TIE process (e.g., as described in Sections 7 and 2.4.3).

g -- 21 d fathead minnow recrudescence assay for freshwater matrices (see Section 7.2(5)). Implement periodic reproduction assessments using appropriate fish and invertebrate species (see e.g., Box 7.1). Coordinate efforts with NPDES WET and bioassessment monitoring. This assay should be used for research purposes only at the present time.

h -- Conduct a pilot investigation using a bioassay that can be used to screen for antibiotic resistance (see Section 7.2(10); Appendix F).

i – Conduct a pilot investigation using PSDs that provide adequate capacity to concentrate the CECs in Table 8.1. These devices should have demonstrated acceptable performance in laboratory or field validation studies, and published guidance on translation of results.

8.1.2.3 Environmental Fate and PEC Models

To the extent appropriate and to assist with assessment and update of the monitoring information collecting during Phase 2, the Panel strongly recommends development or adaptation of environmental fate models (e.g., such as the 1-Box source and fate model utilized by the Panel for PBDEs in Section 3) as tools for summarizing and synthesizing existing knowledge including CEC production and usage, loading and loss rates in receiving water scenarios, and environmental compartment transfer rates. In addition, these screening-level mass-based models can assist in estimating the predicted environmental concentrations (PECs) in effluents coupled with structure-based toxicity assessments. These models serve as an invaluable screening tool for the preliminary analysis of technical and policy issues regarding the environmental system responses to natural processes and evaluation of potential CEC control options. With insights gained from these models, future monitoring and research as part of Phase 3 can be focused on the areas posing the greatest potential risk. Environmental fate models that predict CEC concentrations in various environmental compartments (i.e., surface water, sediments, tissues) will allow managers, as part of Phase 4, to better predict, prioritize, and optimize actions aimed at protecting and/or improving water quality, and ultimately, human and wildlife exposure to CECs. The Panel anticipates that development of new, or, more likely, adaption of existing, environmental fate model(s) will be conducted during Phase 2.

8.1.3 Phase 3 - Assess/Update Monitoring and Response Plans

Phase 3 involves reassessment of the Phase 2 monitoring efforts. The goal is to update the list of CECs based on results of monitoring using conventional and non-targeted methods, and pilot studies using bioassays listed in Table 8.2. In addition, the results of the environmental fate models will be evaluated to assess and prioritize future monitoring needs as well as conduct a preliminary review of the impacts of potential control actions aimed at protecting and/or improving water quality, and ultimately, human and wildlife exposure to CECs that result in an MTQ>1. In essence, the intent is to evaluate the Phase 2 results within the context of a tiered risk-based monitoring and response framework as presented in Figure 8.2. This approach is conceptual and balances the potential risks, including uncertainty, against escalating actions. Phase 3 should be conducted by an independent panel of experts, preferably a single non-project based (i.e., un-biased) entity such as the current Science Advisory Panel.

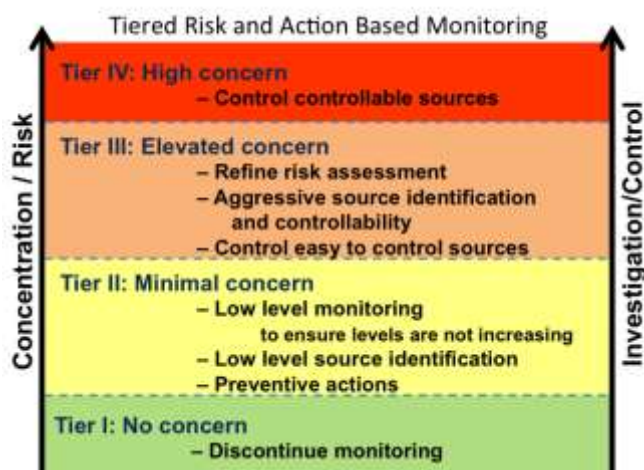


Figure 8.2. Conceptual Tiered Risk and Action Based Monitoring Approach (adopted from Mumley, 2011). (Note: color code adopted from US Forest Service not TSA).

It should be noted that the Phase 1 and 2 monitoring recommendations by the Panel should not be considered for compliance and/or regulatory purposes, but for investigation and potential use for additional follow-up actions. In addition, during Phase 3, consideration should entail topics such as: review of the basis of the (initial) MTL; what is and what is not known about the particular CEC, the CEC's potential health effects at the given concentration, the source(s) of the CEC, as well as possible means of better control to limit its presence, treatment strategies if necessary, and other appropriate actions. In addition, the Panel suggests the following actions relative to updating and confirming the Phase I environmental matrix data as well as the list of priority CECs for monitoring purposes:

1. Collect and review readily available toxicity data and update MTLs;
2. Collect and review California WWTP effluent data and update MECs or PECs;
3. Collect and review stormwater data and MECs or PECs;
4. Update the list of priority CECs to include newly identified CECs where the MEC or PEC/MTL>1 and remove CECs where updated data indicate that the previous Phase 1 MEC or PEC/MTL<1;
5. Review CECs that have come off the monitoring list to see whether use patterns have changed and whether this change warrants their re-listing for monitoring;
6. Review and update guidance on suggested monitoring sampling frequency and location and special studies;
7. Review and update conclusions regarding laboratory analytical methods;
8. Review and update biological and chemical screening methods (see Section 5), and provide guidance on potential new monitoring methods/tools that would significantly enhance conventional chemical monitoring methods (see Section 7);
9. Review results of environmental fate model(s) and provide guidance to the State on potential control actions and plans that should be developed and reviewed for potential State implementation in Phase 4; and
10. Review and update Panel guidance on selecting viable surrogate methods and future investigation needs.

The current Science Advisory Panel (or equivalent) should review and update the list of priority CECs after collection of 2 years of monitoring data.

8.1.4 Phase 4 - Action Plans to Minimize Impacts

If the assessment and update conducted as part of Phase 3 indicates that certain CECs will persist and continue to present significant risks, then during Phase 4 the current Panel (or equivalent)(e.g., as described for Phase 3 in Section 8.1.3) would develop guidance on the development and assessment of specific action plans for consideration by the State for implementation as part of their development of State policies, permits and/or statewide guidance.

9.0 FUTURE RESEARCH NEEDS

Research is needed to develop and test bioanalytical tools that will result in more comprehensive and efficient monitoring programs for CECs in California's receiving waters. High throughput in vitro bioassays, currently being developed for drinking water testing, will allow for screening of multiple CECs using receptors of ecological interest. It will be critical to establish linkage between screening bioassay results and higher order effects, e.g., using gene microarrays to elucidate CEC-specific adverse outcome pathways and whole animal testing. Key data gaps on source contribution, occurrence and toxicity of CECs should be addressed through focused special investigations and the development and application of environmental fate and effects models. The Panel also stresses the need to evaluate the risk posed by CECs relative to other stressors, including priority pollutants and other currently monitored chemicals, to provide decision makers with the information needed to make efficient use of all monitoring resources.

The following is a summary of future research needs identified in response to issues (e.g., data, tool or technology gaps) identified by the Panel within the report. The Panel understands that resources are not available to address each and every issue and need, and that a substantial level of resources are already expended by governmental and private entities around the world on research and development issues that pertain to CECs and monitoring of aquatic systems. Thus, the Panel recommends that the State seek out and capitalize on opportunities to collaborate with local, other State, regional and federal efforts in addressing these issues.

9.1 Develop Bioanalytical Tools for Efficient, Integrated Monitoring and Assessment of CECs

The risk-based screening framework developed and applied by the Panel requires occurrence and toxicological data for individual CECs in a number of exposure scenarios created to represent receiving water conditions. However, a cursory review of the data available and compiled by the Panel reveals substantial data gaps, particularly for occurrence in sediments and tissues (Section 5, Tables 5.4 and 5.5). Clearly, filling these data gaps will be a resource intensive effort, assuming analytical methods are available. Hence, the Panel foresees and recommends a shift away from a chemical-specific monitoring paradigm to one in which biological responses are targeted to address the thousands of chemicals which are potentially present in receiving waters (Section 7). Development of bioanalytical techniques including adaptation of HTP *in vitro* bioassays that target endpoints relevant to ecological receptors and integrate the response of individual CECs or classes of CECs acting with a common mode of action (MOA) is a key first step in realizing this paradigm shift. Moreover, the relevance (and thus utility) of molecular responses measured by HTP *in vitro* bioassays and the elucidation of adverse outcome pathways via gene microarray studies is dependent upon linkages established to higher order effects, e.g., fish reproduction and invertebrate population viability based on life-cycle testing. To realize this paradigm shift, the following issues need to be addressed through research as follows:

Issues:

- 1) Analytical method development cannot keep up with need to monitor newly identified CECs.
- 2) Whole-organism (toxicity) testing and life-cycle is extremely costly.
- 3) Toxicity of mixtures and CEC degradation products remains difficult to assess.
- 4) Current chemical-specific methods do not provide information on unknown CECs, biological response, or potential for toxicity. CECs that work through a common MOA are likely to have additive effects which can be measured by bioassays.

- 5) Although MOA information for some CECs (e.g., pharmaceuticals) is available for humans, there is potential for CECs to have different effects on non-target aquatic organisms. For other CECs (e.g., some personal care products), there is little to no MOA information.
- 6) There is no standardized assessment method for antibiotic resistance (ABR) in receiving water matrices.
- 7) Bioanalytical tools show promise but have not been adapted and/or validated for environmental (i.e., receiving water) matrices, nor have they been adequately linked to effects at higher levels of biological organization.
- 8) Need for validation of bioanalytical methods.

Research needs:

- 1) Develop, adapt and validate HTP *in vitro* bioassays (Section 7) to screen water, sediment and tissue samples for CECs identified by the Panel for monitoring (Table 8.1), with a specific focus on receptors of ecological relevance [Section 7.2(7)]. These assays integrate and measure the activity of chemicals by MOA, e.g., bioassays that target estrogenicity, androgenicity and glucocorticoid activity (Table 7.1) could screen for trace amounts of endocrine disrupting CECs such as 17-beta estradiol and estrone.
- 2) Investigate potential adverse outcome pathways for CECs in Table 8.1, using whole animal exposures and integrated systems toxicology [e.g., gene microarrays, Section 7.2(8)]. Identification of these pathways provide a link between chemical exposure measured by screening techniques (e.g., HTP *in vitro* bioassays) and higher order effects, e.g., reproduction in test or wild organisms. To link the adverse outcome pathways to higher order endpoints, perform 21-d reproductive assay in combination with gene microarrays.
- 3) Develop a marine version of the 21-d reproductive assay for a species of concern in California. The sheepshead minnow, a sensitive estuarine species that can be cultured easily in the laboratory, could be used for this purpose. The USEPA uses this species for estuarine testing.
- 4) Investigate whether fish embryo assays [Section 7.2(5)] reflect full adverse outcome pathways for endocrine insult seen in adults. This would decrease the cost of whole-organism tests.
- 5) Perform testing of simple CEC mixtures using bioassays, starting with the simplest (HTP *in vitro* bioassays) and comparing mixture responses from whole animal testing.
- 6) Develop standardized biological screening assays for quantitation of ABR in receiving water samples (water, sediment and tissue) for antibiotics that have been measured in monitoring studies conducted in California and throughout the US (see Section 7.2[10]). We recommend that the SWRCB convene a workshop of an expert panel of microbiologists to provide advice and oversight on the development and implementation of the ABR methods employed in future monitoring studies, once the initial ABR protocols for assessing ABR in effluent are developed.
- 7) Develop standardized protocols that can extract CECs from water, sediments and tissues and concentrate the resulting extracts into bioassay-compatible solvent systems.

9.2 Filling Data Gaps on Sources, Fates, Occurrence and Effects of CECs

During this transition period from chemical-specific to bioanalytical monitoring, the Panel also sees value in filling data gaps on source contributions, occurrence and toxicity of key CECs, and in developing environmental fate models that can be used to estimate the concentrations of CECs more cost effectively than can be measured, particularly if analytical methods are not available.

Issues:

- 1) Source contributions of CECs in receiving waters of interest throughout the State are ill-defined, due to insufficient data on occurrence (concentrations, frequency) in, e.g., atmospheric deposition, brine discharges, historical sediments and agricultural sources.
- 2) Current monitoring data do not distinguish between aqueous and particle exposure, or account for differences in bioavailability between the two media.
- 3) Little/no occurrence data for CECs that have elevated potential to pose a risk (e.g., progestogens and corticosteroids) in State receiving waters.
- 4) High uncertainty in applying biota sediment accumulation factors (BSAFs) and trophic magnification factors to predict tissue concentrations of bioaccumulative CECs in higher trophic level receptors (e.g., birds and marine mammals).
- 5) Lack of toxicity information for CECs (e.g., NOECs), which leads to lack of credible or highly uncertain MTLs. In many cases studies, NOECs are not available for specific taxa (e.g., invertebrates, fish, birds, marine mammals).
- 6) Lack of ability to predict likely occurrence levels of other CECs being discharged to waters of the State.

Recommendations:

- 1) Design and perform studies to measure concentrations and loadings of CECs in Table 8.1 in natural or background sources (e.g., groundwater, atmosphere) and reference receiving waters.
- 2) Improve and expand the application of conceptual models to estimate occurrence, distribution among aqueous, particulate, sediment and biological compartments, to assist design monitoring efforts and to evaluate CEC control measures. These models should also be used to refine screening evaluations on CEC sources and indirect exposure routes for hydrophobic CECs presented in this report (Section 3.3.2.1 and Appendix C.2).
- 3) Develop a screening-level mass-based model to estimate the predicted environmental concentrations (PECs) in effluents coupled with structure-based toxicity assessments.
- 4) Develop methods (as necessary), and design and perform studies to measure and/or confirm the occurrence of CECs that were not recommended for monitoring by the Panel at this time due to lack of occurrence or toxicity data, but that may be relevant due to increasing use, elevated environmental occurrence and/or high toxic potency, e.g.,
 - i) natural and synthetic hormones (cis-androstenedione, progesterone, levonorgestrel)
 - ii) replacement flame retardants (chlorinated alkylphosphates)
 - iii) current use pesticides (additional pyrethroids and fipronil and its degradates in sediment; herbicides such as diuron)

- iv) bioaccumulative CECs in sediment/tissue matrices as discovered by non-targeted analyses (see Section 2.4.3)
- 5) Identify CECs for which additional toxicity information is needed. Develop a process to track and compile toxicity data as it becomes available. In the absence of toxicity data for specific CECs that appear to have the potential to pose a potential risk, develop a process to establish temporary MTLs, using quantitative structure-activity relationships (QSARs), until toxicity data are developed.
- 6) If the State believes that MTLs based on birds and/or marine mammals are important to develop, the Panel recommends that a subsequent panel with specialized expertise be convened to develop recommendations about the assumptions to be used to derive bird and marine mammal-based MTLs, and to refine and apply the simple bioaccumulation model used by the Panel (Section 3.3.2 and Appendix C.2).

9.3 Balancing the Need to Monitor for CECs with Available Resources

Although the Panel was not asked to characterize the potential risks associated with CECs relative to other water quality parameters for which the State currently has monitoring programs, or to determine where in a ranking of all potential risks to California receiving waters the release of CECs falls, the Panel believes such a ranking would aid the State in allocating available resources most efficiently, i.e., focusing monitoring on the greatest potential of risk to receiving waters and diverting resources, if need be, from lesser to greater sources of potential risk. The Panel suggests the State undertake such an evaluation before developing and implementing a CEC monitoring plan.

Issues:

- 1) The investment needed to monitor for additional chemicals (“CECs”) using existing, conventional analytical methods is incrementally higher than is needed to monitor the current list of “non-CEC” chemicals.
- 2) A re-allocation of existing resources to monitor for CECs will diminish the capacity to monitor for existing parameters in discharge and receiving water monitoring.
- 3) The risks to ecosystem health due to CECs relative to other environmental stressors, including “priority pollutants” and other routinely monitored chemicals and biological vectors, are not well defined.

Recommendation:

- 1) Perform an integrated risk assessment to include all currently monitored chemicals and the CECs recommended by the Panel. The outcome of this risk assessment could guide future investment for monitoring commensurate with the risk posed by each class of monitored chemicals and/or non-chemical stressors.

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APPENDIX A - BIOGRAPHIES

A.1 Panel Members

HUMAN HEALTH TOXICOLOGIST

Dr. Paul Anderson

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Dr. Anderson has over 20 years of experience in human health and ecological risk assessment. Since 2000, Dr. Anderson has led several research efforts investigating the potential presence and effects of pharmaceuticals and personal care products in surface water and other environmental media. His research on constituents of emerging concern (CECs) began with the development of a screening level model (the Pharmaceutical Assessment and Transport Evaluation or *PhATE*[™] model) that predicts the concentration in surface water of pharmaceuticals and other compounds released from wastewater treatment plants (WWTPs) across the U.S. (including the Sacramento and Lower Colorado Rivers). The model has since been corroborated and published in *Environmental Science and Technology*. Dr. Anderson helped develop and continues to oversee the use of a database that summarizes the English language peer-reviewed literature on aquatic toxicity, environmental fate in surface water and treatment plant removal of pharmaceuticals. The database is designed to make historical and current information easily accessible to users. Dr. Anderson and colleagues have used these tools to conduct several evaluations, including an assessment of the potential human health effects of several therapeutic classes of pharmaceuticals in surface waters; the development of a predicted no effect concentration for protection of aquatic receptors from ethinyl estradiol (EE2); a comparison of predicted to measured concentrations of EE2 in surface water; and characterization of the potential ecological risk associated with EE2 in surface water. Recently, Dr. Anderson has expanded his research to include two reviews of existing information and ongoing research efforts, the first on endocrine disrupting compounds (EDCs) and the implications of their presence for wastewater treatment. It described the sources of EDCs in wastewater, their fate in WWTPs, and impacts in the environment as a result of discharges. The second covered the full range of organic EDCs that may occur at trace levels in WWTP effluents. The research included: a review of the different sources and categories of trace organic compounds; how they are measured; their removal in treatment plants; an introduction to the potential ecological and human health effects associated with trace organics in treated wastewater, recycled water, and receiving streams; and an overview of current research needs including a summary of web-links describing major current research initiatives. Dr. Anderson is also an adjunct professor in the Center for Energy and Environmental Studies within Boston University's Geography Department.

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The overall focus of Dr. Schlenk's laboratory has been to evaluate mechanisms of action of chemicals in aquatic and marine organisms. For the past 15 years, Dr. Schlenk has been interested in the estrogenic effects of legacy and emerging chemicals of concern. Initial work began with exploring the stereoselective biotransformation and activation of the legacy contaminant, methoxychlor. His lab helped develop a method to measure the egg yolk protein, vitellogenin in channel catfish and Japanese medaka. This metric was used to evaluate estrogenic activity in wastewater treatment plants in the south and east coasts and waterways of the United States. From there, his laboratory evaluated the effects of β -adrenergic antagonists and other pharmaceutical agents on aquatic fish and invertebrates. Dr. Schlenk's research in California has focused on the impacts of feminization on marine fish reproduction and populations as well as the identification of causal agents in sediments and water receiving oceanic discharge from municipal wastewater treatment facilities, particularly off the coast of Orange County. In addition, his laboratory conducted studies evaluating the long-term effects of recycled water on fish health. Current studies are underway to identify unknown estrogenic compounds in surface waters of the Central Valley and Santa Ana River. Specific agents that have been examined include current use pesticides (such as pyrethroids and herbicides), surfactants and UV-sunscreen agents. It is his goal to understand the modes of action of these compounds alone and in mixtures to determine the interactive roles each may have in endocrine disruption. In 2008, Dr. Schlenk served on the USEPA Science Advisory Board to evaluate potential changes to the Aquatic Life Criteria for Compounds of Emerging Concern. From 2003-2006, he was a member of the Board of Directors for the North American Society of Environmental Toxicology and Chemistry. He is the co-Editor-in Chief of *Aquatic Toxicology* and serves on the editorial boards of *Toxicological Sciences*, *The Asian Journal of Ecotoxicology* and *Marine Environmental Research*. He has been a permanent member of the USEPA FIFRA Science Advisory Panel since 2007, and has participated in proposal review panels for the USEPA, NOAA, and the National Institute of Environmental Health Sciences.

EPIDEMIOLOGIST/RISK ASSESSOR

Dr. Adam Olivieri, P.E.

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M.S., Civil and Sanitary Engineering, University of Connecticut

B.S., Civil Engineering, University of Connecticut

Dr. Olivieri has over 30 years of experience in the technical and regulatory aspects of water recycling, groundwater contamination by hazardous materials, water quality and public health risk assessments, water quality planning, wastewater facility planning, urban runoff management, and on-site waste treatment systems. He is a Registered Civil Engineer and a Registered Environmental Assessor with the State of California. Dr. Olivieri has extensive experience in the area of microbial risk assessment and the application of models to make engineering and public policy decisions. He served as Principal Investigator on the development of a user friendly microbial risk assessment tool (MRAIT) for the Water Environment Research Foundation. He was the co-project director at the Public Health Institute/Western Consortium for Public Health, where he directed the City of San Diego's Health Effects Studies at Mission Valley and San Pasqual, investigating the health risks of potable reuse of recycled municipal wastewater. This project was developed to address the fundamental issues raised by the National Research Council, and consistent with their recommendations involved a comprehensive investigation and comparison of both a recycled and a current potable water supply. The research project involved developing research plans and managing research across a wide base of California's prestigious universities including Berkeley, Davis, Los Angeles, San Francisco, and Scripps, San Diego State University and several laboratories of the California Department of Public Health Services. The project involved research in: a) Infectious Disease Agents – pathogenic viruses, parasites, and bacteria (and indicator organisms), b) Chemical Screening – volatile and semi-volatile organics, metals, PCBs, dioxins, TOC, and TOX, c) Genetic Toxicity Bioassay – Micronucleus tests, Ames, 6-Thioguanine Resistance, and Cellular Transformation Assays, d) Fish Biomonitoring, e) Plant Reliability – performance and mechanical reliability analysis and chemical and microbial agent unit and plant spiking studies, f) Chemical Risk Assessment – carcinogenic and non-carcinogenic, g) Epidemiology – baseline information (reproductive outcomes, vital statistics, and neural tube defects), and h) a Long-Term Health Effects Monitoring Plan. The San Diego Health Effects investigations have been recognized by the Science Advisory Board, the Australian government and the University of New South Wales, and in a special publication by the Water Environment Federation and the American Water Works Association. Dr. Olivieri has served on a number of national technical review panels, e.g., for the National Water Research Institute (evaluating the alternative disinfection options for a wastewater treatment plant and potential public health implications), and Monterey County (CA), which is evaluating groundwater recharge using recycled water. At the request of the US House of Representatives – Subcommittee on Water Resources and Environment, he provided testimony on April 13, 2005 on microbial agents and risk assessment relative to the national wastewater blending issue.

BIOCHEMIST

Dr. Nancy Denslow

Professor

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Education:

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M.S., Biochemistry and Molecular Biology, Yale University

B.S., Chemistry, Mary Washington College

Dr. Denslow's research involves environmental toxicology with a special focus on endocrine disruptors and pharmaceuticals in the environment. Her interests include defining molecular mechanisms of action of endocrine disrupting chemicals that adversely affect reproduction in fish that are exposed to the contaminants in surface waters. Her research covers both sex hormone receptor mediated and independent mechanisms. Favorite model systems include largemouth bass, fathead minnow, sheepshead minnow and zebrafish. Common research tools include traditional toxicology assays, biochemical pathways, histopathology, microarrays, real time PCR, proteomics, tissue culture based assays, transfections and *in vivo* determination of reproductive endpoints. In addition, Dr. Denslow has initiated research to understand the effect of nanomaterials on fish health. These experiments are integrated to look at gill function, histopathology, nanomaterial uptake and nanomaterial characterization. In addition, microarrays and proteomics tools are used to characterize the effects of the exposures. She has published more than 120 peer-reviewed publications and has led research projects supported by NIH/NIEHS, NSF, USEPA, and the US Army Corps of Engineers. Dr. Denslow also serves as Associate Editor for *Comparative Biochemistry and Physiology Part D Toxicogenomics and Ecotoxicology and Environmental Safety*, and received the Pfizer Award for Research Excellence in 2007 and a UFRF professor designation for 2009-2012. Dr. Denslow previously served for 15 years as the Director of the Protein Chemistry and Molecular Biomarkers Core Facility at the University of Florida. She has served on the Executive Board of the Association for Biomolecular Research Facilities (ABRF) and is a member of the Society of Environmental Toxicology and Chemistry (SETAC) and the Society of Toxicology (SOT) serving as senior councilor in the Molecular Biology Specialty Section. She is also a member of the American Association for Biochemistry and Molecular Biology (ASBMB).

CIVIL ENGINEER FAMILIAR WITH THE DESIGN AND CONSTRUCTION OF RECYCLED WATER TREATMENT FACILITIES

Dr. Jörg E. Drewes

Professor

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Postdoctoral Fellow, Arizona State University

Ph.D., Environmental Engineering, Technical University of Berlin, Germany

Dipl. Ing., Environmental Engineering, Technical University of Berlin, Germany

Dr. Drewes has been actively involved in research in the area of water treatment and non-potable and potable water reuse for more than 18 years. For the last 16 years, Dr. Drewes has been conducting research on indirect potable reuse projects in the State of California, including surface spreading as well as direct injection projects. The main focus of these studies has been the fate and transport of trace organic chemicals in these systems. He has led research as the principal investigator (PI) or Co-PI to better understand the rejection of trace organic chemicals during high-pressure membrane treatment (nanofiltration, reverse osmosis) as well as the fate and transport of micropollutants in soil-aquifer treatment systems. A common theme in all these projects was to identify meaningful trace organic compounds that can serve as indicator compounds for system performance assessments. He has also conducted tailored studies to further develop this concept for multiple treatment processes commonly employed in indirect potable reuse followed by more focused efforts for surface spreading and direct injection projects. This indicator concept has been adopted in the Australian Water Recycling Guidelines for Drinking Water Augmentation in 2008. In addition, he has been involved in several studies addressing the occurrence of emerging contaminants in recycled water and to provide guidance to the water industry regarding occurrence, fate and transport, health effects, analytical methods and communication. Dr. Drewes research group is currently working on developing more predictive tools for the fate of trace organic chemicals in various reuse schemes using quantitative structural property relationships (QSPRs) coupled with process models. Dr. Drewes has published more than 160 journal papers, book contributions, and conference proceedings. He was awarded the 2007 AWWA Rocky Mountain Section Outstanding Research Award, the 2003 Dr. Nevis Cook Excellent in Teaching Award, the Quentin Mees Research Award in 1999, and the Willy-Hager Award in 1997. In 2008, he was appointed to the National Research Council Committee on Water Reuse as an Approach for Meeting Future Water Supply Needs. Since 2007, Dr. Drewes has held an Adjunct Professor appointment at the University of New South Wales, Sydney, Australia.

MARINE SCIENTIST FAMILIAR WITH TOXICITY AND OCEAN LIFE

Dr. Geoffrey I. Scott

Director

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M.S., Marine Science, University of South Carolina

B.S., Biology, Wofford College

Dr. Geoffrey I. Scott is an environmental toxicologist with special interest in the ecotoxicology of water chlorination products, urban nonpoint source pollutants (e.g., PAHs/oil spills), and pesticides. Currently, Dr. Scott serves as Director of NOAA's Center for Coastal Environmental Health and Biomolecular Research (CCEHBR) in Charleston, South Carolina. CCEHBR is one of 5 Centers of the National Centers for Coastal Ocean Science within the National Ocean Service of NOAA. CCEHBR also directs research at the Oxford Cooperative Laboratory in Oxford, MD. CCEHBR scientists conduct multidisciplinary environmental research focused on four major themes: Science to manage threats of harmful algal blooms, to understand impacts of coastal pollution, to support coastal & marine spatial planning, and to advance research on climate change impact to coastal ecosystems. Dr. Scott's research has focused on understanding the ecotoxicology of agricultural pesticide and urbanization nonpoint source runoff on estuarine ecosystems, and the health of estuarine organisms as well as methods for measuring bacterial pollution sources emanating from coastal development. Dr. Scott is an Associate Professor in the Medical University of South Carolina's Marine Biomedicine Program, Tenured Associate and Adjunct Professor at the Arnold School of Public Health at the University of South Carolina, Adjunct Associate Professor, The Institute of Human and Environmental Health, Texas Tech. University, Lubbock, TX and Associate Adjunct Professor in the Marine Biology Program at the University of Charleston. Dr. Scott has served on numerous advisory panels to government and industry including: (1) the Interstate Shellfish Sanitation Conference, which regulates the harvesting, processing and shipment of molluscan shellfish in the U.S.; (2) EPA's Advisory Board, Panel on Endocrine Disrupting Chemicals; (3) EPA's Food Quality Protection Act Board, Scientific Panel Member on Genetically Modified Crops and on Atrazine Risk Assessment for Freshwater Areas of the US; (4) EPA's Environmental Technology Verification Program, Water Stakeholder Committee Member; (5) SC Governor's Primary Health Care Task Force; (6) the SC Coastal Pesticide Advisory Committee; (7) United Nations Gulf of Guinea Large Marine Ecosystem Team Member; (8) Research Delegation Exchange with the Black Sea Research Institute; (9) Think Tec Board Member Greater Charleston Chamber of Commerce; (10) Chairman, Bluffton Technical Advisory Committee on Water Quality; (11) EPA's Advisory Board, FIFRA Panel on Climate Change Effects on Pesticide Registration Criteria; and (12) EPA's Advisory Board, FIFRA Panel on Adverse Outcome Pathways Approaches.

CHEMIST FAMILIAR WITH THE DESIGN AND OPERATION OF ADVANCED LABORATORY METHODS FOR THE DETECTION OF EMERGING CONSTITUENTS

Dr. Shane A. Snyder

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Education:

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B.A., Chemistry, Thiel College

Dr. Shane Snyder is a Professor in the College of Engineering at the University of Arizona. He is also the Co-Director of the Arizona Laboratory for Emerging Contaminants. For over 15 years, Dr. Snyder's research has focused on the identification, fate, and health relevance of emerging water pollutants. Dr. Snyder and his team have published over 100 peer-reviewed manuscripts and book chapters on emerging contaminant analysis, treatment, and toxicology. In April of 2008, Dr. Snyder was one of six experts invited to testify before the U.S. Senate regarding pharmaceuticals in US waters. He has since been invited to brief the U.S. Congress three additional times. Dr. Snyder has served two terms on the federal advisory committee to EPA's Endocrine Disruptor Screening Program and was an invited expert panel member for the development of EPA's CCL3. Dr. Snyder is a member of the National Academy of Science's National Research Council Committee on Water Reuse and has served two appointments on the California Chemicals of Emerging Concern Expert Panels. Dr. Snyder is also a visiting professor at the National University of Singapore where he leads research on water reuse technologies and implications for public health.

A.2 Stakeholder Advisors

CHRIS CROMPTON

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Chris Crompton is the Chair of the California Stormwater Quality Association (CASQA), whose membership is composed of stormwater quality management organizations and individuals, including cities, counties, special districts, industries, and consulting firms throughout the state. CASQA's primary mission is to assist water quality programs in California to learn collectively from the individual experiences of its members. Chris is the Manager of Environmental Resources for the Watershed and Coastal Resources Division of the Orange County Resources and Development Management Department, where he oversees coordination of the countywide, municipal NPDES stormwater compliance program.

JIM COLSTON

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Jim Colston is the Chair of Tri-TAC, a Technical Advisory Committee representing three California associations: League of California Cities; California Association of Sanitation Agencies (CASA); and the California Water Environment Association. These agencies collectively treat and reclaim more than two billion gallons of wastewater each day. Tri-TAC's mission is to improve the overall effectiveness and accountability of environmental programs that impact publicly owned treatment works (POTWs) in California by working with State and Federal regulatory agencies and interest groups on matters related to POTWs. Jim currently works in regulatory affairs for the ocean monitoring program at the Orange County Sanitation Districts.

MARK GOLD

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Mark Gold is the Executive Director of Heal the Bay, a nonprofit environmental organization dedicated to making Southern California coastal waters and watersheds, including Santa Monica Bay, safe, healthy and clean. Heal the Bay uses research, education, community action and advocacy to pursue their mission. Mark earned his D.Env. from the UCLA School of Public Health's Environmental Science and Engineering (ESE) Program.

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Amber Mace is the Executive Director of the Ocean Science Trust (OST), a non-profit public organization that strives to connect science to ocean management solutions. Amber serves as the Science Advisor to the California Ocean Protection Council (OPC). Dr. Mace earned a B.A. in Geography from University of California, Berkeley in 1994 and a Ph.D. in Ecology from University of California, Davis and the Bodega Marine Laboratory in 2005. Amber has spent her life along the shores of California and the past ten years working actively to improve communication and collaboration among scientists, resource managers, policy makers, and the public. Effective November 30, Dr. Mace will begin her term as Executive Director of the OPC.

RICK MOSS

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Rick Moss is a cross media liaison with the Integrated Waste Management Board for the State Water Resources Control Board. He currently serves as the Water Board's contract manager for the CEC Science Advisory Panel on Recycled Water. Rick has worked in the environmental protection field since 1981, most recently as Chief of the Office of Military Facilities for the Department of Toxic Substances Control and previously in liaison and management positions for the Air Resources Board and Department of Transportation. He has a BA in Human Ecology from the College of the Atlantic and a MA in Public Policy from the Claremont Graduate School.

GARY DICKENSON

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Gary Dickenson is an Engineering Geologist in the Division of Water Quality of the California Water Resources Control Board. He currently serves as the Water Board's contract manager for the bioanalytical method development project. Gary has worked on issues regarding the state's Recycled Water Policy including contaminants of emerging concern (CECs) and salt and nutrient management. Gary has 20 years experience in environmental consulting specializing in site characterization and remediation.

LINDA SHEEHAN

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Linda Sheehan is the Executive Director of the California Coastkeeper Alliance (CCKA), a non-profit organization that works statewide to protect and expand upon the advances made by local Waterkeeper groups in the areas of water quality and ecosystem protection, and to educate state decision-makers about these issues. Ms. Sheehan brings to CCKA almost 20 years of experience in environmental law and policy matters. She has achieved notable success in protecting the health of coastal and marine waters off California by passing landmark legislation to control polluted runoff, improve coastal water quality monitoring, and limit the introduction of harmful invasive species into coastal habitats. Linda has served as a key stakeholder contact in providing feedback to the SWRCB on the draft Recycled Water Policy.

APPENDIX B - REGULATION, ASSESSMENT, SAMPLING AND MONITORING

B.1 Regulation of Discharges to California's Receiving Waters

The regulation and administration of stormwater, wastewater treatment and disposal, and monitoring in California is carried out by the State Water Resources Control Board (SWRCB) and nine California Water Quality Control Boards (RWB). The SWB consists of five full-time salaried members, each fulfilling a different specialty position. They are appointed by the Governor for four-year terms and confirmed by the Senate. In general, the SWB has overall responsibility for setting statewide policy on the administration of water rights and water quality control in California. The work of the SWB is carried out by a technical, legal and administrative staff which is supervised by an Executive Director. The State Board is located in Sacramento.

In recognition of the regional differences in water quality and quantity, the state is divided into nine regions for the purposes of regional administration of California's water quality control program. The boundaries of the nine Regional Water Boards are generally based on watersheds, also known as hydrologic areas. The nine Regional Water Boards are referred to by specific names, which are: (1) North Coast, (2) San Francisco Bay, (3) Central Coast, (4) Los Angeles, (5) Central Valley, (6) Lahontan, (7) Colorado River Basin, (8) Santa Ana, and (9) San Diego.

Each of the nine regions has a RWB composed of nine part-time members who are appointed by the Governor for four-year terms. The RWBs are responsible for adoption and implementation of water quality control plans (Basin Plans), issuance of waste discharge requirements (WDR), and performing other functions concerning water quality monitoring and control within their respective regions, subject to SWB review or approval. The work of each RWB is carried out by a technical and administrative staff which is supervised by an Executive Officer.

Legislation

Clean Water Act. The Clean Water Act (CWA), officially known as the Federal Water Pollution Control Act, was enacted by Congress in 1972. Ten major bills have subsequently revised the 1972 statute. The objective of the CWA is to "restore and maintain the chemical, physical, and biological integrity of the nation's waters to make all surface waters "fishable" and "swimmable." The US Environmental Protection Agency (EPA) has delegated authority to California to implement provisions of the CWA. One provision of the CWA prohibits discharge of pollutants into waters of the United States unless a permit is issued that complies with the CWA. Under federal law, a discharge permit is officially known as a National Pollutant Discharge Elimination System (NPDES) permit. The State and Regional Water Boards issues WDRs that serve as NPDES permits in California.

Porter Cologne Water Quality Control Act. The Porter Cologne Act legislation was enacted by the California Legislature in 1970. Portions of it became the model for the 1972 CWA amendments. In many respects Porter-Cologne still surpasses the federal act, because it allows the water boards to comprehensively regulate both surface and ground waters. It also allows the water boards to establish requirements for nearly any source of waste discharge, including nonpoint sources and certain other sources exempted from the federal act's permitting requirements. It further provides for the adoption of water quality control plans and the implementation of these plans by adopting waste discharge requirements (WDR) for individual dischargers or classes of dischargers.

Municipal Point Sources

Publicly Owned Treatment Works (POTW) NPDES permits (Orders) are issued by the RWBs pursuant to Clean Water Act (CWA) Section 402 and implementing regulations adopted by the United States Environmental Protection Agency (USEPA) and California Water Code (CWC) Chapter 5.5, Division 7 (commencing with Section 13370). These Orders serve as NPDES permits for point source discharges from Facilities to surface waters. These Orders also serves as Waste Discharge Requirements (WDRs) pursuant to CWC Article 4, Chapter 4, Division 7 (commencing with Section 13260). CWA Section 301(b) and NPDES regulations at 40 CFR 122.44(d) require that permits include limitations more stringent than applicable federal technology-based requirements where necessary to achieve applicable water quality standards. 40 CFR 122.44(d)(1)(i) mandates that permits include effluent limitations for all pollutants that are or may be discharged at levels that have the reasonable potential (RP) to cause or contribute to an exceedance of a water quality standard.

California Toxics Rule and State Implementation Policy. On May 18, 2000, USEPA adopted the California Toxics Rule (CTR) that promulgated new toxics (priority pollutant) water quality criteria for California. The SWB adopted the Policy for Implementation of Toxics Standards for Inland Surface Waters, Enclosed Bays, and Estuaries of California (hereinafter State Implementation Policy or SIP) that became effective on May 18, 2000, with respect to the priority pollutant criteria USEPA promulgated through the CTR. The SIP establishes implementation provisions for priority pollutant criteria, such a determination of which pollutants have RP and require effluent limits and how to calculate the corresponding effluent limits. The SIP provides limited guidance on monitoring requirements. SIP Section 2.3 states that “it is the policy of the SWRCB that individual permit monitoring complement and be coordinated with water body, watershed, and regional monitoring programs to the extent practicable.”

Monitoring and Reporting. NPDES regulations at 40 CFR 122.48 require that all NPDES permits specify requirements for recording and reporting monitoring results. CWC Sections 13267 and 13383 authorize the Regional Water Board to require technical and monitoring reports. The NPDES permit Monitoring and Reporting Program (M&RP) establishes monitoring and reporting requirements to implement federal and State requirements. The M&RP is a standard requirement in almost all NPDES permits issued by the Regional Water Board. It contains definitions of terms, and sets out requirements for reporting of routine monitoring data in accordance with NPDES regulations, the CWC, and Regional Water Board policies. The MRP also defines the sampling stations and frequency, the pollutants to be monitored, and additional reporting requirements. Pollutants to be monitored include all parameters for which effluent limitations are specified. Monitoring for additional constituents, for which no effluent limitations are established, is also required to provide data for future completion of reasonable potential analyses (RPAs).

POTW NPDES Permit Monitoring Program Variability – Inland and Estuarine

There is a wide range in POTW NPDES permit effluent and particularly receiving water monitoring program requirements around the State. A brief summary of selected monitoring requirements from POTW NPDES permits for discharges to San Francisco Bay, Sacramento River, and Los Angeles River is presented below.

San Francisco Bay. On April 15, 1992, the Regional Water Board adopted Resolution No. 92-043 directing the Executive Officer to implement the Regional Monitoring Program (RMP) for the San Francisco Bay. Subsequent to a public hearing and various meetings, the Executive Officer required major permit holders in the Region to report on estuary water quality. These permit holders responded to this request by participating in a collaborative effort through the San Francisco Estuary Institute. This effort has come to be known as the Regional Monitoring Program for Water Quality in the San Francisco Bay Estuary (RMP). NPDES permits for POTWs contain language specifying that Dischargers shall continue to participate in the RMP, which involves collection of data on pollutants and toxicity in water, sediment, and estuary biota of the San Francisco Bay. POTWs are assessed an annual fee based on their prior calendar year's mass loading of copper, nickel, selenium, and chromium. Individual Discharger receiving water monitoring is not required so long as each Discharger adequately supports the RMP. The RMP has been funded by Dischargers at approximately \$3 million per year since 2005.

Sacramento River. The Sacramento Regional County Sanitation District (SRCSD) discharges secondary effluent to the Sacramento River. The SRCSD NPDES permit Order No. R5-2010-0114 reissued on December 9, 2010 would require additional treatment including nitrification, partial denitrification, and filtration to conform with Title 22 disinfected tertiary standards. For this 181 mgd design capacity WWTP the permit requires daily to monthly effluent monitoring for approximately 40 constituents. It also requires effluent characterization monitoring once per month every other year for constituents including: dioxin, 6 pyrethroids, 126 CTR priority pollutants, standard minerals, 22 non-CTR persistent chlorinated hydrocarbon pesticides, and 31 other constituents of concern. Sacramento River receiving water monitoring is required weekly to quarterly at four locations (one upstream and three downstream) for approximately 10 conventional constituents. Additional upstream receiving water monitoring is required once every other year, concurrent with and for the same parameters as for the multi-parameter effluent characterization monitoring described above.

Los Angeles River. The City of Los Angeles 80 mgd design capacity Tillman Water Reclamation Plant (WRP) discharges advanced treated (nitrification/denitrification/filtration) effluent to the Los Angeles River at four locations. The Tillman WRP NPDES permit (Orders No. R4-2006-0091 and R4-2010-0060) is in the process of being reissued. The Fact Sheet (p. F-56) for the 2011 Tentative Order (TO) reissuing the NPDES permit indicates that "monitoring requirements are largely unchanged from the previous Order." The TO requires effluent monitoring for approximately 50 conventional and toxic constituents, plus the remaining USEPA priority pollutants on a weekly to semi-annual basis. Receiving water monitoring is required to be conducted at up to nine sampling locations for up to 50 conventional and toxic constituents, plus the remaining USEPA priority pollutants on a weekly to semi-annual basis. Sediment monitoring is required at two locations for approximately 12 conventional and toxic constituents, plus the remaining USEPA priority pollutants on a quarterly or semi-annual basis. The Discharger is required to participate in the Los Angeles River Watershed Monitoring Program. In coordination with interested stakeholders in the Los Angeles River Watershed, the Discharger shall conduct instream bioassessment monitoring at four stations annually. The Discharger is required to conduct a Special Study to investigate Constituents of Emerging Concern (CECs) in the effluent. A CECs Special Study Workplan is required to be submitted for Executive Officer approval within six months on the effective date of this Order. The TO includes a minimum list of 24 CECs to be monitored annually. The TO (p. E-30) also states that "Once the SCCWRP's recommended list of CECs monitoring in ambient waters, including ocean waters, is finalized, the above list of minimum parameters to be monitored by the Discharger and the sampling frequency may be re-evaluated by the Executive Officer." The sample type and analytical test method are to be proposed by the Discharger. The TO requires an annual reporting and evaluation of the data collected pursuant to this Special Study. The TO also requires that the Discharger propose "a characterization of

all existing CEC data (associated with its effluent or receiving water) that have been collected for various purposes in the past.”

POTW NPDES Permit Monitoring Program Variability – Ocean Plan

Section 13170.2 of the California Water Code directs the State Water Board to formulate and adopt a water quality control plan for ocean waters of California and that the Ocean Plan be reviewed at least every three years. The SWB first adopted the Ocean Plan in 1972. It was amended in 1978, 1983, 1988, 1990, 1997, 2000, 2005, and 2009.

The California Ocean Plan establishes water quality standards for California’s ocean waters and provides the basis for regulation of wastes discharged into the State’s near-coastal waters. The Ocean Plan applies to point and nonpoint source discharges. Currently the Ocean Plan includes three chapters that describe beneficial uses to be protected, water quality objectives, and a program of implementation for achieving water quality objectives. Appendix III to the Ocean Plan includes standard monitoring procedures that provide direction to the Regional Water Boards in developing monitoring programs to accompany discharge permits.

SWRCB staff (August 2011) have prepared a Substitute Environmental Documentation (SED) and Staff Report for proposed amendments to the 2009 Ocean Plan. These proposed amendments to Appendix III, Model Monitoring include question-driven and focus on assuring compliance with narrative and numeric water quality standards, the status and attainment of beneficial uses, and identifying sources of pollution. The proposed Model Monitoring framework has three components that comprise a range of spatial and temporal scales: core monitoring, regional monitoring, and special studies. As noted in Section 3.1.3 Issue Description of the Ocean Plan SED Staff Report (p. 32):

The Ocean Plan does not currently address regional monitoring or standard monitoring and reporting requirements for traditional point sources, stormwater point sources, and non-point source monitoring. Currently, significant differences exist among permit related monitoring efforts along the coast due to the differing quantity and quality among the discharges.

The Southern California Bight (SCB), one of the most densely populated coastal regions in the country, encompasses four wastewater treatment plants discharging over 100 million gallons per day and approximately 15 smaller wastewater treatments discharging directly into the ocean. Over 20 million dollars are spent annually to monitor the influence of these discharges on the marine receiving waters. For the SCB, which encompasses portions of the Central Coast, Los Angeles Santa Ana and San Diego Regions, both major and minor wastewater permittees and MS4 (stormwater) permittees participate in a sophisticated collaborative regional programs in addition to individual permit-specific core monitoring efforts.

Though similar regional monitoring programs are ongoing in other areas such as Monterey Bay, individual point sources are generally smaller and more isolated than those in the SCB, with little consistency between NPDES monitoring programs. The proposed amendments are intended to provide a consistent framework for planning and scaling NPDES receiving water monitoring for ocean waters of California based upon the quantity and quality of effluent. The proposed amendments would be considered for inclusion in Appendix III.

In preparing this proposed amendment staff reviewed and incorporated concepts from the model monitoring method developed by the Southern California Coastal Water Research Project (SCCWRP), in collaboration with the regulated community and regulatory agency staff. The

SCCWRP model monitoring method is question driven, as is the proposed amendment. SCCWRP's model monitoring documents include an approach for large municipal wastewater dischargers, small municipal wastewater dischargers, and stormwater dischargers.

The proposed Appendix III amendments would require monitoring for point sources, stormwater point sources, and non-point sources. Monitoring constituents would include:

- AR bacteria
- Water chemistry
- Sediment chemistry
- Aquatic life toxicit
- Benthic community health
- Bioaccumulation
- Receiving water characteristics

This type, approach, and level of monitoring is similar to that being proposed in other proposed Ocean Plan amendments regarding prohibited discharges to Areas of Special Biological Significance (ASBS). The proposed "special protections" for ASBS discharges includes similar core and regional monitoring requirements as provisions for allowing continuing discharges into ASBS. SWB members heard the staff proposal on October 18 and directed staff to redraft the proposed plan in response public comments.

Northern California Small Ocean Discharger Monitoring Requirements. Sewer Authority Mid-Coastside (SAM) is a small (4 mgd design capacity) secondary treatment plant discharging to the Pacific Ocean in Half Moon Bay. SAM is regulated under NPDES permit Order No. R2-2007-0003, which is currently (late 2011) in the process of being reissued. The SAM permit requires weekly to annually effluent monitoring for approximately 12 conventional constituents plus annual Ocean Plan Table B toxics monitoring. Offshore receiving water monitoring is required at five stations for approximately eight conventional constituents on a quarterly to annual basis.

Southern California Large Ocean Discharger Monitoring Requirements. The Joint Outfall System (JOS) (formerly referred to as the County Sanitation Districts of Los Angeles County) operates the 400 mgd secondary treatment Joint Water Pollution Control Plant (JWPCP). The JOS is regulated under NPDES permit Order No. R4-2006-0042 that is currently (late 2011) in the process of being reissued (Tentative Order R4-2011-XXXX). The extensive effluent and receiving water monitoring programs contained in the TO were based on the 2001 "Model Monitoring Program for Large Ocean Dischargers in Southern California" (Southern California Coastal Water Research Project, Tech. Rep. #357, 101 pp.).

The conceptual framework for the Model Monitoring Program has three components that comprise a range of spatial and temporal scales: (1) core monitoring (effluent and local monitoring); (2) regional monitoring (regional coordinated survey design and sampling techniques); and (3) special studies (focused on refined questions regarding specific effects or development of monitoring techniques).

Discharger participation in regional monitoring programs is required as a condition of the permit. The regional programs that must be conducted under the permit include:

- Future Southern California Bight regional surveys;
- Santa Monica Bay Restoration Project Seafood Safety Survey;
- Central Region Kelp Monitoring Program; and
- Central Bight Water Quality Cooperative Program.

Receiving water monitoring is required to be conducted at multiple stations each in the following general categorical locations:

- Shoreline stations for microbiological monitoring;
- Inshore station for microbiological monitoring;
- Nearshore/offshore stations for microbiological and water quality monitoring;
- Nearshore light energy monitoring stations;
- Bottom stations for benthic sediments monitoring;
- Bottom stations for bioaccumulation monitoring; and
- Bottom stations for fish and invertebrate monitoring (trawl sampling stations).

Effluent monitoring is required for approximately 100 constituents at generally a daily to monthly frequency for conventional constituents and generally quarterly for toxic pollutants.

There is also a requirement to conduct a Special Study of Constituents of Emerging Concern in Effluent. These requirements are similar to those described above included in the LA Tillman WRP NPDES permit.

Municipal Stormwater Discharges

The federal Clean Water Act (CWA)¹³ provides that discharges from point sources to waters of the United States are prohibited, unless authorized by national pollutant discharge elimination system (NPDES) permits (CWA Section 301(a)). In 1987, the CWA was amended to specify the requirements for NPDES permits for stormwater discharges (CWA Section 402(p)).

Consistent with the CWA, California municipalities are required to comply with state (California Water Code (CWC)¹⁴) and federal requirements to control the discharge of pollutants in stormwater runoff from their municipal separate storm sewer systems (MS4s). MS4s are regulated by NPDES permits that contain Discharge Prohibitions, Receiving Water Limitations, and Provisions (e.g., monitoring, commercial and industrial requirements, and inspections, TMDL requirements¹⁵). The Discharge Prohibitions and Receiving Water Limitations require that the stormwater dischargers effectively prohibit the discharge of certain non-stormwater materials, prevent the creation of conditions of nuisance that adversely affect beneficial uses of waters of the state, and comply with applicable water quality standards (WQS).

Compliance with these requirements is achieved through the timely implementation of control measures and other actions to reduce pollutants in the discharge to the “maximum extent practicable (MEP)¹⁶” in accordance with NPDES requirements. The control measures and actions are referred to as Best Management Practices (BMPs). NPDES permits also require MS4s to follow an iterative process as

¹³ Federal Water Pollution Control Act (also referred to as the Clean Water Act or CWA), 33 U.S.C. * 120 I. Statutory references herein are to the CW A.

¹⁴ The California Toxics Rule (CTR) promulgated by USEPA added numeric water quality criteria for a number of constituents (i.e., 30 volatile substances, 58 semi-volatile substances, 15 inorganics, 25 pesticides, and polychlorinated biphenyls (PCBs)) to Water Quality Controls Plans. Subsequently, the State Water Resources Control Board (SWRCB) adopted a State Implementation Plan (SIP) that includes the CTR which states "This Policy does not apply to regulation of stormwater discharges."

¹⁵ A TMDL is a plan that is targeted to reduce a specific pollutant in order to meet water quality standards in a 303(d) listed water body. Once a TMDL is developed, the stormwater NPDES permits must be adopted that are consistent with the TMDL.

¹⁶ The CWA §402(p)(3)(B)(iii) requires that NPDES permits issued to municipalities must include controls to reduce the discharge of pollutants to MEP. The CWA and the Courts have not defined MEP. The Courts have left this discretion to the State. The Phase II regulations offer some guidance on the subject and the SWRCB provided some additional guidance as part of the Phase II general permit. Generally, the MEP definition is met when all BMPs are selected except those that are not technically feasible, where cost exceeds benefits or where selected BMPs serve the same purpose as a rejected BMP.

part of the identification and implementation of additional BMPs, if needed, to address pollutants causing or contributing to the exceedance of water quality standards.

The SWRCB has also adopted a number of decisions (Orders Nos. 91-03, 91-04, 96-13, 98-01, 99-05, and 2001-15) addressing the regulation of municipal stormwater discharges. In addition, the SWRCB has also adopted two statewide general permits regulating the discharge of pollutants contained in stormwater from industrial and construction activities.

At the federal level, a USEPA Environmental Appeals Board Decision (EPA 2002) rejected the requirement that stormwater NPDES permits must include numeric effluent limits to ensure compliance with Water Quality Standards. This conclusion has also been reached in California court decisions (BIA vs SWRCB) and, as a technical matter by a panel of experts assembled by the SWRCB. Thus, this conclusion is well established in federal and California law.

Consistent with the above regulations, the State Water Resources Control Board (SWRCB) and the nine California Water Boards regulate large and small municipal stormwater entering their systems under a two phase system. Phase 1 regulates stormwater permits for medium (serving between 100,000 and 250,000 people) and large (serving 250,000 people) municipalities. The second phase regulates smaller municipalities, including non-traditional small operations, such as military bases, public campuses, and prison and hospital complexes. The largest, single municipal discharger in California is the California Department of Transportation (Caltrans) and their network of highways and road facilities. In addition to Caltrans there are 21 Phase I municipal permits and 125 permittees enrolled in the statewide Phase II municipal permit.

General Industrial and Construction Stormwater Discharges

There are three other permits issued by the State Water Resources Control Board (SWRCB), all with various levels of monitoring required. These permits along with their associated monitoring requirements are briefly described below:

General Industrial Permit - The Industrial Storm Water General Permit Order 97-03-DWQ (General Industrial Permit) is an NPDES permit, issued by the SWRCB that regulates discharges associated with 10 broad categories of industrial activities. The General Industrial Permit requires the implementation of management measures that will achieve the performance standard of best available technology economically achievable (BAT) and best conventional pollutant control technology (BCT). The General Industrial Permit also requires the development of a Storm Water Pollution Prevention Plan (SWPPP) and a monitoring plan. Through the SWPPP, sources of pollutants are to be identified and the means to manage the sources to reduce stormwater pollution are described. The General Industrial Permit requires that an annual report be submitted each July 1. There is an estimated number of 10,000 active permittees in this program area.

Monitoring requirements are tailored to capture the overall impact of stormwater discharge on receiving waters and not the peak impact. At a minimum monitoring is required for four indicators (i.e., pH, TSS, oil & grease, and specific conductance). In addition, monitoring is required based on industrial categories and for specific parameters that indicate the presence of materials that are mobilized by contact with stormwater (e.g., additional monitoring may include one or more of the following: ammonia, Mg, COD, As, CN, Pb, HG, Se, Ag, Fe, Al, Zn (SWRCB website)).

Construction General Permit - Dischargers whose projects disturb one or more acres of soil or whose projects disturb less than one acre but are part of a larger common plan of development that in total disturbs one or more acres, are required to obtain coverage under the General Permit for Discharges of Storm Water Associated with Construction Activity Construction General Permit Order 2009-0009-DWQ. Construction activity subject to this permit includes clearing, grading and disturbances to the ground such as stockpiling, or excavation, but does not include regular maintenance activities performed to restore the original line, grade, or capacity of the facility.

The Construction General Permit requires the development and implementation of a Storm Water Pollution Prevention Plan (SWPPP). The SWPPP should contain a site map(s) which shows the construction site perimeter, existing and proposed buildings, lots, roadways, stormwater collection and discharge points, general topography both before and after construction, and drainage patterns across the project. The SWPPP must list Best Management Practices (BMPs) the discharger will use to protect stormwater runoff and the placement of those BMPs. Additionally, the SWPPP must contain a visual monitoring program; a chemical monitoring program for "non-visible" pollutants to be implemented if there is a failure of BMPs; and a sediment monitoring plan if the site discharges directly to a water body listed on the 303(d) list for sediment. There have been as many as 15,000 active permittees in this program area in the past (SWRCB website).

The permit requires effluent monitoring and reporting for pH and turbidity in stormwater discharges and suspended sediment concentration (SSC) under certain conditions. In addition, the permit calls for receiving water monitoring (e.g., bioassessments) under high risk situations.

Stormwater Monitoring Requirements

There is a wide range in stormwater NPDES permits and particularly receiving water monitoring program requirements around the State. A brief summary of selected monitoring requirements from POTW NPDES permits for discharges to San Francisco Bay, Sacramento River, Los Angeles River and Caltrans is presented below.

San Francisco Bay. The San Francisco Bay Regional Water Quality Control Board (Water Board) achieved a significant milestone in its twenty year effort to regulate urban runoff when it issued the Municipal Regional Storm Water NPDES Permit in October of 2009. This permit, referred to as the MRP ("merp" to insiders), replaces permits previously issued to all municipalities in Alameda, Contra Costa, San Mateo, and Santa Clara Counties, and the Cities of Fairfield, Suisun City, and Vallejo in Solano County. The MRP, which covers 76 local agencies, including cities, counties, and flood management districts, provides an efficient, consistent, and hopefully more effective regulatory mechanism to control pollutants in urban runoff, building on continuous improvements made via previous permits and actions by municipalities. The MRP contains a number of requirements and specifically addresses the following pollutant of concern categories: Pesticides, Trash, Mercury, PCBs, Copper, Polybrominated Diphenyl Ethers (PBDEs), and Legacy Pesticides, and Selenium¹⁷.

In the 1990s, San Francisco Bay Area urban creeks were found to exceed water quality standards for aquatic toxicity, primarily due to urban runoff of organophosphate pesticides (i.e., diazinon and chlorpyrifos). Stormwater and receiving water monitoring conduct by Bay Area stormwater management programs assisted the State of California in determining the sources, pathways and fates

of orthophosphate pesticides to urban creeks. Due to regulatory actions that phased out the uses of these pesticides and the stormwater program sponsored education of Bay Area citizens about the proper use of pesticides to protect water quality, incidents of aquatic toxicity in creeks were significantly reduced by the early 2000s.

In parallel to the decline of organophosphate pesticide use and associated aquatic toxicity in urban creeks, the use of and water quality impacts of pyrethroid pesticides (e.g., permethrin, bifenthrin and cyfluthrin) became evident through evaluations of pesticide use data and receiving water monitoring conducted by stormwater programs and the State of California. Both researchers and municipal stormwater programs have identified pyrethroids as the primary cause of sediment toxicity in receiving waters via monitoring. Pyrethroids that are required to be monitored in receiving water bedded sediments and during storm events via the municipal stormwater NPDES permit in the Bay Area include bifenthrin, cyfluthrin, betacyfluthrin, cypermethrin, deltamethrin, esfenvalerate, lambda-cyhalothrin, permethrin, and tralomethrin. Additionally, monitoring of diazinon, chlorpyrifos, and two pyrethroid replacement pesticides, carbaryl and fipronil, is also currently required.

To address toxicity associated with organophosphate, pyrethroid and other types of pesticides in urban creeks, a comprehensive implementation strategy is currently underway in the Bay Area. Federal, state, and local agencies, and others, are called upon to take actions to reduce the potential for pesticides to runoff into receiving waters. Implementation actions focus on three areas:

1. Regulatory programs: use regulatory tools to ensure that pesticides are not applied in a manner that result in discharges that threaten urban creek beneficial uses.
2. Education and outreach programs: focus on decreasing demand for pesticides that threaten water quality, while increasing awareness of alternatives that pose less risk to water quality.
3. Research: fill information gaps and monitor to measure implementation progress.

Although regulatory processes and educational programs can take many years to reach outcomes, the results of these efforts are starting to become evident.

The Water quality monitoring requirements in the previous permits were general and focused on answering broad questions about sources of pollutants, effectiveness of controls, and receiving water impacts. As a result of monitoring conducted by the municipal stormwater programs and the Water Board through its Surface Water Ambient Monitoring Program, more refined management questions have been developed to guide monitoring requirements in the MRP, which are more prescriptive and expansive compared to previous permits. Specifically, the MRP requires monitoring activities to be conducted in the following categories.

- San Francisco Bay Estuary – Monitoring of the Bay through participation in the Regional Monitoring Program for Water Quality in the San Francisco Bay Estuary (RMP) or equivalent.
- Urban Creek Status Monitoring – Monitoring to assess water quality and the condition of beneficial uses in the urban portions of local creeks and rivers includes stressor and source identification projects triggered by the results of urban creek status monitoring; investigations of stormwater treatment control effectiveness; and geomorphic projects to assess how creeks can be restored or protected to cost-effectively reduce the adverse impacts of pollutants, increased flow rates, and increased flow durations of urban runoff (through the Regional Monitoring Coalition (RMC)).

- Monitoring Projects – Includes stressor and source identification projects triggered by the results of urban creek status monitoring; investigations of stormwater treatment control effectiveness; and geomorphic projects to assess how creeks can be restored or protected to cost-effectively reduce the adverse impacts of pollutants, increased flow rates, and increased flow durations of urban runoff.
- Pollutants of Concern and Long-Term Trends Monitoring – Intended to evaluate inputs of pollutants to the Bay from local tributaries and urban runoff, assess progress toward achieving TMDL wasteload allocations, and help resolve uncertainties associated with loading estimates of pollutants to the Bay.
- Citizens Monitoring and Participation – Requires stormwater programs to encourage citizen monitoring and make efforts to incorporate monitoring data collected by citizens into water quality assessments.

Many of these monitoring activities are coordinated regionally through a regional monitoring coalition. The coalition is expected to provide an efficient, consistent, and cost-effective means of monitoring creeks that is coordinated with the Water Board's Surface Water Ambient Monitoring Program and the RMP's Small Tributary Loading Strategy. Additional benefits include coordinated information management, access, and reporting.

Sacramento River. The current NPDES Permit for municipal stormwater discharges from the Cities of Citrus Heights, Elk Grove, Folsom, Galt, Rancho Cordova, Sacramento, and County of Sacramento (# CAS082597), includes a number of water quality monitoring requirements. These requirements generally fall into the following categories: 1) receiving water monitoring in river and urban tributaries, including water column toxicity and sediment and bioassessment monitoring; 2) urban discharge monitoring; 3) monitoring for water quality based programs (i.e., TMDLs); and 4) special studies designed to evaluate the effectiveness of best management practices. Receiving water monitoring and urban discharge monitoring includes sampling water during a number of dry weather and storm events and analyzing for constituents of concern, including pathogen indicators; nutrients; total and dissolved metals; organophosphate, chlorinated and pyrethroid pesticides; and semi- and non-volatile organics. Standard analytical methods consistent with 40 CFR 122.21(j)(4) or described in the Permit are required.

Los Angeles River. The current NPDES Permit for municipal stormwater discharges from the County of Los Angeles and the cities therein, with the exception of Long Beach, includes a number of water quality monitoring requirements. These requirements generally fall into the following categories: 1) receiving water monitoring in river and urban tributaries, including mass emissions monitoring of constituents of potential concern, water column toxicity testing, and bioassessment monitoring; 2) shoreline pathogen indicator monitoring at bathing beaches; 3) monitoring for water quality based programs (i.e., TMDLs); and 4) special studies designed to evaluate the effectiveness of best management practices. Receiving water monitoring includes sampling water during a number of dry weather and storm events and analyzing for constituents of concern, including nutrients; total and dissolved metals; organophosphate, chlorinated and pyrethroid pesticides; and semi- and non-volatile organics. Standard analytical methods consistent with 40 CFR 122.21(j)(4) or described in the Permit are required.

Caltrans Statewide. Under the previous Caltrans permit (Order No. 99-06-DWQ), the Department conducted a comprehensive, multi-component stormwater monitoring program. The monitoring was conducted at more than 180 sites statewide, yielding more than 60,000 data points. The current draft permit includes case-specific monitoring for the following parameters: conventional pollutants (e.g., pH,

TSS, TDS, temperature, TOC), hydrocarbons (e.g., TPH), total metals, pesticides & herbicides, nutrients, water column toxicity (i.e., acute and chronic) and indicator bacteria.

Regional and Statewide Monitoring Programs

There are several regional water quality monitoring programs within California in addition to a statewide program – the California Surface Water Ambient Monitoring Program (SWAMP) -- for surface waters. These programs differ in the geographical extent and specificity but address many of the same questions regarding the severity, extent and temporal trends associated with contaminants and water/habitat quality, such as:

Are chemical concentrations cause for concern and are associated impacts likely?

- If yes, which chemicals and how should they be monitored?
- What are appropriate guidelines for protection of beneficial uses?
- Do spatial patterns and long-term trends indicate particular regions of concern?

What are the sources, pathways, loadings, and processes leading to contaminant-related impacts in receiving waters?

- Which sources, pathways, and processes contribute most to concentrations of concern?
To impacts on receptors of interest (i.e., humans and wildlife)?
- What management actions are most effective in affecting contaminant sources, pathways, loadings and processes? For limiting potential for adverse impacts on humans and aquatic life due to contamination?

What future sources, concentrations and potential impacts of contaminants should we be concerned about?

Regional Monitoring

San Francisco Bay. San Francisco Bay is the largest Pacific estuary in the Americas (covering up to 4,160 km²) and home to 8 million residents. The San Francisco Estuary Regional Monitoring Program (RMP) is a collaborative effort among the San Francisco Estuary Institute (SFEI), the San Francisco Bay [Regional Water Quality Control Board](#), and the [regulated discharger community](#) dedicated to collect data and communicate information about water quality in the San Francisco Estuary to support management decisions. The RMP, in consultation with its technical and stakeholder advisors, set the direction and focus RMP resources in addressing the management questions listed above (see 6.2). To address these questions, a core monitoring program supplemented with “special topic” studies is vetted, planned, and implemented through a partnership that pools resources and establishes a climate of cooperation and commitment to participation among regulators, dischargers, industry representatives, non-governmental agencies, and scientists. The RMP utilizes its special studies to support an adaptive, long term program of study that addresses the highest priority issues, changing management priorities and advances in scientific understanding. For example, the program collects information to characterize spatial patterns and long-term trends in contamination in water, sediment, bivalves, bird eggs, and fish, and evaluates toxic effects on sensitive organisms and chemical loading. The RMP seeks out data from other sources to provide for comprehensive assessment, and serves as a portal to information about contamination in San Francisco Bay in the form of an [Annual Monitoring Results](#) report, a summary for non-specialists ([Pulse of The Estuary](#)), technical reports, and journal publications. SFEI’s website

(<http://www.sfei.org/>) provides access to RMP products and links to other sources of information about water quality in San Francisco Bay.

Southern California. This region is home to the largest urban population center on the West Coast of the U.S, with more than 16 million people living in proximity to the more than 400 km of coastline stretching from Point Conception to the International border with Mexico. Southern California is home to the nation's largest commercial port, one of the largest US Naval complexes, 15 municipal wastewater treatment facilities, 8 power-generating stations, 10 industrial treatment facilities, and 18 oil platforms that discharge to the open coast (Schiff *et al.* 2001). Eighteen **regional watersheds act as stormwater conduits to the coastal ocean**. More than 60 agencies monitor the condition of local aquatic and marine environments, collectively spending over \$30 million per year. The Southern California Coastal Water Research Project (SCCWRP) organizes and/or participates in several collaborative regional monitoring programs, focusing on coastal watersheds, wetlands and the marine environment of the Southern California Bight (or "Bight"). These programs stress performance based QA/QC provisions that include intercalibration exercises to meet program data quality objectives.

Southern California Bight Regional Monitoring Program ("Bight"). The multi-component "Bight" program has been conducted every 5 years since 1994 (<http://www.sccwrp.org/ResearchAreas/RegionalMonitoring/BightRegionalMonitoring.aspx>). The Coastal Ecology component of the Bight program seeks to determine the spatial extent of contaminant accumulation in marine sediments and assess the effects of this contamination on living marine resources. Sampling efforts are based on a stratified random sampling design, so that data can be extrapolated to estimate conditions in the Bight as a whole. The number and type of strata have varied over the years, with a focus on inshore and offshore habitats as well as permitted discharges and land-based runoff locations. The number of sampling sites has averaged around 400 sites per survey. Like the RMP in San Francisco Bay, a number of special/pilot studies are included to determine the extent and severity of new contaminants or to evaluate new environmental monitoring methods. In 2003, endocrine disrupting chemicals and their effects on fish formed the foundation for current collaborative projects on CECs. In 2008, pilot studies were conducted on PBDEs, pyrethroids and selected PPCPs in Bight sediments.

Stormwater Monitoring Coalition (SMC) Regional Watershed Monitoring Program. In 2008, SCCWRP led the design and implementation of a coordinated and regional watershed monitoring program for stormwater quality. The SMC works with the Los Angeles, San Gabriel and Santa Margarita River Watershed Monitoring Programs, to facilitate greater data collection and provide a regional context to address site- and watershed-specific questions. In contrast to the Bight program, the SMC is focused at the watershed level for southern California's coastal streams and rivers, and asks the following questions:

1. What is the condition of streams in our region?
2. What are the stressors that affect stream condition?
3. Are conditions getting better or worse?

The program examines benthic macroinvertebrates, benthic algae, riparian wetlands, water chemistry and toxicity, and physical habitat as indicators. Sampling takes place across 15 coastal watersheds, with sites characterized by land use and stream order. A total of 450 sites will be sampled over a five-year period (approximately 90 sites per year). All data collected by the SMC will be available to the SWRCB's Surface Water Ambient Monitoring Program (SWAMP) (see also 6.2.2.1).

Statewide and Federal Programs

California Surface Water Ambient Monitoring Program (SWAMP). The California Surface Water Ambient Monitoring Program (SWAMP) was created to fulfill the [State Legislature's](#) mandate for a unifying program that would coordinate all water quality monitoring conducted by the State and Regional Water Boards. SWAMP's mission is to provide resource managers, decision makers, and the public with timely, high-quality information to evaluate the condition of all waters throughout California. To accomplish this mission, SWAMP has identified the pieces necessary to successfully and sustainably meet program goals, which include a Quality Assurance (QA) program, a standardized data storage system, lists of relevant water quality indicators and Standard Operating Procedures (SOPs) for sampling, and a policy to review monitoring plans for each project. In addition, indicators and/or metrics that address specific program narrative objectives have been identified (Table 6.2). For a more complete description of SWAMP, go to http://www.waterboards.ca.gov/water_issues/programs/swamp/about.shtml.

Marine Protected Areas (MPAs) and Areas of Special Biological Significance (ASBS). The Marine Protected Areas (MPAs) in California are designed to help protect marine life and the ocean environment from ecosystem impacts due to coastal development, water pollution, and other human activities. The type of protection can vary from physical habitat, to water quality, to restrictions on fishing. MPAs have taken on special meaning in recent years as a result of the Marine Life Protection Act ([MLPA](#)), which mandates a cohesive network of MPAs to help California's threatened marine ecosystems. A subset of MPAs are known as Areas of Special Biological Significance (ASBS), which are water-quality marine protected areas that the SWRCB has deemed shall be void of waste discharges in order to maintain natural water quality. An important first step to determine the effectiveness of MPAs and ASBS is to define "natural" water-quality conditions, thus baseline assessments are currently being conducted through a collaborative program involving more than 30 regulated agencies.

MARINE and Bivalve Monitoring Programs. [The Multi-Agency Rocky Intertidal Network \(MARINE\)](#) is a partnership formed in 2001 by a group of scientists from local, state, and Federal government agencies, universities, and private organizations who conduct monitoring in rocky intertidal zones along the California coast. Long-term data on habitat quality, species abundance, invertebrate counts, and other survey studies will continue to be gathered biannually during the spring and fall at 89 established monitoring sites. A centralized database that would consolidate disparate sets of historic data with future monitoring results has been established in cooperation with SCCWRP. Information generated by MARINE and maintained in a user-friendly format allows managers to assess the health of critical shoreline habitat, identify human impacts, and evaluate the progress of mitigation measures.

In 2009, SCCWRP entered into a memorandum of understanding with the National Oceanic and Atmospheric Administration (NOAA) to re-focus the long-running National Status & Trends Program on CECs. As a result, the SWRCB and SFEI joined a partnership of multiple local, regional and federal agencies to survey the severity and extent of CEC contamination in bivalves and passive sampling devices (PSDs) at more than 70 coastal and estuarine sites statewide. Results from this pilot study are due in 2012. California's Department of Fish and Game State Mussel Watch Program (SMWP) has been in effect since 1976 and is also designed to detect the presence and concentration of toxic pollutants (e.g., trace elements, pesticides, and PCBs) in estuarine and marine waters using resident or transplanted mussels and clams. The SMWP was designed to provide the SWRCB with long-term information on the existence and relative quantities and trends of toxic pollutants in California waters. Funding cuts have severely limited the extent and effectiveness of these programs in recent years.

National Coastal Assessments. There are a number of national programs that summarize the condition of ecological resources in US coastal waters for Congress and the public. Regional agencies such as SCCWRP and SFEI and statewide programs such as SWAMP are working to determine how the condition of California's resources compares to conditions in the rest of the nation. These agencies have participated in efforts to develop a nationally consistent suite of ecological indicators, such as the Heinz Center [State of the Nation's Ecosystems](#) report, a single framework to establish regional-specific benthic community indices, to compile regional and statewide data for national assessments such as EPA's EMAP and NOAA's National Status & Trends Mussel Watch program.

Table B.1. Water quality indicators for California's regional and statewide monitoring programs.

Question	Beneficial Use	Category	Indicator
Is the water safe to swim?	Water Contact Recreation	Contaminant exposure	Total coliform bacteria Fecal coliform bacteria Enterococcus bacteria Enteric viruses AR indicators
Is the water safe to drink?	Municipal and Domestic Water Supply	Contaminant exposure	Inorganic water chemistry Nutrients Organic water chemistry Total coliform bacteria Cryptosporidium Giardia
Is it safe to eat fish and other aquatic resources?	Commercial and Sport Fishing, Shellfish Harvesting	Contaminant exposure	Fish tissue chemistry Shellfish tissue chemistry Coliform bacteria in shellfish Fecal coliform bacteria in water
Is aquatic life protected?	Aquatic Life	Biological Response	Phytoplankton Chlorophyll-a Benthic infauna Fish assemblage Fish pathology Recruitment of sensitive life-stages Interstitial water toxicity Macroinvertebrate assemblage Periphyton Sediment toxicity Water toxicity
		Pollutant exposure	Acid volatile sulfides/simultaneously extracted metals Debris Interstitial water metal chemistry Reporter Gene System (RGS 450) Organic and inorganic sediment chemistry Total organic carbon Shellfish or fish tissue chemistry Nutrients Turbidity Inorganic and organic water chemistry

Table B.1. Continued

Question	Beneficial Use	Category	Indicator
Is aquatic life protected? (Cont.)	Aquatic Life (Cont.)	Habitat	Dissolved oxygen Sediment grain size and gradations Sediment organic carbon Water flow Water temperature Channel morphology Residual pool volume Instream structure Substrate composition Wetland vegetation Riparian vegetation Electrical conductivity Salinity Hydrogen sulfide Ammonia
Is water flow sufficient to protect fisheries?	Sufficient Flow	Habitat	Water flow Suspended solids Channel morphology Water temperature
		Biological response	Fish assemblage and populations Macroinvertebrate assemblage and populations Periphyton Wetland habitat Riparian habitat
Is the water safe for agriculture use?	Agricultural Supply	Pollutant Exposure	Organic and inorganic chemistry
Is the water safe for industrial use	Industrial Supply	Pollutant Exposure	Organic and inorganic chemistry Total organic carbon Temperature Electrical conductivity
Are aesthetics conditions of water protected?	Non-contact Water Recreation	Pollutant Exposure	Taste and odor Debris and trash

B.2 Quality Assurance/Quality Control for Analytical Methods

Quality control (QC) is the ability to determine and minimize systematic and random errors. A systematic error (or “bias”) is one in which reported values are consistently different from the true value. The ability to reproducibly determine the same value from a given sample is called the precision of the measurement. The ability to determine the true value in an environmental sample is known as accuracy. Random errors are more difficult to track and can affect both the accuracy and precision of an analytical method. Detection of an analyte when it is actually absent is a Type I error (“false positive”), while an error that results in non-detect when the analyte actually is present is a Type II error (“false negative”). Quality assurance (QA) is the step mandated in a particular protocol and/or laboratory to produce accurate and precise analytical data, thus minimizing Type I and Type II errors. Generally, a quality assurance project plan (QAPP) is established before actual environmental testing begins. The QAPP will specify QA/QC procedures that are to be followed and documented at each step of the particular protocol. In environmental monitoring, QAPPs include seven key: problem definition, sample program design, field sampling, sample preparation, chemical analysis, data analysis, and reporting (Batley 1999).

Problem Definition. The initial question in development of a monitoring program can be stated as “What is the problem that requires monitoring?” In defining the problem, it is important to define the goals of a particular monitoring program. In this case, the key question relates to the potential for unregulated CECs to affect aquatic systems. There are several questions within the overarching objective, but the primary focus is to determine which compounds are most likely to be causing an adverse impact. Therefore, the monitoring program should be designed to answer this question or at least provide additional evidence towards determining if an environment problem exists at all. Monitoring for the sake of monitoring will not lead toward an improved environmental condition, but rather, can contribute to environmental demise through increased use of hazardous solvents and disruption of natural habitat through perturbations during extensive sampling regimes. Therefore, it is important to accurately and specifically define the problems to be addressed before the monitoring program is designed and executed.

Sample Program Design. Once defined, a sampling program can be designed to best address the problem. One of the greatest challenges is capturing the representativeness of the true population, i.e., how accurately will the samples collected portray the actual environmental condition? Moreover, will the sampling program capture spatial, temporal, and biological variability? Figure B.1 illustrates how the concentrations of the two pharmaceuticals in Lake Mead (Nevada) varied by distance from a WWTP outfall and the depth of sampling. The discharge of relatively saline wastewater does not always mix with the receiving (fresh) water, resulting in overflow, interflow, or underflow stratification (LaBounty and Horn 1997; LaBounty and Burns 2005, 2007). Thus, a monitoring program with a single collection depth would not accurately portray the actual environmental conditions within this reservoir.

Similarly, temporal variability can also result in dramatic differences in MECs. A recent publication demonstrated that time of day can greatly impact the concentration of certain CECs in WWTP effluent (Figure B.2) (Nelson et al. 2011). This publication and others demonstrate that different days of the week, months, seasons, weather patterns, and even holidays can impact the loading of CECs from WWTPs (Huerta-Fontela et al. 2008, Ort et al. 2010, Delgado-Moreno et al. 2011, Gerrity et al. 2011). The mobility of aquatic organisms and the possibility that exposure to CECs can change due to their mobility/migration should also be considered.

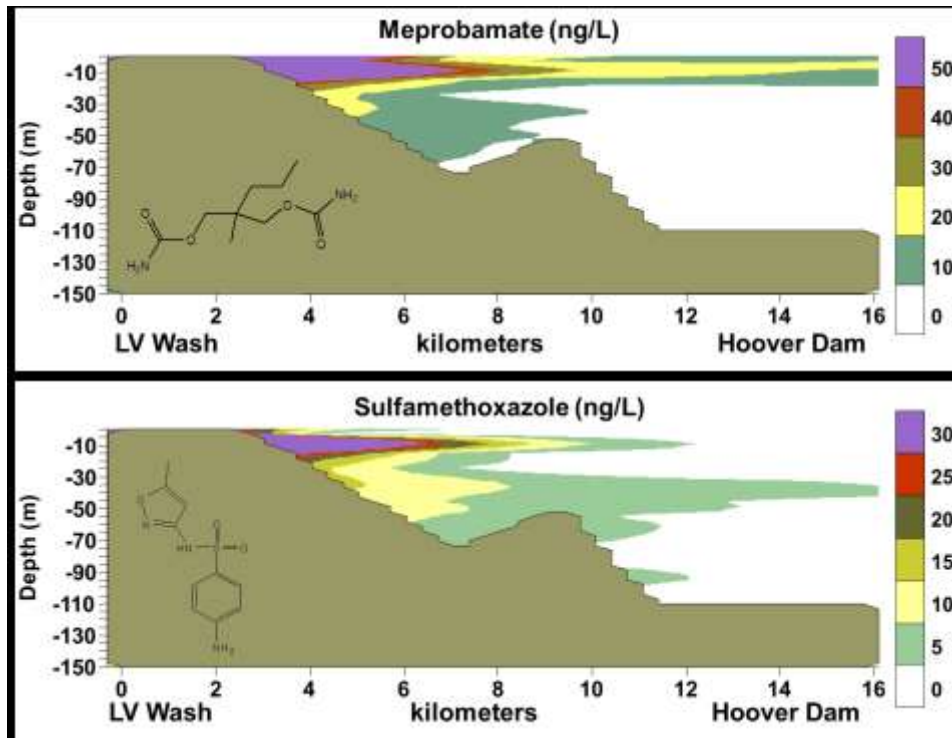


Figure B.1. Monitoring of the pharmaceuticals meprobamate and sulfamethoxazole in Lake Mead, Nevada (depth and longitudinal profiles) (Snyder and Benotti 2010).

Providing adequate statistical power is also an important consideration. Generally, the limiting factor in a strong statistical design will be the cost associated with increasing sample size. While it may be appealing to consider pooling of samples to reduce costs, the statistical power of the sampling program will likely be diminished, and may not have the statistical resolution to adequately determine actual environmental conditions. Therefore, the Panel recommends consulting a statistician with expertise in environmental monitoring before finalizing any sampling program.

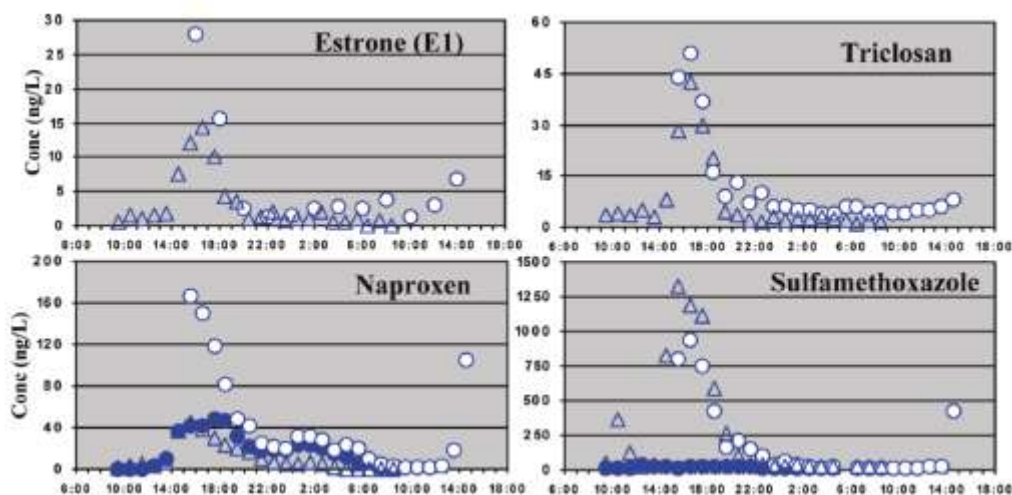


Figure B.2. Diurnal profiles of CECs in treated municipal wastewater effluent (Nelson et al. 2011).

Field Sampling. Field sampling is a critical component to any successful environmental monitoring program, and is the program component where QA is required (Wagner 1995). Grab samples defined as independent discrete samples at a single point in time and space generally provide the highest degree of precision in terms of quantifying a particular chemical or group of chemicals. Compositing is an alternative method of sampling that is often utilized by WWTPs. Sample aliquots are collected at specific times or locations and combined in a common container or are added at a given flow rate to form a composite which integrates the variability in time and/or space to provide a “mean” value for the chemical (s) of interest. Composites can also be part of a pooled sample design, e.g., blood plasma from groups of fish in a certain exposure regime can be pooled to generate the sample volume required for the analyses of interest (Fick et al. 2010a). Sample compositing has many pitfalls and challenges to consider, including sample preservation for labile CECs. Acidification and/or biocide addition as a means for preservation is extremely difficult to accommodate/control when using automated compositing equipment. Another challenge when compositing is cleanliness between and among samples collected. A third challenge is achieving representativeness of field blanks and matrix spikes when using compositing devices. Once again, the primary decision should consider the environmental problem to be addressed and the sample design that best addresses the particular concern/goal.

Passive sampling devices (PSDs) are rapidly gaining favor as an alternative means to collect and pre-concentrate target analytes in environmental media (e.g., water, sediments). PSDs can be calibrated to operate under rapid uptake or equilibrium conditions, taking advantage of preferential partitioning into the device from the media of origin (e.g., water) and offer a range of benefits over conventional sampling and lab concentration protocols (see Box 2.3).

Box B.1. Passive sampling techniques

Passive sampling devices were first designed for lipophilic contaminants like DDT (Petty et al. 1995) and alkylphenols (Bennett and Metcalfe 2000). One such device is known as the semipermeable membrane device (SPMD), which captures the contaminant of interest in the oil-filled reservoir of polyethylene casing. Lipophilic chemicals would partition from water (or sediment) and accumulate to several hundred fold higher concentrations in the oil phase (Petty et al. 2000). Over the years, different PSD designs and materials have evolved and have been used to monitor and predict chemical uptake by aquatic organisms (Bevans et al. 1996, Petty et al. 2004). More recently, the use a polar organic chemical integrative sampler (POCIS) has expanded the realm of chemicals that can be concentrated (Alvarez et al. 2005). The POCIS sampler employs solid-phase extraction (SPE) material similar to those used for isolating polar and non-polar CECs using conventional analytical protocols.

Passive samplers offer several benefits. First, they “monitor” over a pre-specified time period, providing an integrated assessment of concentration, similar to a time-averaged composite sample. A second advantage is ultra-sensitivity. Whereas grab samples are limited by a finite practical volume or mass, passive samplers take advantage of concentration ratios ~1 million, thus requiring much smaller sampler volumes that are easy to deploy, handle and process. A third advantage is cost and sample turnaround time. Passive samplers are constructed of inexpensive materials, do not require power or elaborate field equipment and need only a minimum of post-sampling lab processing. Disadvantages include the necessity for careful pre-calibration, extended deployment/equilibration times, possible interferences due to fouling when sampling productive waters and translation of PSD results into parameters amenable for direct application by data users (i.e., management).

While passive sampler devices offer excellent potential to obtain qualitative and quantitative information on ultra-trace levels of certain CECs, several challenges remain before they can be routinely utilized. It is uncertain how passive samplers would respond to short-term fluctuations such as those reported in treated wastewater effluent (Nelson et al. 2011) or during strong storms. In these situations, or if one is tasked to determine if an instantaneous water quality criterion has been exceeded, then synoptic grab or composite sampling may be more advantageous. If, however, screening for biological activity is desired, then passive samplers can offer the advantage of accumulating substances over longer periods of time and to higher mass. The Panel recommends support for current and future efforts to provide standardized guidance on the use and application of PSD results, particularly as they pertain to CECs in water, sediment and tissue matrices.

Sample Preparation. A unique challenge in monitoring water quality is determining the fraction of chemicals bound to particles versus freely dissolved. Hydrophobic organic chemicals by definition readily sorb to particles, e.g., PBDEs are widely found in SFB (Oros et al. 2005) and southern California coastal and marine sediments (Dodder et al. 2011). This issue becomes especially confounding when considering stormwater and secondary WWTP effluent. Information regarding particle bound CECs is relatively sparse, especially for the above CEC sources (see also Section 3) as they enter the estuarine or marine environment. Moreover, many analytical methods use subjective criteria in deciding whether to filter an aqueous sample or not, such as turbidity or sampling location within a treatment process train (Trenholm et al. 2006). If filtering is carried out, the type and particle retention efficiency of the filter used may not be consistent among methods considered. While SPE-based methods without pre-filtration theoretically load particles onto the solid-phase sorbent, the efficacy of extraction of particle bound contaminants is highly questionable. Other extraction methods involve rigorous solvent techniques such as Soxhlet (Veilens et al. 1992) or accelerated solvent extraction (Martens et al. 2001, Golet et al. 2002). When considering the detection of CECs in sediments near wastewater outfalls and stormwater discharge points, a confounding factor will be determination of loading from freely dissolved CECs versus deposition of particles with adsorbed CECs. The differentiation between dissolved and particle bound CECs are a research need identified by the Panel.

Sample preservation, storage, and transport is another key aspect of ensuring quality monitoring data. Vanderford et al. (2011) details many of the key considerations in sample containers, preservation, and holding times for some CECs. However, specific sample handling conditions should be verified and validated for all compounds targeted for monitoring. Field blanks and matrix spikes are a critical QA/QC component that can identify false positives from contamination and false negatives from sample loss (i.e., degradation during transport). Holding times should be established for all analytical methods and sample matrices. The addition of surrogate standards, preferably stable isotopically labeled analogs of targeted analytes, to environmental samples immediately after collection would allow operators to estimate end-to-end analyte recovery, and final results could be appropriately normalized. Surrogate standards could also be added to sample collection containers in advance; however, great care would be needed to prevent loss due to over filling or pre-rinsing. Blind, randomly sequenced matrix spikes, replicates, and field blanks should also be used to test for laboratory or batch-wise bias.

Chemical Analysis. There are several causes of erroneous analytical data, including matrix interferences, high background, instrument failure, memory effect (injected sample “carry-over”), and operator error. To guard against memory effects, random instrument blanks and replicate sample analyses should be performed. Standard addition -- a known amount of analytical standard is added to a sample extract that has previously been analyzed -- is a practice that can help determine if recovery is compromised, a common occurrence with LC-MS when using electrospray ionization. Moreover,

standard addition can yield important information regarding the degree of suppression within a given sample extract. Increasingly sensitive and selective analytical instruments will be valuable in reducing the amount of extract needed to achieve the desired limit of detection and increasing the number of analytes available for detection. Refinement of analytical methods allows for sample volumes of a few mL or less to be analyzed at detection limits in the ng/L range in many cases. For aqueous samples, automated on-line SPE and solid-phase microextraction (SPME) have revolutionized high-throughput environmental analyses (Canosa et al. 2005, Trenholm et al. 2008, Lopez-Serna et al. 2010). Smaller sample volumes translate into reduced shipping, waste generation/disposal and analytical costs as well as consumption of consumables (e.g., extraction solvents). With the advent of ultra-high performance LC (UHPLC), it is conceivable that water will be analyzed directly without extraction/concentration procedures (Weiss and Reemtsma 2005, Thompson et al. 2009, Bisceglia et al. 2010). In addition, the reliability of next-generation analytical instruments will improve by incorporating diverter valves to minimize system contamination from extraneous materials. Microfluidic developments have produced “lab on a chip” and nanospray/nanopump technologies will likely gain increasing application for environmental analysis. Miniaturized analytical techniques will be capable of analyzing a multitude of environmental samples quickly and efficiently, which allow more samples to be analyzed with far less resources. A more detailed discussion of analytical protocols for CEC analysis can be found in the CEC Recycled Water Panel report (Anderson et al. 2010).

Data Processing and Reporting. Analyzing data is another potential source of error. Most instrumental platforms will integrate peaks based on criteria provided by the operator. However, it is important to manually check peak integration as shifting baselines can result in “noisy” signals that often result in misaligned integration. Additionally, converting peak areas and adjusting to surrogate and internal standards can result in systematic, mathematical errors that are difficult to detect. When possible, certified reference materials (CRMs) should be used to determine if the laboratory values are in alignment with the “true” value. Appropriate laboratory records and standard operating procedures are important in final data calculations and reporting. For instance, sample volume or mass could fluctuate due to spills or extraction failures, in which case, an error would be propagated unless the final analytical concentration is properly adjusted to the actual sample volume/mass. Automated data handling packages such as laboratory information management systems (LIMS) are generally less prone to calculation and data transcription errors; however, care must still be taken to ensure data were accurately transferred from the analytical software. The data reporting stage is also an opportunity to evaluate field, laboratory, and instrumental blanks to determine appropriate reporting limits. Replication and control charts can be extremely valuable in determining whether resulting data are accurate and precise prior to reporting.

Conclusion. Most aspects of QA/QC for environmental monitoring are well understood and properly attained by the majority of well-regarded scientists and commercial laboratories. Ultra-trace analysis (sub-ng/L) is inherently more difficult in terms of potential for Type I and Type II error. However, modern analytical techniques such as isotope dilution and automated on-line solid-phase extraction offer tremendous promise for continually improving analytical data. A detailed QAPP is critical in addressing the question(s) for which the particular study was initiated. Ultimately, through proper planning, QA/QC, and ensuring the samples selected are representative of the population to be monitored, accurate and precise analytical data are possible which allow environmental managers to make the best possible decisions.

Unique Analytical Aspects of Tissue and Sediment Analyses

Although the majority of data concerning CECs in the environment are from aqueous samples, the advancement of analytical protocols has allowed for detection of some CECs (e.g., PBDEs and pyrethroids) in sediment and biological tissues (Maruya et al. 1997, Snyder et al. 2001a, Schlenk et al. 2005). The analysis of CECs in these matrices requires additional analytical considerations, e.g., the need to homogenize sediment and tissue samples. For tissues, samples may be from discrete organs, sections of the organism, or whole bodies. Sediment samples generally require pre-screening to remove rocks and other coarse debris before thorough mixing can take place. Removal of water via freeze-drying or addition of desiccants is often performed prior to extraction to maximize extraction efficiency. Similar to aqueous samples, isotopically-labeled surrogate standards should be added to homogenized samples and followed through the analytical procedure. A known challenge with the organic extraction of solid materials is the efficiency and recovery of the extraction. While the addition of surrogate standards and spike recovery of native compounds provides some information of efficiency, the true extraction of an organic compound embedded within the complex tissue or sediment matrix can be far less effective than those compounds which were spiked. In order to gauge efficiency and method accuracy, parallel analysis of certified and/or standard reference materials (CRMs/SRMs), if available, is highly recommended. The Panel recommends that the State engage in a dialogue with agencies such as the National Institute of Standards and Technology (NIST) to facilitate the creation of CRMs/SRMs for priority CECs in sediment and tissue matrices. When applying a new analytical protocol, it is recommended to extract a representative sample repeatedly, or using different solvent systems to ensure complete recovery. Procedural blanks for tissues and sediments can be more challenging in identifying an appropriate “blank” matrix, such as pre-extracted sodium sulfate or diatomaceous earth. Samples collected from known control or reference sites are helpful in gauging background concentrations. Another challenge with tissues and sediments are the greater number and level of matrix interferences that are co-extracted with the target CECs. Cleanup and/or fractionation steps are typically warranted to isolate the target CECs from matrix interferences as well as co-occurring chemicals. For instance, tissue protocols often employ gel permeation (size exclusion) chromatography to remove protein and lipid interferences in sample extracts. The complexity of chemical residue profiles may warrant additional steps to sub-divide or “fractionate” residue chemicals into distinct fractions for instrumental analysis. Regardless of matrix, QA/QC issues remain of paramount importance in the analysis of environmental samples.

APPENDIX C - CEC SOURCE AND FATE MODELS

C.1 Screening Level Water Mass Balance Model

To better understand the relative importance of the various major sources of water to California's coastal and marine environments, the Panel created a screening-level water mass balance model (SLWMBM) for the Southern California Bight (SCB) based on a series of assumptions and readily available information. The model divided the SCB into three regions, based upon the total distance from the shoreline (Figure C.1), with each region treated as a volume or box of water. The model estimates the amount of water entering each of these regions from five relevant sources: effluent from WWTPs; stormwater; rain water falling directly onto coastal environments; groundwater discharging into the ocean; and ocean currents causing seawater to flow into and out of each of these regions. The Panel acknowledged that other sources of CECs exist that could also be contributing to the CEC load in the water contained in each of these coastal regions. For example, certain areas of the SCB (as well as other parts of the coast of California) have sediments that contain compounds that some people may classify as CECs.

The SLWMBM assumes that the SCB is 300 kilometers (km) long. The three coastal regions are assumed to be 0-1 km ("near-shore"), 0-5 km ("mid-shore"), and 0-10 km ("off-shore") from the shoreline with average depths of 0.05 km, 0.05 km and 0.5 km, respectively. Thus, the total volume of each region is estimated to be 1.5×10^{13} liters (L), 7.5×10^{13} L and 1.5×10^{15} L, respectively (Figure C.1). The total annual WWTP effluent flow into the SCB is estimated to be 1.7×10^{12} L and stormwater runoff is estimated to be 1.1×10^{12} L (Lyon and Stein 2009). Annual rainfall is assumed to be 25 centimeters resulting in 7.5×10^{10} L, 3.8×10^{11} L and 7.5×10^{11} L of rainwater entering each of the regions, respectively. Groundwater is assumed to discharge into the SCB at a rate of 5 cm per day (Swarzenski and Izbicki 2009), equivalent to 0.05 m³/m and the discharge is assumed to occur within the first 100 meters (m) of shoreline along the entire 300 km length of the SCB. This results in an assumed annual groundwater discharge of 5.5×10^{11} L. Exchange of water within each of these three coastal regions caused by ocean currents is estimated for four possible current velocities: 0 km/day, 1 km/day, 5 km/day and 10 km/day. The latter was selected as an upper bound of velocity based on reported velocities of eddies within the SCB. These result in annual inflow (and outflow) of 0 L, 1.8×10^{13} L, 9.1×10^{13} L and 1.8×10^{14} L, respectively for the 0-1 km coastal region (Figure C.1)).

Similarly, the annual water exchange due to ocean currents is assumed to be 0L, 9.1×10^{13} L, 4.6×10^{14} L, and 9.1×10^{15} L, respectively, for the 0-5 km coastal region and 0L, 1.8×10^{15} L, 9.1×10^{15} L and 1.8×10^{16} L, respectively, for the 0-10 km coastal region. For screening purposes, the model assumes complete and instantaneous mixing of each input within each coastal region. The Panel acknowledges that this is an important simplifying assumption and that near field effects may occur that would not be predicted by the SLWMBM. Nevertheless, the Panel believes the SLWMBM has great utility in being able to identify those sources of water (and CECs) to coastal systems that have the greatest potential to cause an effect.

The Panel believes that for the vast majority of CECs associated with surface water inputs to the coastal system, focusing on the five water sources listed above should provide an adequate characterization of relative importance of major CEC contributions. For compounds that are known to be present in coastal sediments and that have been shown to pose a potentially unacceptable risk in the past (e.g., PCBs or DDT and their degradation by-products), more refined modeling will be necessary to determine the relative importance of sediments versus surface water inputs (see discussion below).

Screening Level Water Mass Balance Model (SLWMBM) for the Southern California Bight (SCB)

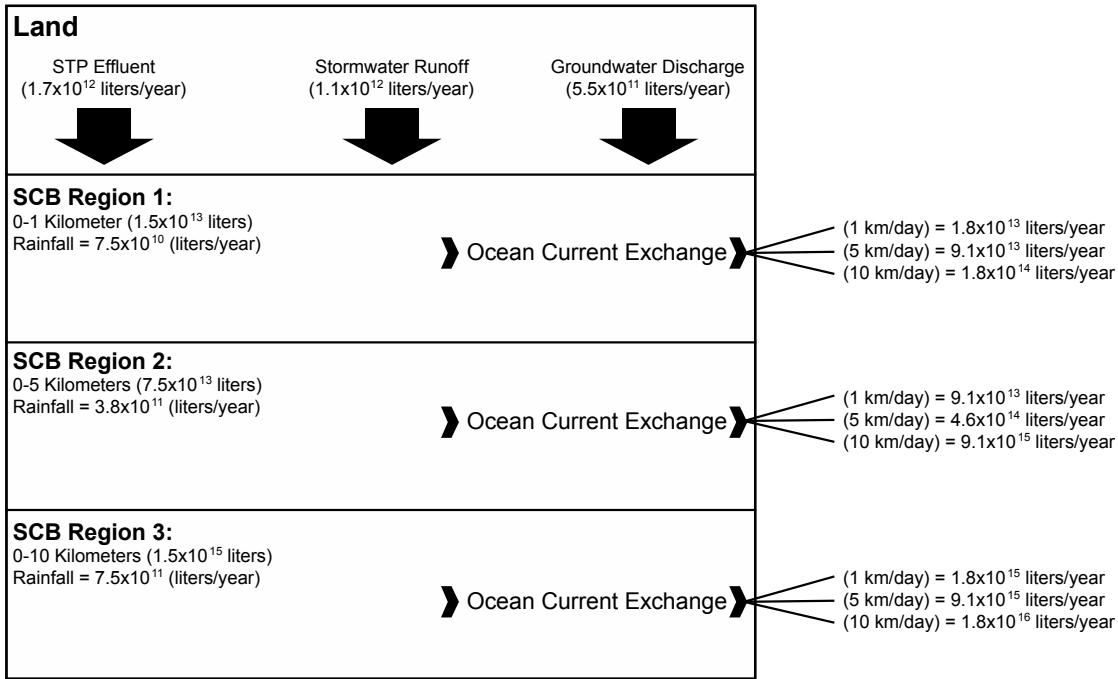


Figure C.1. Screening level mass balance model for the Southern California Bight.

The relative importance of each source was estimated by calculating the dilution factor for each source for each coastal region. Within each region, dilution factors were estimated for the four different assumed ocean current exchange volumes. Table C.1 summarizes the dilution factors. The Panel notes that several observations become apparent from a comparison of dilution factors, keeping in mind that these dilution factors assume complete and instantaneous mixing within each of the 3 modeled regions.

- **Mid- and off-shore regions.** Very large dilution exists for inputs to the mid- and off-shore regions. For example, a recent study on CECs in treated effluent and receiving seawater from large WWTP outfalls in the SCB suggested outfall dilution factors of ~ 1000 (Vidal-Dorsch et al. 2011). These dilution factors are large enough to suggest that investigating effects associated with “off-shore” discharges is not a high priority at this time. Investigation of potential effects associated with CECs should first focus on inland fresh water and near-shore coastal releases. If potential effects are found to potentially exist with those discharges, then further assessment of effects associated with off-shore discharges may be warranted.
- **Rainfall.** Large dilution factors exist for rainfall in all coastal regions. Unless a CEC is found to be present at substantially higher concentrations in rainfall than either in WWTP effluent, stormwater or groundwater, direct rainfall is not likely to represent an important source of CECs to inland fresh waters or coastal waters. Few data are available on the magnitude of dry and/or wet deposition of CECs in this region (see also Section 5).

- Near-shore. In the freshwater inflow region to coastal waters, the lowest dilution is predicted for WWTP effluents, followed by stormwater and then groundwater, though even in the near-shore coastal region, dilution factors are relatively large (between 10 and 400) when the water exchange by ocean currents is accounted for and assumed to be instantaneous within a particular coastal zone of the SLWMBM. (Note that in marine environments the potential for near-field effects at discharge locations is not ruled out by the results of the SLWMBM and that in inland freshwaters relatively low dilution would also be associated with WWTP discharges and under low river flow conditions into effluent dominated rivers.)
- A cursory review of the near-shore dilution factors suggests that the greatest potential for effects is associated with waterways dominated by WWTP effluents because they have the lowest dilution factors. However, that may not be the case for at least two reasons. First, WWTP effluents are not generally discharged immediately adjacent to the shoreline but are rather released off-shore, often beyond the 1 km distance that defines the near-shore coastal region in the SLWMBM. Both stormwater and groundwater are discharged in the immediate vicinity of the shoreline. Second, inputs of effluent from WWTPs and groundwater to the coastal system are generally continuous and, thus, assuming complete mixing, the dilution factors shown in Table C.1 may well be representative of the relative long-term impacts of these two sources. Stormwater in most regions of the State does not represent a continuous discharge. In the SCB, the vast majority of the annual stormwater input may occur on a few days with heavy rainfall. During periods when storm events are occurring, substantially lower dilution of stormwater may be occurring in the near-shore coastal region than suggested in Table C.1. The Panel recognizes that this limited amount of dilution may only be present for the few days during and immediately following a storm event. However, the Panel believes these relatively short-term, potentially high CEC concentration events should be evaluated closer to determine whether they may pose a risk to aquatic receptors. This evaluation would also be applicable to the potential effects of CECs in stormwater on inland fresh waters.

Table C.1. Dilution Factors for CEC sources in three coastal regions using a screening level water mass balance model (SLWMBM).

Dilution Factors for Different Coastal Regions					
Ocean Current (km/day)	Rainfall	WWTP Effluent	Stormwater	WWTP and Stormwater	Groundwater
Near-Shore Coastal Region (0-1 km)					
0	200	9	14	5	27
1	440	20	30	12	60
5	1400	63	97	38	190
10	2600	120	180	71	360
Mid -Shore Coastal Region (0-5 km)					
0	200	44	68	27	140
1	440	98	150	59	300
5	1400	310	480	190	970
10	2600	580	900	350	1800
Off- Shore Coastal Region (0-10 km)					
0	2000	880	1400	540	2700
1	4400	2000	3000	1200	6000
5	14000	6300	9700	3800	19000
10	26000	12000	18000	7000	36000

Beyond providing insight about the relative importance of different sources of water to the SCB, the SLWMBM could also be combined with the information about the concentrations of CECs in the various sources of CECs to the SCB. With that information the Panel, or others, could develop a mass balance for key CECs to better understand the relative contributions of the primary input sources to the SCB. For inland waters, measured concentrations of CECs in WWTP effluents and runoff could be used directly to understand the relative importance of those two sources (assuming minimal dilution in an effluent dominated river during low flow conditions).

Summary. The observations about the differences in dilution across the three coastal regions, the Panel's expectation of minimal dilution of WWTP effluents and runoff in inland waters under worse case conditions, the differences in the nature of the inputs (continuous vs. discontinuous), the different types of CECs that might be in each, and the locations of the inputs, led the Panel to create three scenarios for which to evaluate the potential effects of CECs on inland and coastal systems. The three scenarios encompass what the Panel believes represent the broad range of settings where potential effects from CECs may be of concern to regulatory agencies and the public.

C.2 One-box Mass Balance Model of Contaminant Sources, Loading and Fate in San Francisco Bay - Screening Example using Polybrominated Diphenyl Ethers (PBDEs) as a Surrogate Chemical of Emerging Concern (CEC)¹⁸

BACKGROUND

San Francisco Bay ("Bay") is an estuary situated in the middle of the California Coast and is a tidally complex system characterized by broad shoals and narrow channels (FAA and SF 2003). It can be further divided into two components: the "North Bay" extending from the outlet to the Pacific Ocean at the Golden Gate through the Central Bay northward to San Pablo Bay, Carquinez Strait, Suisun Bay and ending then ending at the Sacramento-San Joaquin River Delta ("Delta") to the east. The second component, known as the "South Bay", extends from the Central Bay at the Bay Bridge southward to its terminus at the Guadalupe River and Coyote Creek watersheds of the Santa Clara Valley (Figure C.2).

Numerous factors affect flows in the Bay, but water depth is the most important (Cheng et.al. 1993). Roughly 90% of the freshwater inflow to the Bay comes from the Delta (Cheng et.al. 1993) and flows through the North Bay resulting in a well-mixed to partially mixed estuary (FAA and SF 2003). In the North Bay the ratio of freshwater inputs to the tidal prism¹⁹ is less than 1% during low-flow conditions (summer) and ~20% during the high flow, winter season (FAA and SF 2003). Very little freshwater flows into the South Bay with flow properties controlled to a greater extent by exchange of water with the Central Bay (FAA AND SF 2003). Due to its relatively shallow mean depth, the South Bay is considered a well-mixed body of water.

In 2008, the San Francisco Bay Regional Water Quality Control Board established a Total Maximum Daily Load (TMDL) for Polychlorinated Biphenyls (PCBs) using a one-box model created by the San Francisco Estuary Institute (SFEI)²⁰. The entire Bay was modeled as a single box -- partitioned into water and

¹⁸ We appreciate the assistance of Courtney Siu (EOA, Inc.) and Dr Jay Davis (SFEI –RMP).

¹⁹ Tidal prism = volume of water exchanged between the ocean and the San Francisco Bay estuary. Average estimates range from 25% to 30% of the entire Bay volume (~1.5 billion m³)(Smith 1966, Cheng et al. 1993).

²⁰ First-order, mass balance models are an important tool in summarizing and synthesizing existing knowledge on contaminant loads, system losses, and environmental compartment transfer rates. These models are useful for

sediment -- which receives inflows from the Delta, municipal and industrial flows, and stream/runoff flows from the surrounding watersheds. Losses included outflow to the ocean, degradation in sediment and water, volatilization, and burial in sub-surface sediment. Re-suspension, diffusion of dissolved PCBs and deposition were also included as processes influencing PCB transfer (Davis 2003, Davis et al. 2007). This model can be used to estimate mass of PCBs lost from the Bay over time and the resulting changes in average PCB mass in water and sediment.

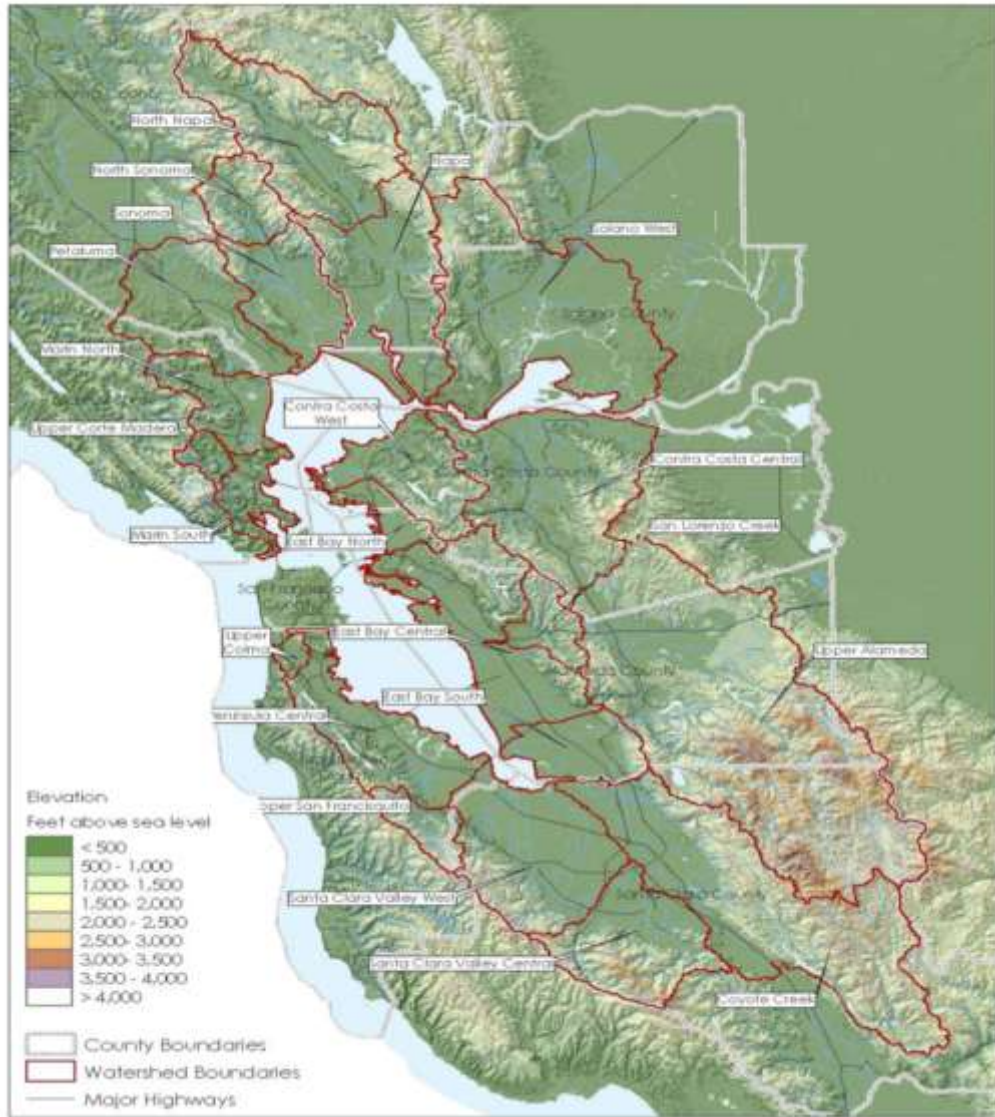


Figure C.2. Watersheds draining into the San Francisco Bay estuary (source - Modeling the Contribution of Copper from Brake Pad Wear Debris to the San Francisco Bay Prepared by A. S. Donigian, Jr. and B. R. Bicknell AQUA TERRA Consultants, October 2, 2007).

analysis of technical and policy issues regarding the environmental system responses to natural processes and contaminant control actions.

The Panel utilized the one-box model, after slight modification described in detail below, as a screening tool for investigating the sources and fate of model and/or target CECs, by changing chemical-specific parameters such as K_{ow} and the Henry's law constant to represent a surrogate CEC (e.g., PBDEs) and adjusting source loading estimates. The model outputs include the mass of CEC surrogate in Bay water and sediment, which after conversion to average sediment and water concentrations, were to be used for:

- a. Screening against chronic and acute toxicity thresholds (Section 6)
- b. input into indirect exposure (e.g., food-web) models

CONCEPTUAL ONE-BOX MODEL

Davis (2003) created the SFEI one-box conceptual source and fate model based on the sources and processes shown in Figure 3.1. The original model computes the mass of the PCBs in the water and active sediment layer through external loads, degradation, tidal flow losses, and the exchange between these layers and the surrounding layers (air and buried sediment).

Equations and Inputs

The conceptual model is based on two governing equations that solve for the mass of PCBs in the sediment and in water, both of which rely on the conservation of mass.

$$\frac{\Delta M_w}{\Delta t} = L + k_{SW1}M_S + k_{SW2}M_S - k_V M_W - k_O M_W - k_{WR} M_W - k_{WS1} M_W - k_{WS2} M_W \quad (1)$$

$$\frac{\Delta M_S}{\Delta t} = k_{WS1} M_W + k_{WS2} M_W - k_{SW1} M_S - k_{SW2} M_S - k_B M_S - k_O M_W - k_{SR} M_W \quad (2)$$

where

M_w	= the mass of PCB in water [kg]
t	= the time step
L	= the external load of PCB to the water column [kg/yr]
k_{SW1}	= the solids re-suspension rate constant [d^{-1}]
M_S	= the mass of PCB in sediment [kg]
k_{SW2}	= the sediment to water diffusion rate constant [d^{-1}]
k_V	= the volatilization rate constant [d^{-1}]
k_O	= the outflow rate constant [d^{-1}]
k_{WR}	= the degradation in water rate constant [d^{-1}]
k_{WS1}	= the solids settling rate constant [d^{-1}]
k_{WS2}	= the water to sediment diffusion rate constant [d^{-1}]
k_B	= the burial rate constant [d^{-1}]
k_{SB}	= the degradation in sediment rate constant [d^{-1}]

Initial concentrations, external loads, flows and Bay parameters were estimated from the literature and published studies by SFEI (Table C.2). It is critical to recognize that this model relies on simplifying a large dataset into average inputs for a number of physical features of the Bay and transfer processes (e.g., water temperature and volume, sediment layers, chemical concentrations in the aqueous and particle phases, exchange rates, etc.). Thus, model outputs are estimates and contain a large amount of uncertainty (Davis 2003). The outputs, however, provide a means of investigating long-term trends in

the ultimate fate of chemical contaminants such as CECs relative to source loading assumptions and parameter variability. In this regard, the model is useful as a screening tool for CECs.

Model user specified parameters include external contaminant load (in kg/year), tide configuration (off, on but not scaled, or on and scaled), attenuation (on or off) and whether or not to plot the concentrations over time. Using the inputs shown in Table C.2, the long-term PCB concentrations predicted in the TMDL final report (CRWQCB 2008) can be replicated for various loading scenarios (Figure C.3). The total PCB mass noted on the y-axis represents the sum of the PCB mass in the water and the active sediment layer²¹.

Partitioning of Source Loading Inputs

The original one-box model included a single source loading input estimate that represented municipal and industrial loads, wet and dry weather stream loads and Delta loads. The one-box model was modified to include separate loads from the following categories:

1. Municipal POTW²² loads from secondary treatment facilities
2. Municipal POTW loads from advanced treatment facilities
3. Stream base loads (dry period)
4. Stream storm loads (wet period)
5. Industrial wastewater loads
6. Delta loads

Table C.2. Inputs and parameters for the San Francisco Bay one-box model for PCBs.

Parameter		Value	Units	Source
SA _W	Surface Area of Water	1.10E+09	m ²	Jassby 1992
SA _S	Surface Area of Sediment	1.285E+09	m ²	Davis 2003
D _W	Average Water Depth	5.3	m	Davis 2003
D _S	Depth of Active Sediment Layer	0.15	m	Davis 2003
V _W	Volume of Water	5.50E+09	m ³	Jassby 1992
V _S	Volume of Sediment	SAS*DS (1.9275 x 10 ⁸)	m ³	Davis 2003
T _W	Water Temperature	15	C	Davis 2003
F	Water Outflow (If Tides Are On)	Qdelta + Qtide	L/d	Code
C _{PW}	Concentration of Particles In Water	8.50E-05	kg/L	Davis 2003
C _{SS}	Concentration of Solids In Sediment	0.726027	kg/L	Code
d _{PW}	Density of Suspended Sediments	1.1	kg/L	Krank and Milligan 1992
d _{SS}	Density of Sediment Solids	2.0	kg/L	Code
OC _{PW}	Organic Carbon Content of Suspended Sediment	0.030		Davis 2003
OC _{SS}	Organic Carbon Content of Bottom Sediment	0.010		Davis 2003
d _{OC}	Density of OC	1.0	kg/L	Code

²¹ San Francisco Bay sediments are divided into active and buried layers. The active layer freely exchanges PCBs with the water column and biota, while the buried sediment layer is assumed to be not available for exchange. The depth of the active layer is dependent on bioturbation and mixing driven by tides and storms (Davis 2003).

²² Also known as municipal wastewater dischargers

Table C.2 Continued

Parameter		Value	Units	Source
V _{EW}	Water Side Evaporation Coefficient	0.649	m/d	Davis 2003
V _{EA}	Air-Side Evaporation Coefficient	423.0	m/d	Davis 2003
V _{SS}	Solids Settling Rate	1.0	m/d	Davis 2003
V _d	Water-to-Sediment Diffusion Coefficient	0.0024	m/d	Gobas et al. 1995
V _b	Sediment Burial Coefficient	0	m/d	Cappiella et al. 1999
pH	ph of Water	7.80		Code
H ₂₉₈	Henry's Law Constant	3.94	Pa-m ³ /mol	Code
K _{OW}	Octanol-Water Partition Coefficient	5495409.0		Code
WS	Average Wind Speed	10.6	mph	Davis 2003
A	Tidal Flushing Ratio [Q _{tide} / Q _{delta}]	3.75		Code
C _{bay}	Average Concentration in Bay Water	426.5	pg/L	SFEI 2007
C _{YBI}	Average Concentration in Water at Yerba Buena Island	315	pg/L	SFEI 2007
C _{sed}	Average Concentration in Sediment	4.65	ng/g	SFEI 2007
C _{ocean}	Average Concentration in Ocean Water	24	pg/L	Connolly et al. 2005
Atten	Attenuation Rate	3.39E-05	1/d	Code
K _{WR}	Degradation in Water	3.40E-05	1/d	Davis 2003
K _{SR}	Degradation in Sediment	3.40E-05	1/d	Davis 2003
k _v	The Volatilization Rate Constant	SA _w * F _{DW} * V _E / V _w	1/d	Code
F _{DW}	Fraction of Dissolved PCB in Water	1 / (1 + (C _{PW} * OC _{PW} * K _{OW} / d _{PW}))	1/d	Code
V _E	Volatilization Coefficient	1 / (1 / V _{EW} + 1 / (K _{AW} + V _{EA}))	m/d	Code
K _{WS1}	Sediment Settling	A _w * V _s * (1 - F _{DW}) / V _w	1/d	Code
K _{WS2}	Water-to-Sediment Diffusion	SA _s * V _d * F _{DW} / V _w	1/d	Code
K _{SW1}	Solids Resuspension	(FL _{RS} / C _{SS}) * (1 - F _{DS}) / (1000 * V _s)	1/d	Code
K _{SW2}	Sediment-To Water Diffusion	SA _s * V _d * F _{DS} / V _s	1/d	Code
FL _{RS}	Resuspension Flux	FL _s - FL _b	kg/d	Code
FL _s	Solids Settling Flux	1000 * C _{PW} * V _s * SA _w	kg/d	Code
FL _b	Sediment Burial Flux	1000 * C _{SS} * V _b * SA _s	kg/d	Code

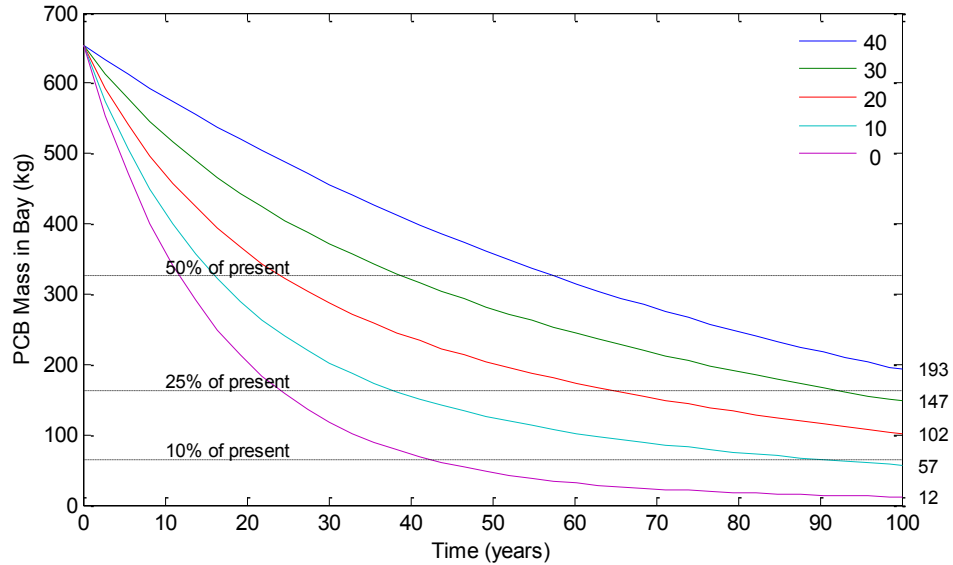


Figure C.3. Long-term PCB mass in the Bay (water + sediment) for different loading scenarios: 40, 30, 20, 10 and 0 kg/year. Estimated flows and concentrations from all external sources are included in Table C.3.

Table C.3. Partitioned inflows to San Francisco Bay and their respective PCB concentrations.

Inflow	Discharge [L/d]	Source	Concentrations [pg/L]	Source
POTWs With Secondary Treatment	2.35×10^9	Total Design Flow for all POTWs with secondary treatment ²³	3600	CRWQCB 2008
POTWs With Advanced Treatment	9.79×10^8	Total Design Flow for all POTWs with advanced treatment	210	CRWQCB 2008
Stream Base Flows	1.51×10^9	Bay tributaries 1980-2005 flows during April 16-Oct 15 ²⁴	9000	Back calculated ²⁵ from L = 10 kg/yr
Stream Storm Flows	4.55×10^9	Bay tributaries 1980-2005 flows during Oct 16-Apr 14	9000	Back calculated from L = 10 kg/yr
Industrial Flows	7.90×10^7	Calculated from TMDL report from L = 0.035 kg/yr	1200	CRWQCB 2008
Delta Flows	6.82×10^{10}	Calculated from average annual water discharge pas Mallard Island 1971-2000 (Oram et al. 2008)	600	CRWQCB 2008

²³ Bay Area Clean Water Agencies, average daily POTW flows for 39 plants for the period 1999 – 2002 and POTW design flows (Amy Chastain spreadsheet dated 2/8/2011).

²⁴ Modeling results used for the preparation of the report entitled "Modeling the Contribution of Copper from Brake Pad Wear Debris to the San Francisco Bay", AQUA TERRA Consultants, October 2007.

²⁵ For storm + base flows, L = 20 (from CRWQCB 2008)

While the greatest difference in loads is seen for delta flows, 14.9 kg/yr falls within the range of values estimated for 2002 and 2003 of 6.0 ± 2.0 and 23 ± 18 kg/year, respectively (CRWQCB 2008).

The resulting mass of PCBs in Bay water and sediments forecast for the next 100 years, with scaled tides²⁶ and attenuation²⁷ operational, are shown in Figure C.4. The line represents the mass of PCBs with an external load of 40.4 kg/year, which includes the partitioned loads in Table A3-4 and the load from the tides. Also shown are lines representing the remaining mass in the sediment for 50, 25, and 10%. As shown, the mass of PCBs in sediments is estimated to be 50% of the current annual loads over a 50-year interval into the future (assuming annual loads of ~40 kg/year). This figure illustrates that the one-box model with the partitioned loads is generally consistent with the output from the aggregated load input model utilized by the RWQB for their TMDL report.

The estimated fate of the PCBs in the system over time is shown in Figure C.5. Recall, that the combined mass of PCBs in the water and sediment is the total mass of PCBs in the Bay. After 30 years, roughly 75% of the total mass of PCBs is estimated to have left the Bay primarily due to tidal exchange and other minor processes (e.g., degradation and volatilization).

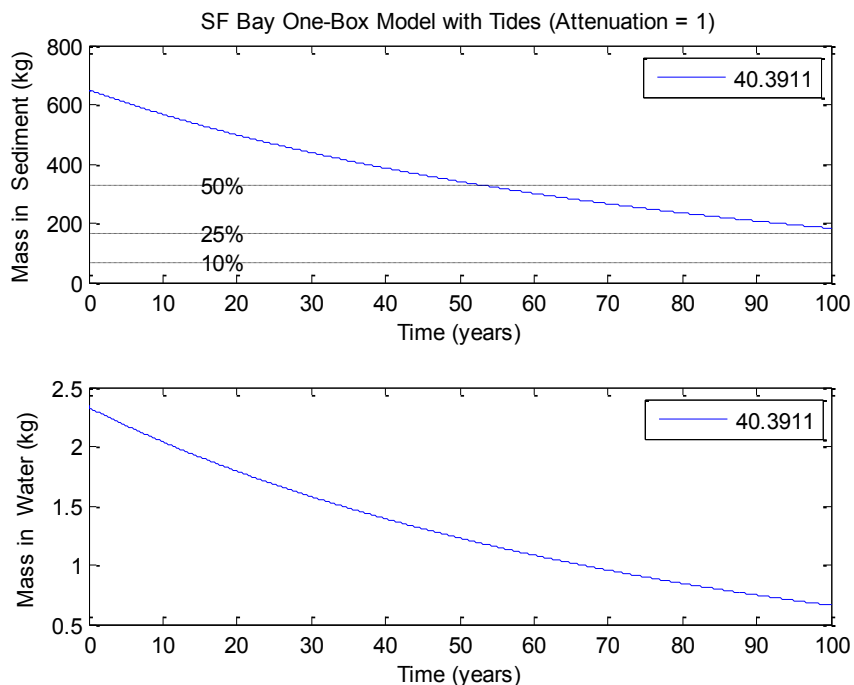


Figure C.4. Mass of PCBs in San Francisco Bay sediments and water forecast for the next 100 years with attenuation and scaled tides. Fifty, 25 and 10% of the current mass are shown as dotted lines.

²⁶ PCB water concentrations in the Central Bay, the segment with a direct connection to the Pacific Ocean, are consistently lower than the Bay-wide average concentration, partially due to dilution by ocean water which does not occur uniformly for the North and South Bay components. Application of the outflow scaling factor is a means of accounting for the spatial heterogeneity of PCB concentrations (SFEI 2007).

²⁷ PCB loads can be expected to decrease due to degradation, volatilization, and burial occurring in watershed soils and sediments, reduced emissions due to existing management efforts, and erosion of less highly contaminated material. An attenuation half-life accounts for these processes (SFEI 2007).

Table C.4. Comparison of estimated partitioned loads and PCB TMDL loads.

Source	Partitioned Loads (kg/yr)	TMDL Loads (kg/yr)
All POTWs (secondary and advanced treatment)	3.2	2.3
All Stream Flows (base and storm)	19.9	20
Industrial Flows	0.034	0.035
Delta Flows	14.9	11
Total	38	33

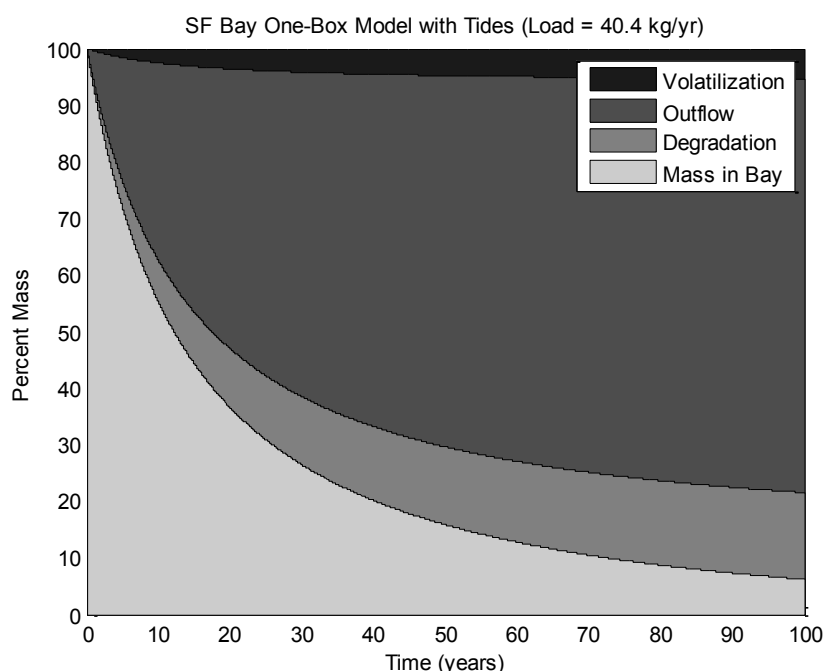


Figure C.5. Prediction of PCB mass loads in Bay sediments and water over time due to various modeled loss processes (e.g., tidal exchange, degradation and volatilization).

Adaptation of the One-Box Model to Screen for CECs: Polybrominated Diphenyl Ethers (PBDEs) in the Bay

Further modification of the one-box model for PCBs can be made to address the fate of polybrominated diphenyl ethers (PBDEs), a class of CECs with similar physicochemical and environmental properties. Such modifications, however, rely on information from the literature and are thus subject to large uncertainty and variability. For the purpose of screening, PBDE 47 (2,2',4,4'-tetrabromo) was chosen because it is a dominant congener observed in the Bay (Oros et al. 2005) as well as other California coastal aquatic ecosystems (Meng et al. 2009, Dodder et al. 2011). The concentration of PBDE 47 has been measured through San Francisco Bay's Regional Monitoring Program for Water Quality (RMP). Concentrations for PBDE 47 in Delta outflows, municipal discharge (POTWs) and stream flows, as well as annual loads were taken from Oram et al. (2008). Chemical specific model parameters such as degradation rates, evaporation and diffusion coefficients, K_{ow} and Henry's law constants were modified for PBDE 47. The adjustments to the one-box model for PBDE 47 are shown in Table C.5.

The base and storm flow concentrations (C_{base} and C_{storm} , respectively) were back-calculated from a reported total annual load of 2.9 kg/yr from small Bay tributaries and an annual runoff flow of 1.05×10^9 m³ (Oram et al. 2008). The resulting annual concentration is 2800 pg/L for all flows. The ratio of concentrations of PBDE 47 during storm flows (a rising hydrograph) to all flows is approximately 2 to 1 for Guadalupe River and Coyote Creek. For base flows (falling hydrograph), this ratio is 0.7 to 1 (base to all). Using these ratios, C_{base} is 1960 pg/L and C_{storm} is 5600 pg/L.

The above concentrations in Table C.5 coupled with the partitioned discharge flows shown in Table C.3 generate an estimated annual external load of 21.7 kg, which falls within the range estimated previously (between 11 and 28 kg; Oram et al. 2008). Using the values from Table C.5, the model was run for PBDE 47 with scaled tides and no attenuation. The expected PBDE 47 mass in sediment and water forecast for 100 years is shown in Figure C.6. Including the tidal loading, the total annual load for PBDE 47 is estimated at 23.0 kg/y. Under the assumed current loading scenario, the mass in both the sediments and water reach a steady state after ~10 years. System losses to degradation, outflow and volatilization are shown in Figure C.7, with degradation serving as the primary loss process. Concentrations of PBDE 47 in sediment and water vs. time are shown in Figure C.8.

Table C.5. Inputs and parameters for the San Francisco Bay one-box model for PBDE 47.

Parameter		Value	Unit	Source
		BDE 47		
K_{WR}	Degradation Rate in Water	0.0046	1/d	Wania and Dugani 2003
K_{SR}	Degradation Rate in Sediment	0.0012	1/d	Wania and Dugani 2003
V_{EW}	Water -Side Evaporation Coefficient	0.67	m/d	Cetin and Odabasi 2005
V_{EA}	Air-Side Evaporation Coefficient	251	m/d	Cetin and Odabasi 2005
V_d	Water-to-Sediment Diffusion Coefficient	0	m/d	Oram et al. 2008
$\log K_{ow}$	Octanol-Water Partitioning Coefficient	6.81	----	Mackay et al. 2006
H_{298}	Henry's Law Coefficient	0.56	Pa-m ³ /mol	Cetin and Odabasi 2005
C_{ocean}	Average Concentration in the Pacific Ocean	13.7	Pg/L	Oram et al. 2008
C_{bay}	Average Concentration in Bay Water	54.9	pg/L	Oram et al. 2008
C_{ybi}	Average Concentration at Yerba Buena Island	46.8	pg/L	Average Concentration in the Central Bay (Oram et al. 2008)
C_{sed}	Average Concentration in sediment	0.4	pg/L	Oram et al. 2008
C_{delta}	Average Concentration in Delta Flows	200	pg/L	Average Concentration at Mallard Island (Oram et al. 2008)
C_{Base}	Average Concentration in Bay Tributary Base Flows	1960	pg/L	Back-calculated from annual load in runoff and tributary flows (Oram et al. 2008)
C_{Storm}	Average Concentration in Bay Tributary Storm Flows	5600	pg/L	Back-calculated from annual load in runoff and tributary flows (Oram et al. 2008)
C_{POTW}	Average Concentration in POTW flows	5200	pg/L	North 2004 ²⁸

²⁸ Calculated from total PBDE concentrations ranging from 0.004-29ng/L, with BDE 47 accounting for 36%.

Conservation of Mass – Evaluating the One-Box Model

Annual mass conservation was evaluated for the BDE one-box model. When added to the system, loads are assigned to the water, sediment, as degraded in water or sediments, as transported out of the Bay or volatilizing into the atmosphere. The sum of the mass assigned to these destinations should equal the initial input. The initial concentrations in the water and sediments were set to zero, and an annual load was set to 10 kg/year for evaluation purposes. A snapshot of five different years is shown in Figure C.9 to show the fate of PBDE 47 at different points in time.

At year 1, the majority of mass is associated with sediment with the next highest proportion leaving via the Golden Gate (Pacific Ocean) through water column exchange. Over time, degradation in sediment plays an increasingly important role in terms of loss processes, whereas the outflow loss stays somewhat constant and less is added to the Bay sediments and water.

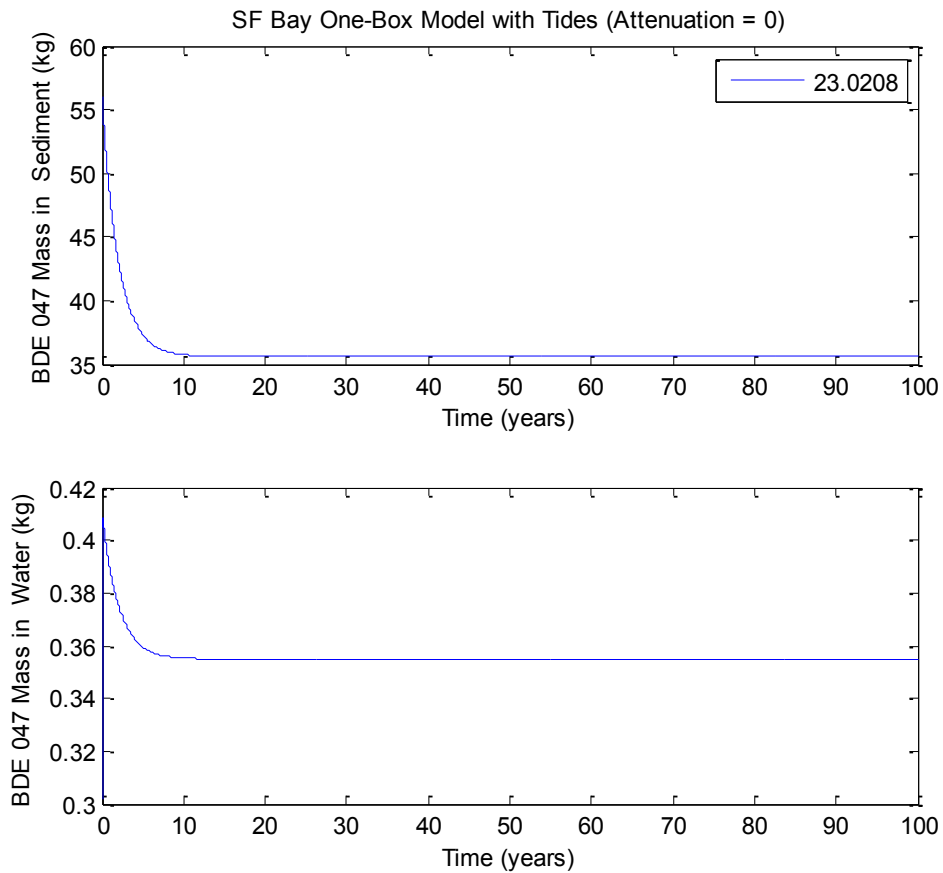


Figure C.6. Mass of PBDE 47 in San Francisco Bay sediments and water forecast for the next 100 years.

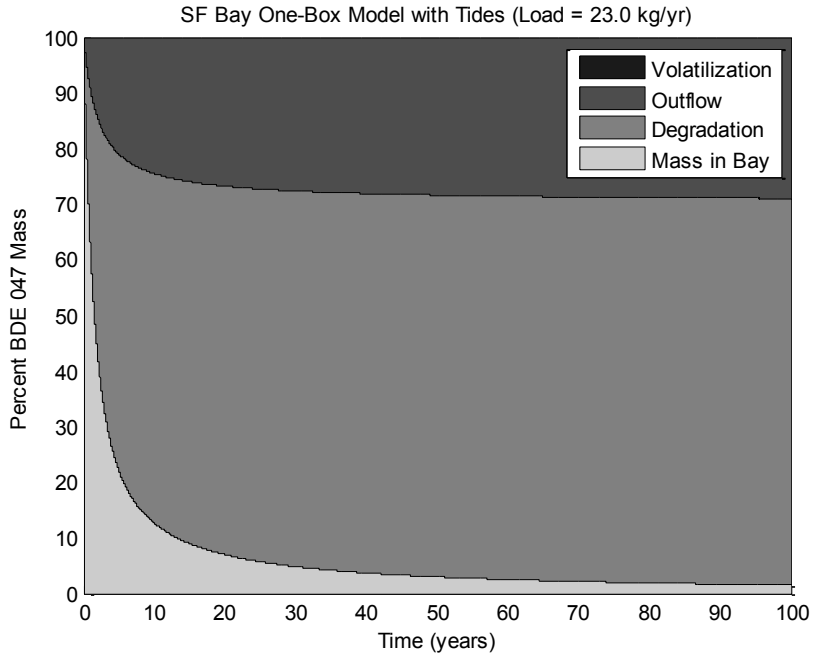


Figure C.7. Prediction of PBDE 47 mass loads in Bay sediments and water over time due to various modeled loss processes (e.g., tidal exchange, degradation and volatilization).

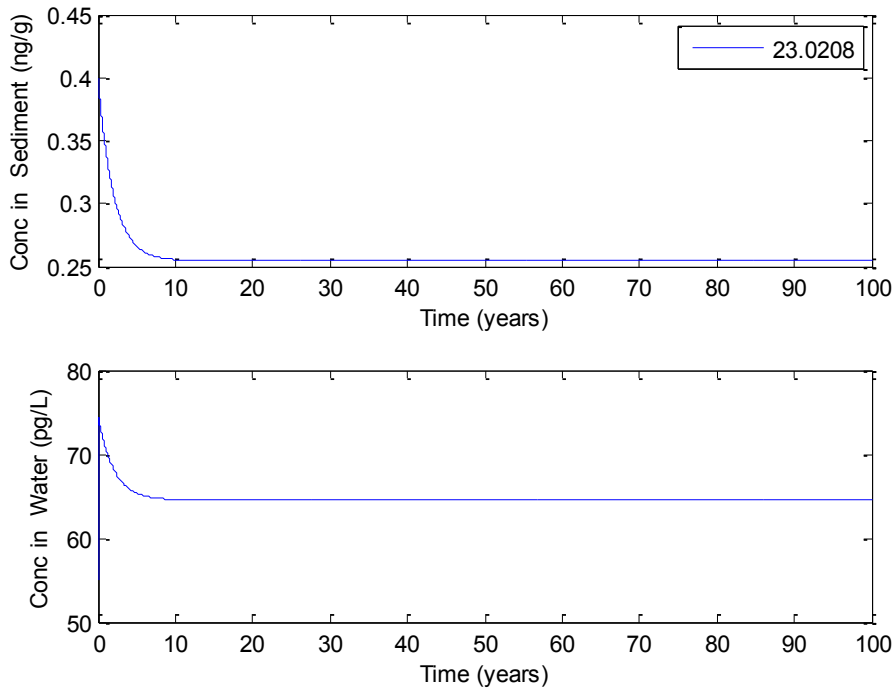


Figure C.8. Predicted concentration of PBDE 47 in Bay sediments and water over time.

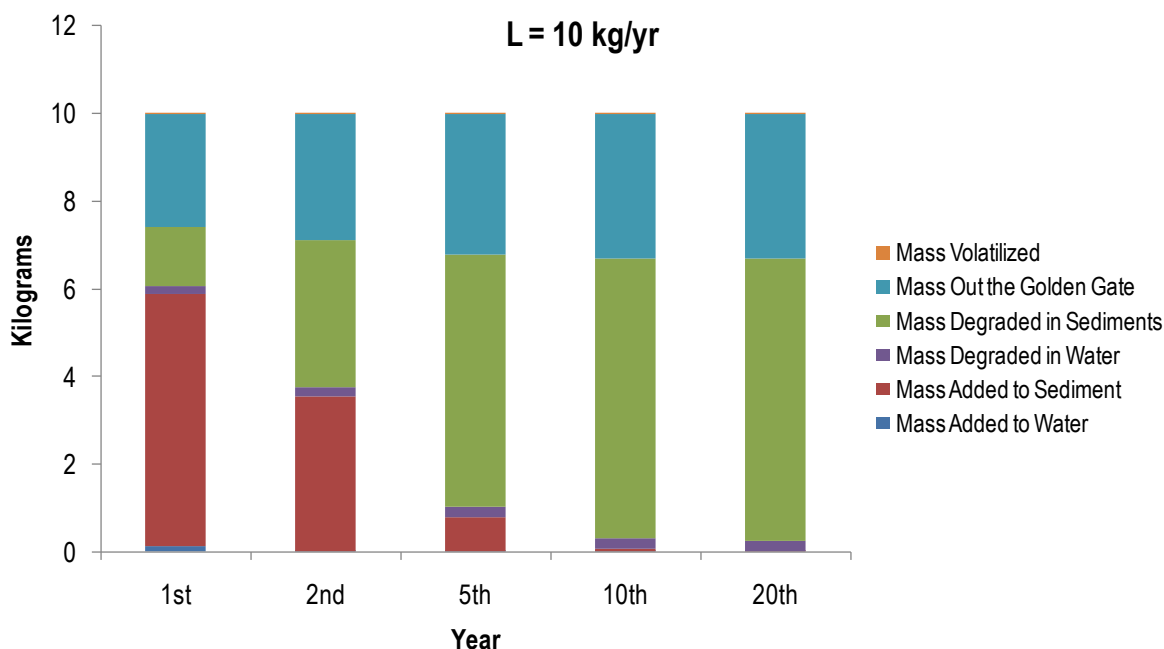


Figure C.9. Compartmentalization of the fate of PBDE 47 in San Francisco Bay over a 20 year period, assuming initial concentrations of zero in sediment and water.

Sensitivity Analysis of the Henry's Constant and Octanol-Water Partitioning Coefficient for PBDE 47

Because little to no physicochemical data are available for most CECs, the one-box model can be utilized as an initial screening tool to generate estimated or predicted environmental concentrations (PECs) in sediment and water. Using this example for PBDE 47, a simple sensitivity analysis for two chemical properties -- Henry's Law Constant and Octanol-Water Partitioning Coefficient (K_{ow}) -- was conducted.

Henry's Law Constant. Figure C.10 illustrates the model-predicted mass of PBDE 47 ($\log K_{ow}$ held constant at 6.81) in San Francisco Bay for a 25 year period for a range of Henry's Law Constant values (0.01 to 3 Pa-m³/mol, which brackets the PBDE 47 value of 0.56 Pa-m³/mol). Little change in the mass of PBDE 47 in sediment and water is predicted by the one-box model. Whereas slightly more mass is predicted to be volatilized (highlighted in red) when the Henry's Law Constant of 3 Pa-m³/mol is assumed, degradation and outflow remain the major loss processes (Figure C.11).

Octanol-Water Partition Coefficient. Figure C.12 illustrates the model-predicted mass of PBDE 47 (Henry's Law Constant held constant at the PBDE 47 value of 0.56 Pa-m³/mol) in San Francisco Bay for a 25 year period for a range of $\log K_{ow}$ values. A much more pronounced effect on total mass remaining is predicted for a model compound with 3 < $\log K_{ow}$ < 6. There appears to be a relatively small difference in remaining mass for large $\log K_{ow}$ values (i.e., 6 -10) as well as for small $\log K_{ow}$ values (i.e., <3), suggesting that these values might represent reasonable upper and lower bound thresholds. When one compares model output for a model CEC with a fixed Henry's Law Constant similar to PBDE 47 (0.56 Pa-m³/mol) but varies $\log K_{ow}$ over a larger range (e.g., 3 to 10)(Figures C.13 through C.16), three observations are apparent:

1. The rate at which total mass in the Bay declines decreases with increasing CEC hydrophobicity (i.e., as K_{ow} increases).

- Volatilization of CECs is a minor loss process that decreases with increasing hydrophobicity. This process is insignificant compared to degradation and outflow for CECs with $k_H < 0.3 \text{ Pa}\cdot\text{m}^3/\text{mol}$.
- Degradation in sediment and outflow are the major loss processes for CECs with the relative contribution of degradation increasing with increasing CEC hydrophobicity.

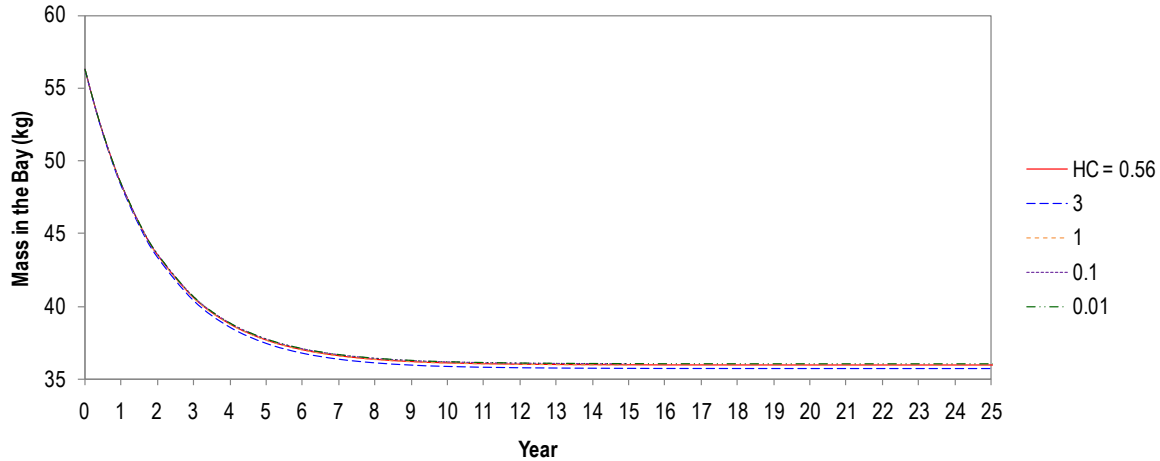


Figure C.10. Total mass of a model hydrophobic CEC ($\log K_{ow} = 6.81$) in the Bay using the one-box model for values of Henry's Law Constant ranging between 0.01 and $3 \text{ Pa}\cdot\text{m}^3/\text{mol}$.

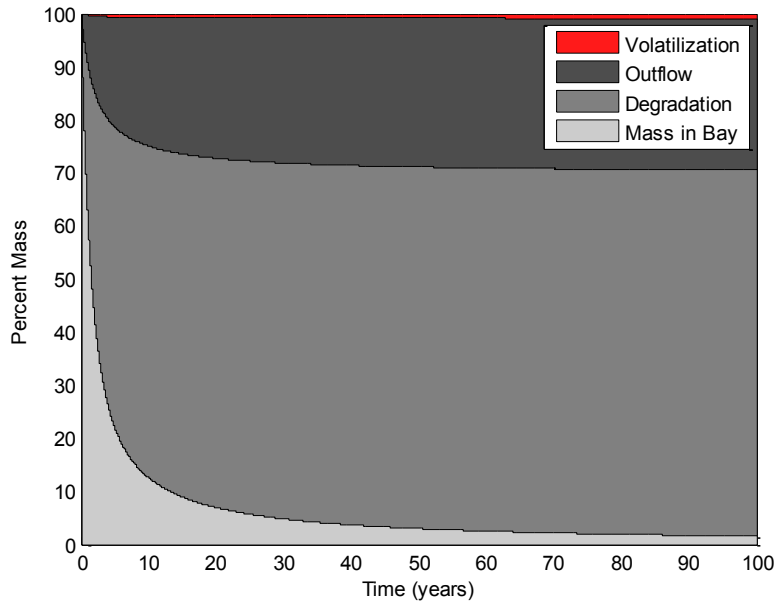


Figure C.11. Loss of a model hydrophobic CEC ($\log K_{ow} = 6.81$) with a theoretical Henry's Law Constant of $3.0 \text{ Pa}\cdot\text{m}^3/\text{mol}$ over time.

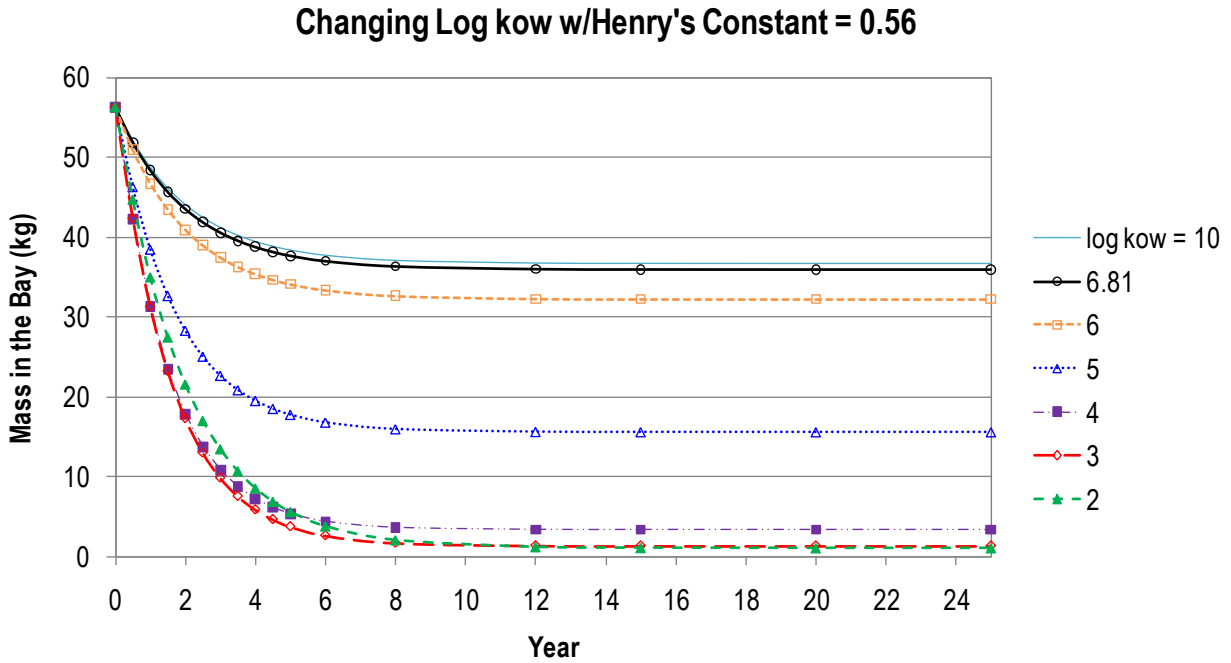


Figure C.12. Total mass of a model hydrophobic CEC (Henry's Law Constant = $0.56 \text{ Pa}\cdot\text{m}^3/\text{mol}$) in the Bay using the one-box model for values of the octanol-water partition coefficient ranging between 10^2 to 10^{10} .

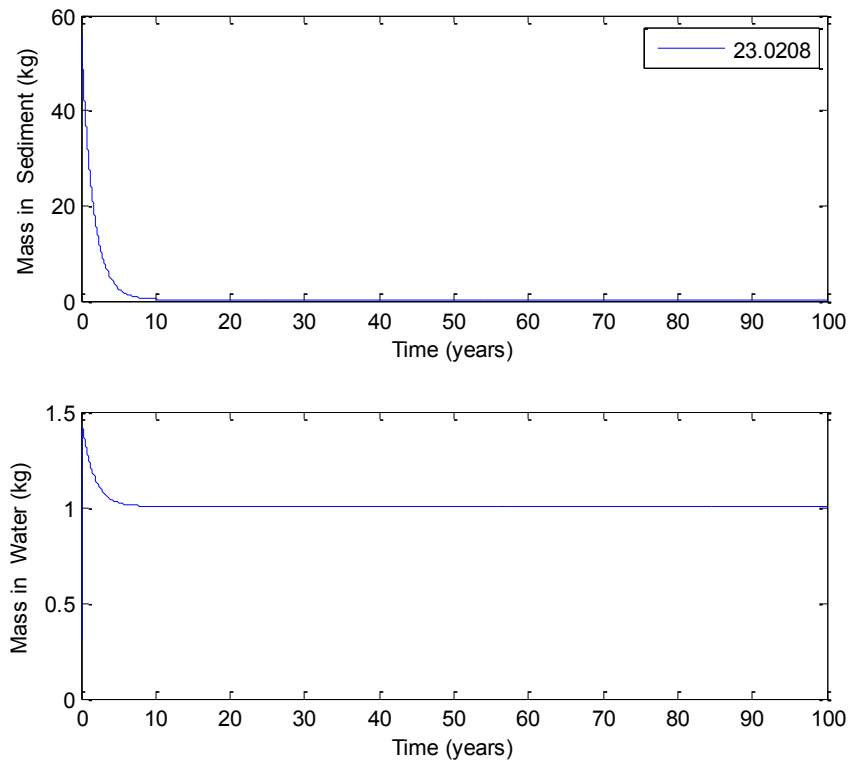


Figure C.13. Mass of a model CEC water (Henry's Law Constant = $0.56 \text{ Pa}\cdot\text{m}^3/\text{mol}$; log K_{ow} = 3) in (top) sediments and (bottom) water.

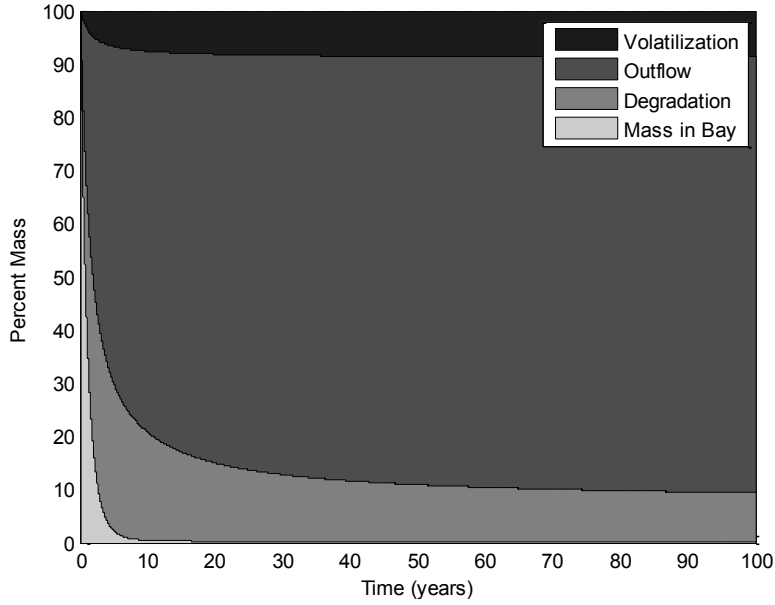


Figure C.14. Prediction of mass loads for a model CEC (Henry's Law Constant = $0.56 \text{ Pa}\cdot\text{m}^3/\text{mol}$; $\log K_{ow} = 3$) in Bay sediments and water over time due to various modeled loss processes (e.g., tidal exchange, degradation and volatilization).

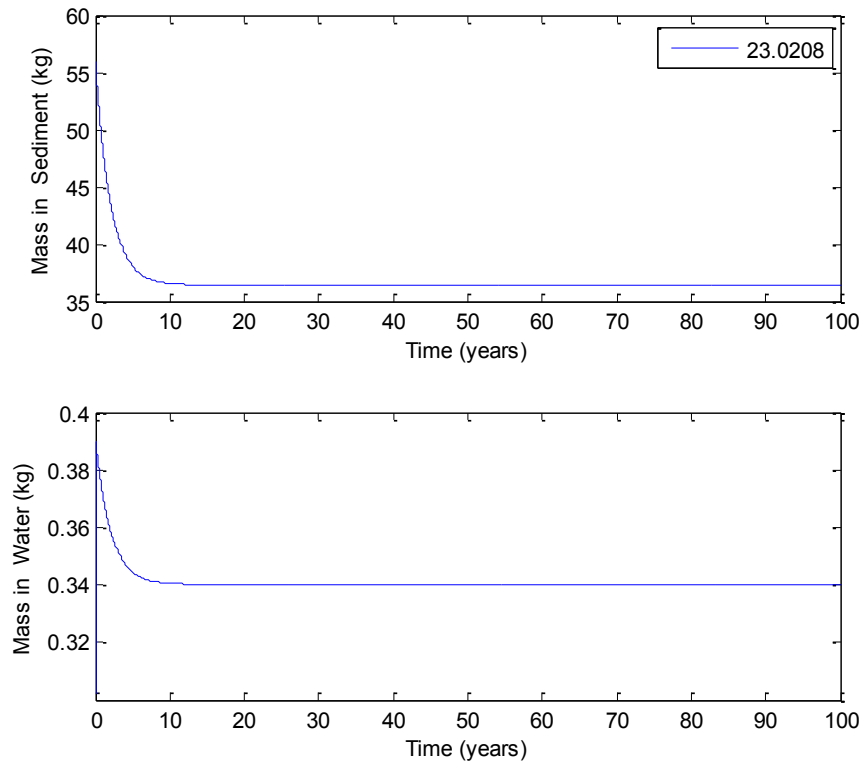


Figure C.15. Mass of a model CEC water (Henry's Law Constant = $0.56 \text{ Pa}\cdot\text{m}^3/\text{mol}$; $\log K_{ow} = 10$) in (top) sediments and (bottom) water.

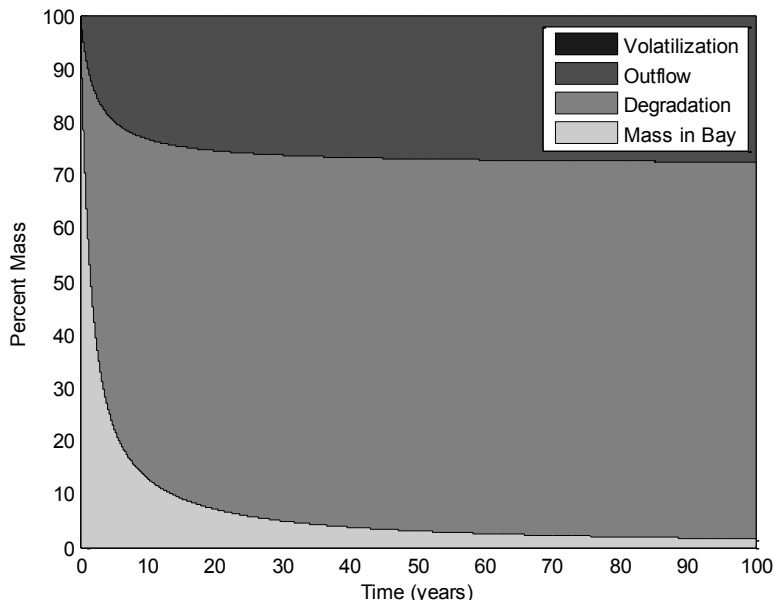


Figure C.16. Prediction of mass loads for a model CEC (Henry's Law Constant = 0.56 Pa-m³/mol; log K_{ow} = 10) in Bay sediments and water over time due to various modeled loss processes (e.g., tidal exchange, degradation and volatilization).

PBDE 47 One-Box Results as Surrogate for CECs

To assist with the screening of model CECs for toxicity and to assist with evaluating food web implications, the concentrations in sediments and water²⁹ after 5, 10 and 40 years were estimated for a model CEC of similar volatility to PBDE 47 (Henry's Law Constant = 0.56 Pa-m³/mol) but with log K_{ow} values of 3, 5, and 10, respectively (Tables C.6 and C.7). Review of the box-model estimates contained in Table C.6 for higher log Kow values indicates that they are well within the range of SFEI sediment monitoring results.

Table C.6. Concentration (ng/g) of a model CEC (Henry's Law Constant = 0.56 Pa-m³/mol) with different log K_{ow} values in Bay sediments after 5, 10 and 40 years.

Year	Concentration in Sediments [ng/g]		
	log K _{ow} = 3	log K _{ow} = 5	log K _{ow} = 10
5	0.0191	0.1216	0.2722
10	0.0027	0.1070	0.2613
40	0.0019	0.1062	0.2603

²⁹ The concentration estimates in the sediment assumed an active layer volume of 1.9275 x 10⁸ m³ (Table A3-2). The concentration estimates in the water assumed a water volume of 5.50 x 10⁹ m³ (Table A3-2).

Table C.7. Concentration (pg/L) of a model CEC (Henry's Law Constant = 0.56 Pa·m³/mol) with different log K_{ow} values in Bay water after 5, 10 and 40 years.

Year	Concentration in Water [pg/L]		
	log K _{ow} = 3	log K _{ow} = 5	log K _{ow} = 10
5	186.5	136.7	62.7
10	183.0	134.1	61.9
40	182.8	134.0	61.9

Because suspended particle loads associated with seasonal stormwater inputs can have a profound impact on sediment concentrations (Figure C.17) and loads of particle reactive CECs, the one-box model can be run to investigate the effect of various base vs. storm flows (i.e., simulate dry, wet and very wet conditions)(Tables C.8 through C.10).

Table C.8. Concentration (ng/g) of a model CEC (Henry's Law Constant = 0.56 Pa·m³/mol) with different log K_{ow} values in Bay sediments after 5, 10 and 40 years after varying base and storm flow concentrations (C_{base} = 1; C_{storm} = 1).

Year	Concentration in Sediments [ng/g]		
	log K _{ow} = 3	log K _{ow} = 5	log K _{ow} = 10
5	0.0183	0.0763	0.1649
10	0.0018	0.0592	0.1447
40	0.0011	0.0583	0.1428

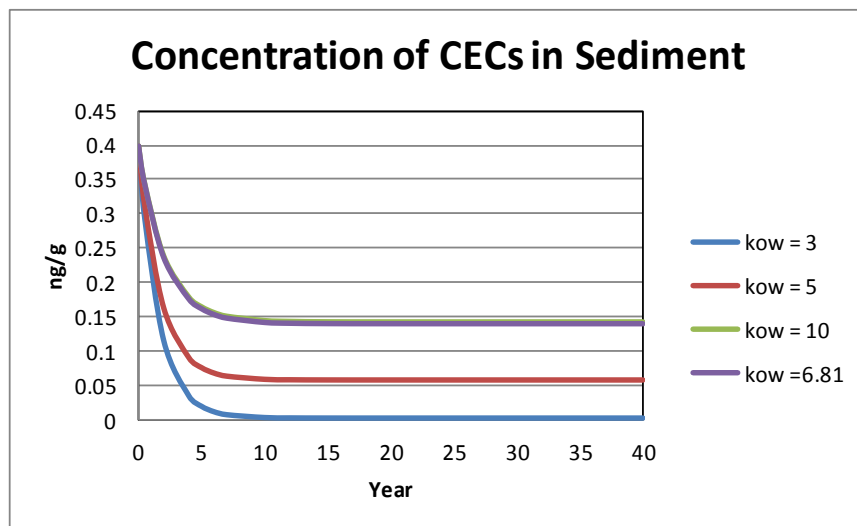


Figure C.17. Concentration of CECs in sediments for C_{base} = 1; C_{storm} = 1.

Table C.9. Concentration (ng/g) of a model CEC (Henry's Law Constant = 0.56 Pa·m³/mol) with different log K_{ow} values in Bay sediments after 5, 10 and 40 years after varying base and storm flow concentrations (C_{base} = 1960; C_{storm} = 1).

Year	Concentration in Sediments [ng/g]		
	log K _{ow} = 3	log K _{ow} = 5	log K _{ow} = 10
5	0.0810	0.0810	0.1761
10	0.0642	0.0642	0.1569
40	0.0633	0.0633	0.1551

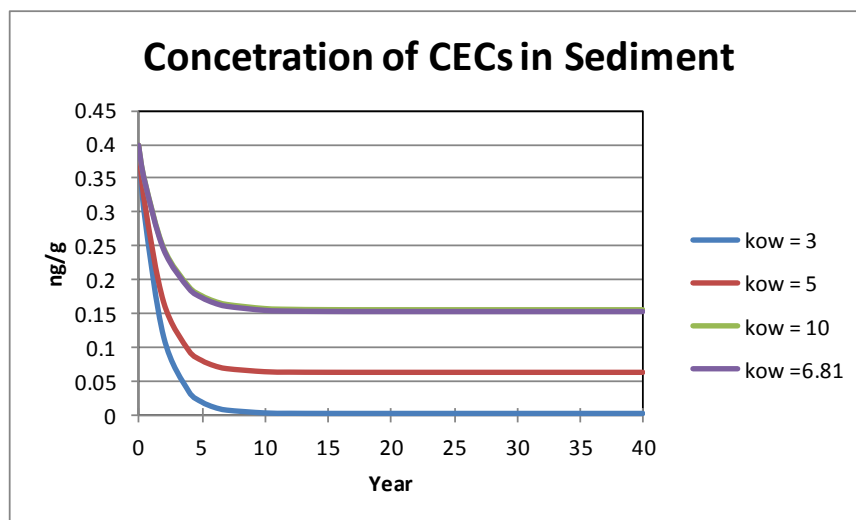


Figure C.18. Concentration of CECs in Sediments for C_{base} = 1960; C_{storm} = 1.

Table C.10. Concentration (ng/g) of a model CEC (Henry's Law Constant = 0.56 Pa·m³/mol) with different log K_{ow} values in Bay sediments after 5, 10 and 40 years after varying base and storm flow concentrations (C_{base} = 5600; C_{storm} = 1).

Year	Concentration in Sediments [ng/g]		
	log K _{ow} = 3	log K _{ow} = 5	log K _{ow} = 10
5	0.0190	0.1169	0.2610
10	0.0026	0.1020	0.2492
40	0.0018	0.1012	0.2480

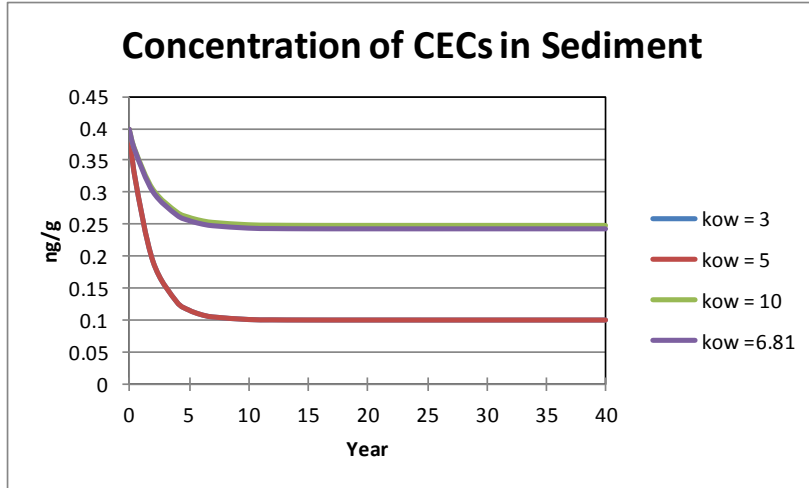


Figure C.19. Concentration of CECs in sediments for $C_{base} = 5600$; $C_{storm} = 1$.

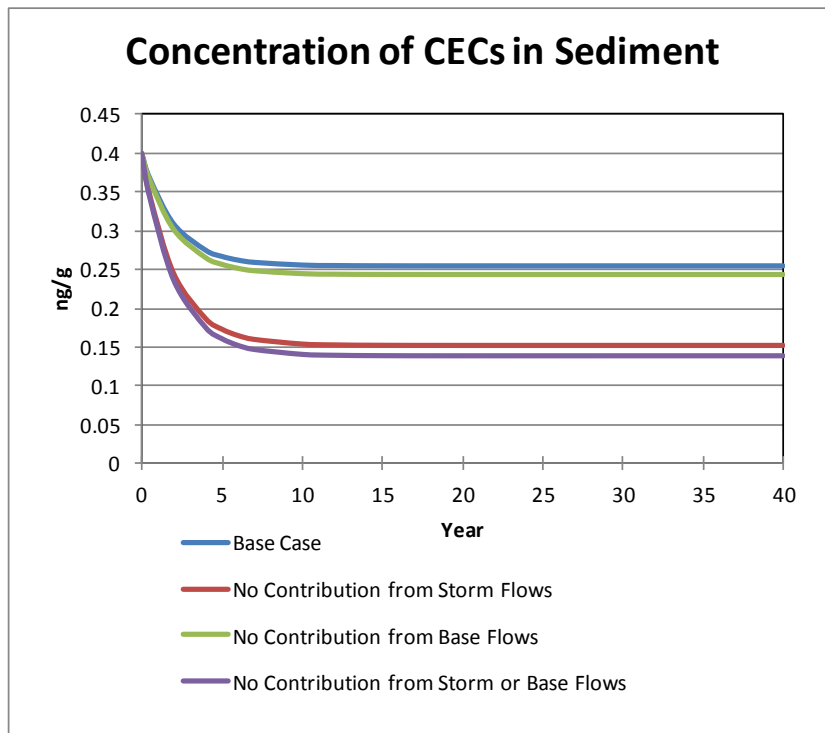


Figure C.20. Concentration (ng/g) of a model CEC (Henry's Law Constant = $0.56 \text{ Pa}\cdot\text{m}^3/\text{mol}$; $\log k_{ow} = 6.81$) in Bay sediments over time in the presence/absence of base flow and stormwater source contributions.

In addition, a general estimate of the range of initial dilution for source inputs throughout the entire San Francisco is on the order of 10:1 to 100:1, based on the one box modeling assumptions. An “average” estimate for initial dilution employed within the CEC screening framework utilizes a conservative estimate of 10:1 dilution. This conservative assumption is consistent with the policy contained in the San Francisco Bay Basin Plan. Initial and secondary mixing dilution in San Francisco Bay has been documented at levels equal to greater than 100:1.

Please note that potential acute impacts associated with the episodic nature of near shore stormwater runoff have not been investigated as part of the CEC framework, nor has consideration been given to the question of site-specific mixing zones.

As noted previously, the one-box model can be used to investigate the contribution of various sources to the estimated sediment and water concentrations. For example, the estimated sediment concentrations shown in Table C.6 were used to investigate, using a by-difference approach, the percent stormwater, municipal wastewater and other discharges (i.e., background sediment, delta flows, ocean input, and stream base flows) represent of the estimated sediment concentrations. For this specific analysis, stormwater represents approximately 40% of the sediment concentration, municipal WWTPs represent approximately 27%, and other flows account for the remaining 33%.

Linking the One-Box Model to Biotic Levels

PBDEs have been reported to be present in both abiotic media and biota (Table C.12). When paired sediment and biota data are available, the concentrations of PBDE 47 presented in Table C.6 can be combined with BSAFs to predict tissue concentrations of PBDE in biota potentially affected by PBDEs released to California receiving waters. The Panel identified a study of paired sediment and tissue data (flatfish livers) from the southern California Bight (Maruya et al. 2011) in which the authors estimated wet weight BSAFs of 91 and 64 (kg sediment/kg tissue) for PBDE 47 and PBDE 99, respectively. The data can also be used to derive a combined PBDE 47 and PBDE 99 wet weight BSAF of 81 (kg sediment/kg tissue). The Panel assumed the BSAFs derived for turbot livers could be used to estimate PBDE concentrations in all fish tissues. Predicted tissue concentrations are provided below in Table C.11. These predicted concentrations can then be compared to the tissue-based MTLs to determine if CECs such as PBDE should be monitored because of concerns associated with potential exposures of higher trophic level biota (including humans) via the food chain.

Table C.11. Concentration of PBDE 47 (ng/g) in fish tissue after 5, 10 and 40 years for Henry’s Law Constant = 0.56 Pa·m³/mol assuming a log K_{ow} of 5 and BSAF of 90.

Year	Concentration in Tissue [ng/g]
5	11
10	9.6
40	9.6

PBDEs may pose risks to the environment (Shaw and Kannan 2009) with levels of PBDEs in air ranging from 1.4-980 pp/m³. In the Pacific Northwest, mean concentrations of 12.8 (range = 1.4-36.9) pp/m³ have been measured which were composed primarily of congeners 99 and 209. In seawater, concentrations ranging from 0.0002-0.513, averaging 0.49 µg/L were measured in San Francisco Bay, which were predominantly congeners 47 and 209. In sediments, concentrations ranging from <0.2-212 ng/g dw were measured, with highest concentrations measured in San Francisco Bay (mean concentration = 11.9 ng/g dw) and in New York (mean concentration = 7.1 ng/g dw), with predominant congeners of 47, 99 and 209 observed. In marine invertebrates PBDE concentrations ranging from 6.7 - >14,000 ng/g have been reported, with highest levels observed in mussels from California with mean concentrations of 13,500 ng/g (range = 13,100-14,000 ng/g) compared to levels ranging from 46-714 ng/g in other regions of the US and from 6.7-1841 ng/g in mussels from Canada. The dominant congeners in mussels were 47, 99 and 100. High PBDE levels were also observed in other bivalves such as oysters ranging from 19 – 11,100 ng/g. Highest oyster concentration of PBDE were measured in CA in San Francisco Bay with mean concentrations of 5,360 ng/g (range = <DL - 11,100 ng/g). In other invertebrates, much lower PBDE concentrations were observed ranging from 9.4 (zooplankton) – 93 (worms) ng/g with dominant congeners of 47 and 99. In fish, PBDE concentrations ranging from 18 -337 ng/g have been reported, with dominant congeners of 47, 99 and 100 observed. In piscivorous birds, PBDE concentrations in eggs ranging from 5 to 369 ng/g have been detected with highest concentrations observed in CA in San Francisco Bay (2,160-9,420 ng/g) and Canada (486-5,359 ng/g) with dominant congeners of 47, 99 and 100. In the plasma of piscivorous birds, a concentration of 4,755 ng/g was reported in CA at Santa Catalina with dominant congeners of 47, 99, 100, while much lower plasma levels were reported in Canada (57-801 ng/g). In seals and sea lions, PBDE concentrations ranged from 3.2-5,778 ng/g, with highest levels measured in California and lowest concentrations measured in Alaska, with dominant congeners of 33 and 183. PBDE levels in bottle-nose dolphins, ranging from 120 - 7, 850 ng/g, were measured throughout the US, with dominant congeners of 47, 99 and 100 being observed. Similarly, PBDE levels in killer whales, ranging from 36 -12,600 ng/g were measured throughout the US, with highest concentrations observed in California (12,600 ng/g) and much lower concentrations observed in Alaska and Canada (36-3,300 ng/g) with 47, 99 and 100 being the dominant congeners. In human adipose tissue concentrations ranged from 17-9,630 ng/g (measured throughout the US) with dominant congeners of 99 and 153 being observed. In California, concentration of PBDE in human adipose tissue averaged 41 ng/g (range = 17.2 – 462 ng/g). This brief review indicates that PBDE are present at relatively low concentrations in the abiotic portions of the environment (air, water and sediments) and can be bioconcentrated to higher levels in a variety of biota, ranging from invertebrates to humans (Table C.12).

Table C.12. Concentrations of PBDEs in various aquatic ecosystem compartments.

Media	Location	Mean Conc. (Range)	Comment
Air	Pacific NW 2003	12.8 (1.4-36.9) pp/m ³	99 & 209 DI
	Great Lakes 2002-04	100 (13-980) pp/m ³	47 & 209 DI
	Mid West 2002-04	19 (6.4-44) pp/m ³	47 & 99 DI
	SE US 2002-04	30 (2.7-165) pp/m ³	47 & 209 DI
Seawater	CA SF Bay 2002-06	0.490 (0.0002-0.513) µg/L	47 & 209 DI
Sediments	CA SF Bay 2002-06	11.9 (<0.2-212) ng/g dw	47 & 99 DI
	CA 2004-07	7.1 (<DL -88) ng/g dw	NR
	NY 2004-07	14.4 (2.9-41.3) ng/g dw	NR
	Other US Sites 2004-07	0.2-4.9 ng/g dw	NR
	Canada 2006	0.3-2.6 ng/g dw	99, 47, 209 DI
Marine Invertebrates			
-Worms	Canada 1999-00	59-93 ng/g (WA)	47 & 99 DI
-Oysters	CA SF Bay 2002	5,360 ng/g (<DL -11,100)	47 & 99 DI
	Other US Sites 2004-07	19-302 ng/g (WA)	NR
-Mussels	CA SF Bay 2002	13,500 (13,100-14,000) (WA)	47, 99, 100 DI
	Other US Sites 2004-07	46-714 ng/g (WA)	NR
	Canada 2006	6.7-1841 ng/g (WA)	47 & 99 DI
-Shrimp	Canada 1999-00	27 ng/g (muscle)	47 & 100 DI
-Zooplankton	Canada 1999-00	9.4 ng/g (WA)	47 & 99 DI
Estuarine/Marine Fish			
	GA 2004-05	337 ng/g lw (WA)	47 & 99 D
	Other US Sites 04-05	26-89.5 ng/g lw (WA)	47 & 99 DI
	Canada 20006	18-82 ng/g lw (WA)	47, 99 100 DI
Piscivorous Birds			
-Eggs	Canada 1979	5 ng/g lw	47 & 99 DI
	Canada 1985-1990	130-485 ng/g lw	47 & 99 DI
	Canada 1994-2002	486-5,359 ng/g lw	47, 99 100 DI
	US 1993-2007	30-8,627 ng/g lw	NR
	CA SF Bay 2000-02	2,160-9,420 ng/g lw	47, 99, 100 DI
-Plasma	CA Santa Catalina 2003	4,755 ng/g lw	47, 99, 100 DI
	Canada 2001-03	57-801 ng/g lw	47, 99, 100 DI
Sea Lions & Seals			
	CA 1993-03	5,778 ng/g lw (blubber)	M; NR
	AK 2003	3.2-15 ng/g lw	M/F; NR
	Galapagos	35 ng/g	Pup; 33, 183 DI
Bottle Nose Dolphins			
	US 1987	200 (180-220) ng/g lw (blubber)	F; NR
	US 2000-04	120-7,850 ng/g lw (blubber)	M/F/J; 47, 99, 100 DI
Killer Whales			
	AK 2003-04	36-3,300 ng/g lw (blubber)	M; NR
	CA 2003-04	12,600 ng/g	M; NR
	Canada 1993-96	203-1,014 ng/g lw (blubber)	47, 99, 100 DI
Polar Bears			
	Canada 1999-02	14 (4.3-46) ng/g lw (Adipose)	F; 47, 99, 153 DI
	AK 1994-02	6.7-6.8 ng/g lw (Adipose)	M/F; 47, 99 DI

Table C.12. Continued

Media	Location	Mean Conc. (Range)	Comment
Humans			
-Breast Adipose	CA 1996-98	29 (5.2-196) ng/g lw	F; 99 DI
	CA 1996-99	41 (17.2-462)	F; 99 DI
	NY 2003-04	398 (17-9,630)	M/F; 153 DI
-Breast Milk	MA 2004-05	30 (4.3-264)	F; 47 DI
	Canada 1992	3.1 (0.8-28.5)	F; 99 DI
-Serum	CA 1959-67	<DL	F
	CA 1997-99	51 (<10-511)	F, 47 OIM
	CA 1999-01	21 (5.3-320)	F; 153 DI
	CA 2003-04	461 (X ranged from 62-461)	M/F; 100, 154 DI
	Mexico 2006	Mean levels ranged from 2.7-15.7	M/F
	Nicaragua 2002	Mean levels ranged from 22-438	M/F; 47, 99, 209 DI

APPENDIX D - TOXICITY DATA

NOECs by Scenario

Effluent Dominated Inland Waterway (Scenario 1)

This section provides information used for NOEC determination for CECs that exceeded MTQs of 1 for the effluent dominated inland waterway exposure scenario (Section 3.3.1). Exposure to aqueous phase CECs in freshwater is the basis for the following NOECs (see Section 6).

(1) Diclofenac (CAS number 15307-86-5) NOEC = 1000 ng/L. Endpoint: kidney damage and morphological changes in kidney and intestine in fish.

Diclofenac is a non-steroidal anti-inflammatory agent whose primary MOA is to inhibit prostaglandin synthesis. Experiments with adult carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) showed kidney damage at 1 µg/L (Triebkorn et al. 2004, 2007). In a study by Mehinto et al. (2010), rainbow trout were exposed to aqueous concentrations of diclofenac ranging from 0.5 to 25 µg/L for 21 days. Changes in tissue morphology for intestine and kidney and changes in gene transcription were measured. Diclofenac induced morphological changes in the intestine and kidney in exposed animals. In the kidney morphological changes included an increase in the number of developing nephrons, loss of bowman space (seen at 5 µg/L) and tubular necrosis (seen at 25 µg/L). The LOEC for a decrease in expression of COX1 mRNA in the kidney and in the liver was 0.5 µg/L and in the gills was 1 µg/L. A decrease of COX2 mRNA was also measured with LOEC at 0.5 µg/L for the liver and 1 µg/L for the kidney. CYP1A mRNA was increased in the liver (LOEC 0.5 µg/L) and kidney (LOEC 1 µg/L).

(2) 17-beta estradiol aka "E2" (CAS number 50-28-2). PNEC = 2 ng/L. Endpoint: various fish. Caldwell et al. (in press) conducted a comprehensive review of the literature and identified fish reproduction as the most sensitive endpoint. A species sensitivity distribution using all fish reproduction studies was used to derive a hazardous concentration of 4 ng/l to which an assessment factor of 2 was applied to derive a PNEC of 2 ng/L and is the PNEC adopted by the Panel.

(3) Estrone aka "E1" (CAS number 53-16-7). PNEC = 6 ng/L. Endpoint: testis-ova in medaka.

Following their comprehensive review of the available literature, Caldwell et al. (in press) concluded that insufficient data were available to construct a species sensitivity distribution for estrone from which a PNEC could be derived. Instead they used *in vivo* vitellogenin (VTG) induction studies to determine the relative potency of the steroid estrogens to induce VTG. The studies that this value is based on were carefully performed and predict a conservative value. Based upon the relative differences between *in vivo* VTG induction, Caldwell et al. (in press) derived a PNEC of 6 ng/l for estrone and is the PNEC adopted by the Panel.

(4) Ibuprofen (CAS # 15687-27-1). NOEC = 1000 ng/L. Endpoint: egg production in medaka.

Ibuprofen is a non-steroidal anti-inflammatory drug commonly used as an analgesic. It inhibits both forms of the cyclooxygenase (COX) enzyme in humans. Flippin et al. (2007) exposed medaka (*Oryzias latipes*) to 1-100 µg ibuprofen/L for 6 weeks (only nominal concentrations were reported). With increasing exposure, pairs spawned less frequently but produced more eggs when they did spawn. The frequency of egg production decreased with increasing concentrations. The NOEC in this study was 1

µg/L. All concentrations tested reduced COX activity in medaka, showing that ibuprofen apparently functions the same way in fish as it does in humans. This change in activity was predicted based on the actions of ibuprofen in mammals.

In another study, adult zebrafish were treated for up to 28 days with solutions of ibuprofen in water. At the conclusion of the test, a sample of blood was removed from the gill and tested for genotoxicity using the Comet Assay and an assay for apoptosis. Losses of DNA integrity and increases in apoptosis were measured with a concentration of 66.4 ng/L (Rocco et al. 2010).

In a third fish study, a full life-cycle toxicity test was performed with *O. latipes* using the OECD embryolarval test (TG 210). Endpoints measured were gross development, vitellogenin induction, histological manifestations and reproductive success. For early life-stages, development and growth were the main endpoints. Fertilized eggs were exposed to ibuprofen at six concentrations ranging from 0.01 to 1000 µg/L for the life-cycle test. Survival of adult fish (120 dph) was significantly lower than for controls at 1 µg/L (LOEC)(Han et al. 2010).

In an experiment performed by Pomati et al. (2004), ibuprofen was tested to see if it would alter growth of the duckweed *Lemna minor* using a standard EPA test. *L. minor* was exposed to four concentrations: 1, 10, 100 and 1000 µg/L (all nominal). Each experiment was carried out in triplicate for 7 days. Growth was quantified by the number of fronds in the test containers, and was affected at all concentrations tested by the end of the experiment and exhibited a clear dose response. The 1 µg/L exposure had no negative response for the first 5 days but by day 7, growth dropped by 14%.

(5) Chlorpyrifos (CAS # 2921-88-2). LOEC = 50 ng/L. Endpoint: survival of *Ceriodaphnia dubia*; for *P. macrodactylus* LC50 was 10 ng/L

Chlorpyrifos is a high volume organophosphate insecticide used heavily in the US on crops that is metabolically activated by cytochrome P-450-dependent monooxygenases, which creates a metabolite that is a strong inhibitor of acetylcholinesterase (Belden and Lydy 2000). Sherrard et al. (2002) exposed *Ceriodaphnia dubia* to nine concentrations of chlorpyrifos (0.05 to 0.13 µg/L), using a static renewal test system. Actual concentrations were determined analytically. The LOEC for mortality in this study was 50 ng/L. A NOEC was not determined. In saltwater, the LC50 for *Palaemon macrodactylus* was 10 ng/L (Baron and Woodburn 1995).

(6) Galaxolide (CAS # 1222-05-5). aka HHCB or 1,3,4,6,7,8-hexahydro-4,6,6,7,8-Hexamethylcyclopenta[g]-2-benzopyran. NOEC = 7000 ng/L. Endpoint: copepod metamorphosis.

Galaxolide is a high production synthetic musk used in soaps, perfumes, cosmetics, laundry detergents and shampoos, and is found at relatively high concentrations in WWTP effluents. Breitholtz et al. (2003) measured larval development rate in copepods (*N. spinipes*) and other endpoints with a full life-cycle test starting with 10 to 14 nauplii per condition with eight replicates per group. Test media (0.002 to 0.2 mg/L) were exchanged 70% every second day and actual concentrations of test substance were measured at the beginning and end of the tests. Larval development was measured as changes that accompany metamorphosis at the first copepodite stage. In total the exposure was for 22 days. The lowest concentration that significantly reduced larval development rate was 20 µg/L. The NOEC for this study was 7 µg/L.

(7) Permethrin (CAS number 52645-53-1). PNEC = 10 ng/L.

Bifenthrin (CAS # 82657-04-3). PNEC = 4 ng/L.

Permethrin and bifenthrin are synthetic pyrethroid pesticides used widely in the US. Proposed aquatic life criteria have been published using species sensitivity distributions and acute-chronic ratios in freshwater species (Fojut et al. 2012). Forty studies were used for bifenthrin and 155 were used for permethrin to determine the values. While the panel only listed two compounds, other pyrethroids had similar PNEC values and should also be evaluated for monitoring (see Section 8).

(8) Bisphenol A (CAS number 80-05-7). PNEC = 60 ng/L.

A total of 61 studies yielded 94 NOECs and a toxicity dataset which suggests that mortality, and effects on growth, development and reproduction are most likely to occur between 0.0483 and 2280 µg/L. This finding is within the range for aquatic adverse estrogenic effects reported in the literature. A PNEC of 0.06 µg/L was calculated. The 95% confidence interval was found to be (0.02, 3.40) µg/L (Wright-Walters et al. 2011).

Coastal Embayment (Scenario 2)

Aqueous exposure

Seven of the 9 compounds discussed in Scenario 1 (effluent dominated inland waterway) also had MTQ>1 for Scenario 2 (coastal embayment).

Sediment exposure

Protocols for sediment toxicity testing have been established by NOAA and EPA and sediment quality guidelines have been established for a number of regions. For marine outfalls, toxicity tests usually use marine amphipods, e.g., *Ampelisca abdita*. These amphipods are usually abundant in the environment and it is relatively easy to run tests with spiked sediments to determine the sensitivity of amphipods or other marine benthic species to individual chemicals. However, in addition to determining direct toxic effects on the benthic species, it is also important to determine sublethal effects and bioconcentration factors, as many sediment associated chemicals can be transmitted to larger prey fish and ultimately to birds or marine mammals. Thus, determining bioaccumulation and biomagnification factors are important for risk assessment since the most susceptible organisms may occupy higher trophic levels. Few studies have been done with marine sediments in California. An important study performed by Bay et al. (2011) quantified CECs in sediments in the Southern California Bight and also looked at effects in the hornyhead turbot (*Pleuronichthys verticalis*), a flatfish associated with bottom sediments and which feeds on organisms that may bioaccumulate CECs from sediments. In the Bight, concentrations of CECs in marine waters are low (less than one part per trillion) and well below concentrations expected to produce toxic effects, but concentrations of CECs in the sediments were in the part per billion range. The same CECs were available to marine biota as levels in the livers of hornyhead turbot were also in the parts per billion range for phthalates, PBDEs and nonylphenol. Below we summarize some of the CECs that have been found in sediments and their effects on marine invertebrates.

(1) PBDE-47 (CAS number 5436-43-1). NOEC = 3 µg/kg. PBDE-99 (CAS number 60348-60-9). LOEC = 3 µg/kg. Endpoint: polychaete larval settlement and growth.

PBDEs are fire retarding chemicals thought to disrupt the thyroid axis. The main metabolites of PBDEs are hydroxylated, bind to thyroid hormone transport proteins inhibiting the transport of thyroid hormones, reducing the amounts of total and free thyroid hormones in blood (Meerts et al. 2000). PBDEs can also disrupt thyroid hormone metabolism such as sulfation, deiodination and glucuronidation (Legler and Brouwer 2003).

Lam et al. (2010) spiked clean sediments with PBDE 47 at concentrations of 0.5 and 3 µg/kg dry wt, and exposed larvae from three polychaetes, *P. cornuta*, *P. vexillosa* and *Capitella spp.* Endpoints measured were larval settlement and growth after 24 h. *P. vexillosa* and capitellids had significantly ($p<0.05$ and <0.001 , respectively) increased settlement in sediments spiked at 3 µg/kg, whereas *P. cornuta* had significantly decreased settlement ($p<0.05$). After 4 weeks, juvenile *P. vexillosa* and *Capitella sp.* were significantly longer than controls at the high concentration ($p<0.001$) while length of the *P. cornuta* was significantly shorter ($p<0.02$).

Other studies suggest that the toxicity from PBDE-99 is similar in magnitude to PBDE-47. Sensitive endpoints are tail resorption and metamorphosis involved in tadpole development which depend on thyroid hormone (Kawahara et al. 1991). To assess the effects of PBDE 47 and PBDE 99, Balch et al. (2006) either fed tadpoles a commercial mixture, known as DE-71 or injected them IP with each of the pure compounds and also with DE71. The feeding study started at 2 weeks after hatch (stage 50 to stage 66), while the IP injections (1 and 100 µg/tadpole) occurred at stage 58 of development. For the

diet, 5 concentrations (0.1 ng DE71/gm feed to 5,000 µg DE71/gm feed) were tested and absolute tail resorption, tail resorption rate during metamorphosis and the percentage of tadpoles within a treatment that completed metamorphosis were determined as endpoints. The estimated time for 50% of the population to reach stage 66 (completion of metamorphosis) was increased by 2 days and 1 day, respectively, for tadpoles injected with 1 µg/tadpole of PBDE 47 and PBDE 99 and by 2 days for tadpoles injected with 0.6 µg DE71 per tadpole. The feeding study also showed increased time (3 days) to reach stage 66 for tadpoles fed with 1 µg DE71/gm diet. The proportion of tadpoles reaching metamorphosis was significantly reduced at a DE 71 dietary concentration of 1 µg/gm. Thus, the NOEL for the diet was 0.01 µg DE71/gm diet. Tail resorption and rate of tail resorption NOEL values for IP applied PBDEs was 1 µg/tadpole for either BPDE 47 or BPDE 99 and 0.6 µg/tadpole for DE71.

(2) Permethrin (CAS number 52645-53-1). LOEC = 73 µg/kg. Bifenthrin (CAS number 82657-04-3). LOEC = 5.2 µg/kg. Endpoint: growth of *Hyalella azteca*.

Amweg et al. (2005) conducted 10-d toxicity tests with the freshwater amphipod, *Hyalella azteca* (USEPA 2000a) with a variety of pyrethroids, including permethrin and bifenthrin. *H. azteca* is the most sensitive species tested to date (Amweg et al. 2005). Analytical methods were used to quantify actual concentrations, and sediments from several areas that contained detectable pyrethroids were also collected and tested in triplicate for amphipod survival and growth. Bifenthrin (10-d LC₅₀ of 4.5 µg/kg) was more toxic than permethrin (10-d LC₅₀ of 90 µg/kg). The LOEC for growth was 5.2 µg/kg for bifenthrin and 73 µg/kg for permethrin. The collected sediments also showed toxicity to *H. azteca*, suggesting that pyrethroids present were bioavailable and toxic.

Ocean discharge of WWTP effluent (Scenario 3)

Aqueous exposure

No compounds had monitoring trigger quotients greater than one for this Scenario.

Sediment exposure

Two PBDE congeners (47 and 99) and phthalates were found to have toxicity which contributed to a monitoring trigger quotient above 1. These chemicals are discussed above under sediment exposure under Coastal Embayment (Scenario 2).

(1) Bis(2-ethylhexyl)phthalate (CAS # 117-81-7, DEHP). NOEC = 1,300 µg/kg.

Butylbenzylphthalate (CAS # 85-68-7, BBP). NOEC = 63 µg/kg.

di-n-butylphthalate (CAS # 84-74-2, DBP). NOEC = 1,400 µg/kg

Endpoint: amphipod mortality.

Phthalates are a class of plasticizers used to increase the flexibility of high molecular weight polymers. Several different ester formulations including the 3 named above are high volume production chemicals. The concentrations of phthalates in marine waters are low (Vidal-Dorsch et al. 2011), however, their concentrations were detectable in marine sediments collected near WWTP outfalls (Maruya et al. 2011). To determine the range of sediment toxicity, Vidal and Bay (2005) used survival data from the Los Angeles Contaminated Sediments Task Force database from 117 dredging, monitoring and research studies conducted in the SCB between 1984 and 2001. Toxicity data were from 10-d amphipod survival tests for marine amphipods, including *A. abdita*, *Rhepoxynius abronius*, *Eohaustorius estuarius* and *Grandidierella japonica*. In their approach, the authors used three different sediment quality guidelines based on empirical measurements and one that is based on mechanistic measurements, to derive toxicity values. The low apparent effects threshold (LAET) calculated corresponds to the 10th percentile of the distribution observed in toxicity are the values listed in the NOEC column for phthalates. These include DEHP at 1,300 µg/kg; BBP at 63 µg/kg; and DBPat 1,400 µg/kg.

(2) p-nonylphenol, aka 4-nonylphenol or 4-NP (CAS # 84852-15-3). NOEC = 1,400 µg/kg. Endpoint: unknown

An interim sediment quality guideline (ISQG) was obtained from the Canadian Council of Ministers of the Environment and was the lowest value. Canadian provisional interim sediment quality guidelines) for nonylphenol in marine sediments were developed using an equilibrium partitioning (EqP) approach. Provisional ISQGs of 1.4 mg/kg dw, were recommended for the protection of marine life. Fay et al. (2000) used *A. abdita* as the test organism in sediment spiking experiments, carried out in glass jars containing 5 g wet sediment spiked with 4-NP using the standard USEPA amphipod toxicity test and 60 ml overlying filtered seawater and the exposures were for 10 days. The organisms were allowed to burrow into the sediments. The end point measured was mortality. The 10-d LC50 observed for 4-NP was 98.7 mg/kg and 100% mortality occurred at a concentration of 243 mg/kg (Fay et al. 2000, CCME 2002). In a second study by Hansen et al. (1999), the polychaete *Capitella sp.*, an organism that feeds in sediments was used in a 78-day exposure with spiked sediments. The endpoints of the study were chronic effects on growth, fecundity and survival and included time to first reproduction, times between reproductive evtns, number of broods per individula and number of eggs per individual. Nominal sediment concentrations of 0 to 185 µg NP/g dry sediment were used in the spiking experiments. A NOEC of 52 mg NP/kg sediment and a LOEC of 174 mg NP/kg sediment were recorded.

Exposure through fish tissue

(1) PBDE-47 (CAS # 5436-43-1). PBDE-99 (CAS #60348-60-9). NOEC = 289 µg/kg Endpoint: egg laying in kestrels.

Birds of prey can be exposed to PBDEs in their diet. Several studies performed on the American Kestrel suggest that PBDEs can affect several endpoints, such as thyroid hormone and vitamin A binding to transthyretin (Fernie et al. 2005), courtship behavior (Fernie et al. 2008), and egg shell thickness and reproduction (Fernie et al. 2009).

Fernie et al. (2009) exposed kestrels via the diet by feeding them DE-71-contaminated cockerels (injected with DE-71 to achieve concentrations that are similar to those found in wild birds). Two exposures were carried out, one at 1.6 ppm and the other at 0.3 ppm. Exposure began at least 3 weeks before pairing and continued through courtship, egg laying and incubation. Egg shell quality was assessed from the first egg produced per pair. Chemical analyses were carried out for the 7 most prevalent congeners in DE-71 (BDE 27, 47, 100, 99, 154, 153 and 183), which made up >94% of the total PBDE concentration in eggs for both exposures. The mean egg concentration for the two exposures were 289 and 1131 µg/kg wet weight, respectively. Egg laying was significantly delayed for the higher concentration. Egg volume, length, width and weight were significantly reduced in both treatments compared to controls. The eggshells of the high exposure group were significantly thinner than controls and the eggshells of the low exposure group were significantly lighter. The high-exposure kestrel pairs had poorer fertility, hatching, and fledging success, compared to control pairs. However, it should be mentioned that these effects were for total PBDEs in the DE71 mixture. BDE-99 and -47 were 46% and 9.1% of the total, respectively. Together they accounted for 55% of the total active ingredient.

(2) Perfluorooctane sulfonate (CAS # 1763-23-1, PFOS). PNEC = 1000 µg/kg

In the definitive reproductive studies, adult Mallard and Bobwhite Quail were exposed to nominal dietary PFOS concentrations of 0, 10, 50, or 150 mg/kg feed for up to 20 weeks and sacrificed at week 21. Adult birds were given a treated diet for up to 10 weeks prior to photostimulation and the onset of egg-laying. Endpoints monitored in the study included growth, behavior, and histopathology of adult and offspring. Reproductive endpoints included egg production, fertility, hatchability, and hatching survival and growth. Concentrations of PFOS were measured in the diet, liver, and serum of adult and juvenile birds and in eggs during the study (Newsted et al. 2005).

Human Health and Mammalian Effects

The Panel also considered the need to develop monitoring trigger levels (MTLs) based on the potential effects of CECs released to receiving waters on human health. For most CECs considered, the potential for human health exposure occurs if receiving water is used as a potable water supply and people are exposed by drinking this supply. The Panel assumed such potential exposures are limited to freshwater settings (i.e., Scenario 1). Because the focus of the CEC Recycled Water Panel was identification of CECs for monitoring in reused water (i.e., potable water supplies), this Panel did not evaluate potential drinking water exposures again as part of Scenario 1. This Panel also judged potential direct contact exposures to CECs in receiving waters (e.g., while swimming or wading) to be small enough to not warrant quantitative evaluation. Such exposures are anticipated to be small because frequency of contact is low for most people and dilution is expected to be high in coastal waters (see Section 3.3.2.1).

The other potential human health exposure pathway the Panel considered was exposure to CECs via the consumption of aquatic organisms. While most CECs are not expected to bioaccumulate in aquatic biota (i.e., finfish and shellfish), CECs with a log K_{ow} greater than 3, that remain largely un-ionized in receiving

waters and are not rapidly metabolized by aquatic organisms, have the potential to bioaccumulate. While this Panel did not have the resources to conduct an exhaustive review of the bioaccumulation potential of all the CECs evaluated in this report, the Panel selected PBDEs as a model bioaccumulative CEC to demonstrate how such a compound might be evaluated for inclusion in a monitoring program.

For PBDEs the establishment of an allowable concentration in fish consumed by humans is based on the Fish Contaminant Goal (FCG) of 310 µg/kg recently derived by the State of California (<http://oehha.ca.gov/fish/gtllsv/pdf/PBDEs052311.pdf>). The FCG assumes an adult weighing 70 kilograms eats 32 grams of fish per day and that the allowable intake (i.e., reference dose, or RfD) for PBDEs is 1×10^{-4} (mg/kg-day). If MECs or PECs for PBDEs in fish are greater than the FCG, then PBDEs should be considered for monitoring. It should be noted that exceedance of the FCG does not indicate an unacceptable fish consumption risk is posed by PBDEs. The State of California in its derivation states FCGs "...provide a starting point for OEHHA to assist other agencies that wish to develop fish tissue-based criteria with a goal toward pollution mitigation or elimination. FCGs prevent consumers from being exposed to more than the daily reference dose (RfD) for non-carcinogens or to a risk level greater than 1×10^{-6} for carcinogens (not more than one additional cancer case in a population of 1,000,000 people consuming fish at the given consumption rate over a lifetime). FCGs are based solely on public health considerations relating to exposure to each individual contaminant, without regard to economic considerations, technical feasibility, or the counterbalancing benefits of fish consumption are based solely on public health considerations relating to exposure to each individual contaminant, without regard to economic considerations, technical feasibility, or the counterbalancing benefits of fish consumption." (<http://oehha.ca.gov/fish/gtllsv/pdf/PBDEs052311.pdf>). The Panel believes this approach can be used to derive FCGs for other CECs, as long as CEC-specific RfDs (or cancer slope factors, if relevant) are available.

The Panel was not able to identify allowable concentrations of PBDEs in fish for protection of marine mammals that could serve as MTLs for PBDEs for marine mammals. The Panel believes such marine mammal-based MTLs could be derived using the same general approach as used to derive FCGs for protection of human health. The key differences would be in the selection of an aquatic biota consumption rate and an allowable daily intake of a CEC for marine mammals. Both would likely be higher for marine mammals than for humans. For example, as noted above, the FCG assumes a daily fish consumption rate of approximately 0.0005 grams of fish per gram of human body weight (32 grams-day/70 kilograms). Harbor seals are reported to consume fish at approximately 100 times that rate (0.05 g/g-day from USEPA 1993 Wildlife Exposure Factors Handbook, EPA/600/R-93/187). Thus, based on the difference in fish consumption rate alone, a marine mammal-based MTL might be 100 times lower than the MTL based on the FCG. However, the RfD used to derive the FCG is based on a study of neurobehavioral effects in mice from which both a NOAEL and LOAEL was available and to which an uncertainty factor of 3000 was applied for the protection of human health (<http://oehha.ca.gov/fish/gtllsv/pdf/PBDEs052311.pdf>). Although the Panel has not attempted to derive an allowable intake for marine mammals, it expects that a smaller safety factor, possibly substantially smaller, would be used to establish such an allowable daily intake for marine mammals. If an uncertainty factor of 30 (instead 3000) were used, the human and marine mammal-based MTLs would be identical. If a smaller safety factor was used for marine mammals, then the human-based MTL would be lower than the marine mammal-based MTL. If the State believes that MTLs based on marine mammals are important to develop, this Panel recommends that a subsequent panel be convened to develop recommendations about the assumptions to use to derive marine mammal-based MTLs.

Additional Chemicals of Toxicological Concern

There are new compounds that have been recently discovered to have robust toxicologic effects in aquatic species, but for which there may be very scant occurrence data. It is critical to start collecting occurrence data for these to make sure they do not pose a risk in California receiving waters. In particular, progestogens and glucocorticoids have come to the attention of Europeans and new work is currently being pursued on both the effects and occurrence side on these chemicals.

(1) Progestogens: Levonorgestrel (CAS # 797-63-7). Drospirinone (CAS # 67392-87-4). Norethindrone (CAS # 51-98-9).

Progestogens are widely used in birth control and for agricultural animals. Zeilinger et al. (2009) treated fathead minnows in a 21-d reproductive study with three doses of levonorgestrel (0.8, 3.3, and 29.6 ng/L) or drospirinone (0.66 µg/L, 6.5 µg/L and 70 µg/L). The lowest dose of levonorgestrel reduced egg production significantly after one week and shut it down after 2 weeks. Males became more aggressive and less interested in tending a nest and females showed masculinization tendencies at the highest dose. Thus the LOEC for levonorgestrel is 0.8 ng/L and the NOEC <0.8 ng/L. Drospirinone appeared to be less toxic. There were no changes at the lowest dose but the two higher doses showed complete inhibition of egg production by week 2. Thus the NOEC for drospirinone is 0.66 µg/L. The predicted concentration of levonorgestrel in effluents was <1 ng/L (Fick et al. 2010b). Other studies with frogs (Säfholm et al. 2011) and fecundity in fish also point to very low sensitivities in the range of environmental concentrations (Paulos et al. 2010).

(2) Glucocorticoids: prednisolone (CAS # 8056-11-9). beclomethasone (CAS # 5534-09-8).

Glucocorticoids and synthetic corticosteroids are common pharmaceuticals used to treat a variety of conditions such as asthma, rheumatic disease, inflammatory bowel disease and inflammatory illnesses (Kugathas and Sumpter 2011). Little is known about their occurrence with scant reports in the environment at concentrations of about 1 ng/L. Prednisolone is mainly used for treating allergic disorders, skin conditions, ulcerative colitis, among others and beclomethasone is primarily used for the treatment of asthma in children. The main endpoints measured to date are biochemical, for example the reduction of blood leukocyte counts in fish or a dose-dependent increase in blood glucose levels (Kugathas and Sumpter 2011). Both of these endpoints can be associated with adverse outcomes, e.g., immune-suppression could result from low blood leukocyte counts resulting in a higher susceptibility to disease, and low blood glucose levels are associated with hypoglycemia. Glucocorticoids are in general detected at <1 ng/L concentrations in surface waters and WWTP effluents (Kostich et al. 2010), but in the range that could have biological effects on aquatic receptors.

Table D.1. Toxicity Data for Non-Fish Receptors.

Compounds--CA measured	CAS number	PNEC (mg/L)	Repro growth survival (mg/L)	Other organism (mg/L)	Chronic value Daphnids (EcoSAR) WERF (mg/L)	Chronic value algae (EcoSAR) WERF (mg/L)	MOA (EDC, immuno develop, misc)	BCF (PBT profiler) (L/kg)	Kow	Tissue residue threshold	REF
1.Acetaminophen	103-90-2		Daphnia NOEC 9.2		78.864	70.194		3.2	0.46		Kühn et al. 1989
2.AHTN (tonalide)	21145-77-7				0.019	0.159		2200	5.8	10 mg/kg	Balk and Ford 1999
3.amphetamine	300-62-9				11.871	24.232		4.5	1.76		
4.Atenolol	29122-68-7			EC10 Lemna 0.019	228	179		3.2	0.16		Brain et al. 2006
5.Atorvastatin	134523-03-8			Lemna EC10 0.026							Brain et al. 2006
6.Atrazine	1912-24-9			.002 micro/ meso	3.235	7.044	EDC	9.8	2.61		Hall et al. 1997
7.Diphenyl-ketone (benzophenone)	119-61-9				1.207	3.182	EDC	8.1	3.18		
8.Bisphenol A	80-05-7	6.10 ⁻⁵			1.061	3.103	EDC	72	3.32		Wright-Walters et al. 2011
9.Butylated hydroxyanisole	25013-16-5										
10.Butylated hydroxytoluene	128-37-0			1.7 Tetrahymena EC50							Yoshioka et al. 1985
11.Butylbenzyl phthalate	85-68-7		63 µg/kg NOEC		0.142	0.745	EDC	880	4.73		Vidal and Bay 2005
12.Carbamazepine	298-46-4		0.025 Repro NOEC 7d C. dubia 33 µg/kg sediments		4.615	9.402		15	2.45	33 µg/kg	Ferrari et al. 2004 Dussault et al. 2008

Table D.1. Continued

Compounds--CA measured	CAS number	PNEC (mg/L)	Repro growth survival (mg/L)	Other organism (mg/L)	Chronic value Daphnids (EcoSAR) WERF (mg/L)	Chronic value algae (EcoSAR) WERF (mg/L)	MOA (EDC, immuno develop, misc)	BCF (PBT profiler) (L/kg)	Kow	Tissue residue threshold	REF
13.cis-androstenedione	63-05-8				3.41	7.87	EDC	26	2.75		
14.chlorpyrifos			0.00005 LOEC C. dubia								Sherrard et al. 2002
15.desulfinyl fipronil							EDC				
16.diazepam	439-14-5				29.957	39.92		30	1.43		
17.diazinon	333-41-5				0.63	2.259		170	3.81		
18.Diclofenac	15307-86-5				1395.69	1228.369		3.2	0.7		
19.Dilantin	57-41-0				61.46	55.212					
20.bis(2-ethylhexyl) phthalate	117-81-7			1.3 mg/kg sediment Amphipod			EDC				Vidal and Bay 2005
21. 17-alpha estradiol	57-91-0				0.405	1.58	EDC	240	4.01		
22. 17-beta estradiol	50-28-2				0.405	1.58	EDC	240	4.01		
23. estrone	53-16-7			0.410 EC50 copepod develop	1.719	4.647	EDC	51	3.13		Andersen et al. 2001
24.fipronil	120068-37-3		<5.10 ⁻⁶ NOEC Mysid	0.0098 NOEC Daphnia			EDC				USEPA 1996
25.furosemide	54-31-9		LC50 D.magna 60.62		12.924	22.106		3.2	2.03		Isidori et al. 2006
26. Galaxolide (HHCB)	1222-05-5		0.007 NOEC Copepod metamorph		0.016	0.14		13000	5.9	100 mg/kg	Balk and Ford 1999 Brieholtz et.al. 2003
27.Gemfibrozil	25812-30-0		0.078 C.dubia NOEC	0.1 NOEC Cnidaria morph			EDC				Quinn et al. 2008 Isidori et al. 2007

Table D.1. Continued

Compounds--CA measured	CAS number	PNEC (mg/L)	Repro growth survival (mg/L)	Other organism (mg/L)	Chronic value Daphnids (EcoSAR) WERF (mg/L)	Chronic value algae (EcoSAR) WERF (mg/L)	MOA (EDC, immuno develop, misc)	BCF (PBT profiler) (L/kg)	Kow	Tissue residue threshold	REF
28.Hydrocodone	125-29-1				9.37	16.95		9.3	2.16		
29.ibuprofen	15687-27-1			0.001 Lemna NOEC	3.511	13.134		3.2	3.97		Pomati et al. 2004
30. Iopromide	73334-07-3		>1g/L								Steger-Hartmann et al. 1999
31.Meprobamate	57-53-4				76.619	75.357		3.2	0.7		WERF dataset
32. metformin	657-24-9		64.0 EC50 D.magna		11243.257	2755.258		3.2	-2.64		Cleuvers 2003
33.miconazole	22916-47-8				0.015	0.152	EDC	13000	6.25		
34.DEET (N,N-diethyl-meta-toluamide)	134-62-3				5.835	10.623		9.5	2.18		
35. naproxen	22204-53-1				15.247	40.215		3.2	3.18		
36. p-nonylphenol	84852-15-3		0.044 Daphnia NOEC	1.4 mg/kg sediment NOEC	0.014	0.121	EDC	7200	5.92		LeBlanc et al. 2000 CCME 2002
37. NP1EO	27986-36-3				0.028	0.215	EDC	88	5.58		
38.NP2EO	26027-38-2						EDC				
39.octocrylene	6197-30-4			0.021 algae NOEC							Rodil et al. 2009
40. octylphenol	27193-28-8		0.013 Copepod EC50 Develop		0.038	0.249	EDC	340	5.5		Andersen et al. 2001
41. o-Hydroxy atorvastatin	214217-86-6		0.653 Dapnia ECOSAR LC50								

Table D.1. Continued

Compounds-- CA measured	CAS number	PNEC (mg/L)	Repro growth survival (mg/L)	Other organism (mg/L)	Chronic value Daphnids (EcoSAR) WERF (mg/L)	Chronic value algae (EcoSAR) WERF (mg/L)	MOA (EDC, immuno develop, misc)	BCF (PBT profiler (L/kg)	Kow	Tissue residue threshold	REF
42. p-Hydroxy atorvastatin	214217-88-6										
43. Oxybenzone BP-3	131-57-7										
44. PBDE -47	5436-43-1			.001 frog NOEL develop 0.003 mg/kg sediment larval growth	0.007	0.087			6.77	0.289 µg/g wt wt egg	Balch et al. 2006 Fernie et al. 2008Lam et al. 2010
45. PBDE -99	60348-60-9			.001 frog NOEL develop	0.007	0.092			6.84		Balch et al. 2006 Kawahara et al. 1991
46.permethrin	52645-53-1	10.10 ⁻⁶		0.0052 mg/kg sediment Amphipod	0.01	0.105		450	6.5		Fojut et al. 2012 Amweg et al. 2005
47.PFBA	375-22-4		LC50 411 Daphnia ECOSAR								
48.PFDA	335-76-2		LC50 0.013 Fish ECOSAR								
49.PFDoA											
50.PFHxS											
51.PFNA											
52.PFOA											
53.PFOS				0.021 C. tentans NOEC growth	Egg development					1000 µg/kg egg wet wt	Newsted et al. 2005

Table D.1. Continued

Compounds--CA measured	CAS number	PNEC (mg/L)	Repro growth survival (mg/L)	Other organism (mg/L)	Chronic value Daphnids (EcoSAR) WERF (mg/L)	Chronic value algae (EcoSAR) WERF (mg/L)	MOA (EDC, immuno develop, misc)	BCF (PBT profiler) (L/kg)	Kow	Tissue residue threshold	REF
54.PFOSA					10	.3					
55.PFUnA											
56.Progesterone			0.01 NOEC gender shift Daphnia				EDC				Kashian and Dodson 2004
57.sulfamethoxazole			NOEC algae 0.059; C dubia 0.25								Ferrari et al. 2004
58. TCEP	13674-84-5				5.079	10.966		3.3	2.59		
59.TCPP	13674-84-5		Daphnia NOEC 13.0 21 d repro								Kühn et al. 1989
60.Testosterone	58-22-0		0.01 NOEC fecundity Daphnia		1.34	3.921	EDC	72	3.32		Kashian and Dodson 2004
61.triamterene	396-01-0				56.004	61.888		3.2	0.98		
62.triclocarban	101-20-2			0.056 Mysid NOEC	0.108	0.61	EDC	1200	4.9		Langdon et al. 2010
63. triclosan	3380-34-5			0.7 Algae NOEC 0.154 LC50 P. pugio (shrimp)	0.125	0.665	EDC	370	4.76		Orvos et al. 2002 DeLorenzo et al. 2008
64.Trimethoprim	738-70-5			16.0 Algae	72.062	77.347		3.2	0.91		Lutzhof et al. 1999

Table D.2. Toxicity Data for Fish.

Compounds—CA measured	Chronic Value Fish (ECOSAR) WERF (mg/L)	LC50	NOEC	LOEC	Effect Concentration	Comments	Reference
Acetaminophen	229.872	814 mg/L				FHM	Brooke et al. 1984
AHTN (tonalide)	0.012		10-1000 µg/L in Zebrafish		33 µg/L in Zebrafish		Carlsson and Norrgren 2004
Amphetamine	18.281	28.8mg/L				FHM	Geiger et al. 1988
Atenolol	731		1 mg/L in FHM		3.2 mg/L in FHM		Winter et al. 2008
Atorvastatin			200 mg/L			cytotoxicity -- rainbow trout hepatocytes	Ellesat et al. 2010
Atrazine	4.741		50 µg/L in Rainbow Trout		340 µg/L in Rainbow Trout		Davies et al. 1994
Diphenylketone (Benzophenone)	1.45		3.3 mg/L in FHM		6.3 mg/L in FHM		Call and Geiger 1992
Bisphenol A	1.239		120-130 µg/L in FHM		280-300 µg/L in FHM	PNEC-60 ng/L	Brian et al. 2007 Wright-Walters et al. 2011
Butylated hydroxyanisole		1 mg/L				rainbow trout	Cope et al. 1997
Butylated hydroxytoluene			LC50: 13.5-17.5 mg/L in Medaka (Killifish)				Tsuji et al. 1986.
Butylbenzyl phthalate	0.105		140 µg/L in FHM		360 µg/L in FHM		LeBlanc 1984
Carbamazepine	7.119		30.6 mg/L		EC50: 86.5 mg/L	Zebrafish-- Embryo deformities: tail, scoliosis, growth, yolk sac	van den Brandhof et al. 2010

Table D.2. Continued

Compounds—CA measured	Chronic Value Fish (ECOSAR) WERF (mg/L)	LC50	NOEC	LOEC	Effect Concentration	Comments	Reference
cis-androstenedione	4.778	LC50: 10.5 mg/L in Mosquitofish				40 ng/L Mosquitofish gonopodium	Hunsinger and Howell 1991 Stanko and Angus 2007
Chlorpyrifos			50 µg/L in FHM	75 µg/L in FHM			Sherrard et al. 2002
Desulfinyl fipronil		NO DATA					
Diazepam	64.027	LC50: 12.7 mg/L in Eastern Mosquitofish					Nunes et al. 2005
Diazinon	0.629		6 mg/L in FHM	12.5 mg/L in FHM			Burkepile et al. 2000
Diclofenac	3035.701		1.5 mg/L 1 µg/L	2.95 mg/L	EC50: 5.3 mg/L	Zebrafish--Embryo deformities: tail, scoliosis, growth, yolk sac Tissue histopathology	van den Brandhof et al. 2010 Triebkorn et al. 2004, 2007
Dilantin (phenytoin)	3.35	250 uM		31.25uM		Zebrafish embryo toxicity test-deformities	Weigt et al. 2011
bis(2-ethylhexyl) phthalate		LC50: 160 µg/L in FHM	500 µg/L in Rainbow Trout				Spehar 1986 Adams et al. 1995
17-alpha estradiol	0.379		1 ng/L in FHM				Lange et al. 2001
17-beta estradiol	0.379		0.041 µg/L in FHM	.107 µg/L in FHM		2 ng/L PNEC	Larkin et al. 2007 Caldwell et al. (In press)
Estrone	2.133		0.00074 µg/L in Rainbow Trout	0.0033 µg/L in Rainbow Trout; 6 ng/L PNEC			Thorpe et al. 2003 Caldwell et al. (In press)
Fipronil			0.51 µg/L in SHM	7.61 µg/L in SHM			Wirth et al. 2004.
Furosemide	22.799			611.08 ng/L		Comet assay -- amount of DNA in tail	Rocco et al. 2010
Galaxolide (HHCB)	0.01		1 mg/L in Zebrafish				Carlsson and Norrgren 2004

Table D.2. Continued

Compounds—CA measured	Chronic Value Fish (ECOSAR) WERF (mg/L)	LC50	NOEC	LOEC	Effect Concentration	Comments	Reference
Gemfibrozil			851.9 µg/L in Goldfish	1500 µg/L in Goldfish			Mimeault et al. 2005
Hydrocodone	15.835	NO DATA					
Ibuprofen	3.492		1 µg/L egg production medaka	66.4 ng/L			Flippin et al. 2007
Iopromide		LC50: 10000 mg/L in Zebrafish					Steger-Hartmann et al. 1999
Meprobamate	206.825	NO DATA					
Metformin	88323.07	NO DATA					
Miconazole	0.007				100 µM	Similar to the effect of ketoconazole on trout microsomes. Inhibition of Cy2K1, Cyp1A1, Cype 3A27	Miranda et al. 1998
DEET (N,N-diethyl-meta-toluamide)	9.811	72.1 mg/L				Rainbow Trout mortality	USEPA 2000b
Naproxen	18.323		793ng/L			Fathead Minnow full life-cycle - mixture with 7PPPC	Parrott and Bennie 2009
"					10 µM	EROD inducer PLHC cells	Thibaut and Porte 2008
"					300 µM	EROD inducer -- trout cells	Gagné et al. 2006

Table D.2. Continued

Compounds—CA measured	Chronic Value Fish (ECOSAR) WERF (mg/L)	LC50	NOEC	LOEC	Effect Concentration	Comments	Reference
p-nonylphenol	0.007	17 µg/L				Winter Flounder	Lussier et al. 2000
"				5 µg/L		Atlantic Salmon	Mortensen and Arukwe 2007
"			7.4 µg/L	14 µg/L		FHM	Ward and Boeri 1991
NP1EO	0.019						
NP2EO							
Octocrylene						No toxicity data -- but occurrence in fish	
Octylphenol	0.024		3.2 µg/L	3.2 µg/L		Zebrafish	Cruz-Li 2004
"				4.5 µg/L		Atlantic Salmon	Bangsgaard et al. 2006
o-Hydroxy atorvastatin			200 mg/L			cytotoxicity -- Rainbow Trout hepatocytes	Ellesat et al. 2010
p-Hydroxy atorvastatin			200 mg/L			cytotoxicity -- Rainbow Trout hepatocytes	
Oxybenzone (BP-3)				16 µg/L	620 µg/L	Medaka -- LOEC for egg hatching 21-day exposure	Coronado et al. 2008
PBDE -47	0.003		50 µg/L	100 µg/L		Fundulus mortality	Key et al. 2009
PBDE -99	0.003					No data on fish	

Table D.2. Continued

Compounds—CA measured	Chronic Value Fish (ECOSAR) WERF (mg/L)	LC50	NOEC	LOEC	Effect Concentration	Comments	Reference
Permethrin	0.004	1.2 µg/L				Shortnose Sturgeon	Dwyer et al. 2000
"		7.8 µg/L				Sheepshead Minnow	Schimmel et al. 1983
"		4.5 µg/L				Bluegill	Mayer and Ellersieck 1986
PFBA							
PFDA			0.128 ppm (NOEL)	0.64 ppm (LOEL)	214 ppm	Plasma Vtg from dietary exposure of juvenile Rainbow Trout	Tilton et al. 2008
PFDoA							
PFHxS							
PFNA					50 ppm Vtg effect	Plasma Vtg from dietary exposure of juvenile Rainbow Trout	
PFOA			16 ppm	80 ppm	458 ppm	Plasma Vtg from dietary exposure of juvenile Rainbow Trout	
PFOS							
PFOSA	17.5						
PFUnDA					250 ppm Vtg induced	Plasma Vtg from dietary exposure of juvenile Rainbow Trout	

Table D.2. Continued

Compounds—CA measured	Chronic Value Fish (ECOSAR) WERF (mg/L)	LC50	NOEC	LOEC	Effect Concentration	Comments	Reference
Progesterone		no data					
Sulfamethoxazole			1000 mg/ml			Acute toxicity -- Zebrafish	Isidori et al. 2005
TCEP -- Tris (2-chloroethyl) phosphate		200 mg/L				ebrafish	MSDS -- Nullifire Limited, Torrington Avenue, Coventry, CV4 9TJ, England.
TCPP -- Tris (chloroisopropyl) phosphate	7.49	30 mg/L				Guppy	MSDS -- Bayer Material Science
Testosterone	1.565				0.1 µg	Enzyme regulation	Prendas and Metcalf 1996
Triamterene	138.228	NO DATA				No information	
Triclocarban	0.76	40 µg/L		3.6 µg/L		Bluegill	Monsanto Co. 1992
"				10 µg/L		Fathead Minnow LOEL	Monsanto Co. 1992
Triclosan	0.092	220 µg/L				Zebrafish mortality	Tatarazako et al. 2004
"		288 µg/L	15.1 µg/L	31.6 µg/L		Rainbow Trout mortality	Orvos et al. 2002
Trimethoprim	181.889					No effects observed at highest concentration tested in fish	Gagné et al. 2006

Table D.3. Antibiotic/Antimicrobial MIC and NOEC values.

	Highest MIC ^a (µg/ml)	Moderate Observed MIC (µg/ml)	Lowest MIC (µg/ml)	NOEC µg/L
Antibiotic				
Cell Wall Inhibitors				
Ampicillin	≥32	8	1 ^b	<1,000
DNA/RNA Inhibitors				
Azithromycin	≥8	4	0.25	250
Ciprofloxacin	≥4	<1	0.06	<60
Clarithromycin	8	2	Most Sensitive MIC 0.25 ^b	<250
Erythromycin	≥32	8	Highly Sensitive MIC NR	<8,000
Tetracycline	≥16	8	0.01	<10
Metabolic Inhibitors				
Sulfathiazole	≥500	10-500	0.25 ^b	<250
Sulfamethoxazole	76	76	Highly Sensitive MIC	<76,000 ^c
Trimethoprim	≥16	4		<4,000 ^b
Sulfamethizole	512	128	32-64 ^b	<32,000 ^b
Antibacterial Agent				
Triclosan	80	64	0.025 ^b	25
Triclocarban	80	0.20	0.200 ^b 0.0001 Microtox LOEC	0.1-200 ^b

^a MIC = Minimum Inhibitory Concentration that prevents bacterial growth

^b value based upon most sensitive MIC value

^c value based on a single MIC value

Antibiotic Evaluations

Safety factors were based on the both amount/quality of data provided for each antibiotic and the range of MICs for an antibiotic. The range of MICs provides an indication for the variability of response of bacteria to each antibiotic among bacteria. A safety factor of 100 was used for antibiotic that had a range (High, Moderate and Most Sensitive) of MIC values. This safety factor of 100 was applied to account for uncertainty associated with the use of indicator bacteria that are not aquatic (safety factor = 10) and for the uncertainty of development of ABR from plasmids or other molecular constituents (safety factor = 10). For antibiotics with less than 3 independent MICs a safety factor of 1000 (the safety factor of 100 mentioned above plus an additional safety factor of 10 to account for incomplete acute toxicity (MIC) data) was used to derive the MTLs discussed in Appendix D. NOECs were derived from most sensitive MICs (lowest concentration causing toxicity) and were set at concentrations less than the most sensitive MIC value reported (Section 4).

(1) Ampicillin (CAS number 69-53-4) NOEC <1,000 µg/L. The MICs ranged from 1,000 to 32,000 µg/L; the lowest MIC value was 1,000 µg/L, which was used as a LOEC. The NOEC was estimated to be <1,000 µg/L. The ratio between the highest and lowest MIC values was 32 (32,000 µg/L/1,000 µg/L), indicating the variability of bacterial response to exposure to this antibiotic.

(2) Ciprofloxacin (CAS number 87521-33-1) NOEC <60 µg/L. The MICs ranged from 60 to <4,000 µg/L; the lowest MIC value was 60 µg/L, which was used as a LOEC. The NOEC was estimated to be <60 µg/L. The ratio between the highest and lowest MIC values was 67 (4,000 µg/L/60 µg/L), indicating the variability of bacterial response to exposure to this antibiotic.

(3) Azithromycin (CAS number 83905-01-5) NOEC <250 µg/L. The MICs ranged from 250 to <8,000 µg/L; the lowest MIC value was 250 µg/L which was a MIC for the most sensitive microbial species, which was used as a LOEC. The NOEC was estimated to be <250 µg/L. The ratio between the highest and lowest MIC value was 32 (8,000 µg/L/250 µg/L), indicating the variability of bacterial response to exposure to this antibiotic.

(4) Clarithromycin (CAS number 81103-11-9) NOEC <250 µg/L. The MICs ranged from 250 to 8,000 µg/L; the lowest MIC value was 250 µg/L, which was the MIC used as a LOEC for the most sensitive microbial species. The NOEC was estimated to be <250 µg/L. The ratio between the highest and lowest MIC value was 32 (8,000 µg/L/250 µg/L), indicating the variability of bacterial response to exposure to this antibiotic.

(5) Erythromycin (CAS number 114-07-8) NOEC <8,000 µg/L. The MICs ranged from 8,000 - 32,000 µg/L. The MIC 8,000 µg/L was used as a LOEC for the most sensitive species. The NOEC was estimated to be <8,000 µg/L. The ratio between the highest and lowest MIC was 4 (32,000 µg/L/8,000 µg/L), which is indicative of the variability of bacterial response to this antibiotic.

(6) Tetracycline (CAS number 60-54-8) NOEC <10 µg/L. The MICs ranged from 8,000 to 16,000 µg/L, and the most sensitive species MIC of 10 µg/L was used as a LOEC. The NOEC was estimated to be <10 µg/L. The ratio between the highest and lowest MIC was 1600 (16,000 µg/L/10 µg/L), which is indicative of the variability of bacterial response to this antibiotic.

(7) Sulfathiazole (CAS number 72-14-0) NOEC <250 µg/L. The MICs ranged from 250 to 500,000 µg/L. The MIC for the most sensitive species, 250 µg/L, was used as a LOEC. The NOEC was estimated to be

<250 µg/L. The ratio between the highest and lowest MIC was 2,000 (500,000 µg/L/250 µg/L), which is indicative of the variability of bacterial response to this antibiotic.

(8) Sulfamethoxazole (CAS number 723-46-6) NOEC <76,000 µg/L. Only one MIC of >76,000 µg/L was reported. The MIC for the most sensitive species of 76,000 µg/L was used as a LOEC. The NOEC was estimated to be <76,000 µg/L. No assessment of the variability of bacterial response to this antibiotic can be made at this time.

(9) Sulfamethizole (CAS number 144-82-1) NOEC <32,000 µg/L. The MICs ranged from 32,000 to 512,000 µg/L. The MIC for the most sensitive species of ranged 32,000 – 64,000 µg/L and the lowest MIC value of 32,000 was used as a LOEC. The NOEC was estimated to be <32,000 µg/L. The ratio between the highest and lowest MIC was 16 (512,000 µg/L/32,000 µg/L) which is indicative of the variability of bacterial response to this antibiotic.

(10) Trimethoprim (CAS number 738-70-5) NOEC <4,000 µg/L. The MICs ranged from >16,000 to 4,000 µg/L. The lower MIC value reported for the most sensitive species of 4,000 µg/L was used as a LOEC. The NOEC was estimated to be <4,000 µg/L. The ratio between the highest and lowest MIC was >4 (>16,000 µg/L/4,000 µg/L) which is indicative of the variability of bacterial response to this antibiotic.

(11) Triclocarban (CAS number 101-20-2) NOEC ranged from 0.100 (Microtox) - <200 µg/L (Most Sensitive MIC = only Most Sensitive MIC value was use). The MICs ranged from 200 to 80,000 µg/L, and a LOEC of 100 ng/L based on a Microtox LOEC was reported, but was not used because the effect was based on a sublethal physiological response rather than the toxicity values reported in MIC elsewhere in the report. The lower MIC value reported for the most sensitive species of 200 µg/L was used as a MIC LOEC. The MIC NOEC was estimated to be <200 µg/L. The ratio between the highest and lowest MIC was 400 (80,000 µg/L/200 µg/L), indicating the variability of bacterial response to exposure to this antibiotic.

(12) Triclosan (CAS number 3380-34-5) NOEC <25 µg/L. The MICs ranged from 25 to 80,000 µg/L. The lowest MIC value reported for the most sensitive species of 25 µg/L was used as a LOEC. The NOEC was estimated to be <25 µg/L. The ratio between the highest and lowest MIC was 3,200 (80,000 µg/L/25 µg/L) indicating the variability of bacterial response to exposure to triclosan.

Antibiotics are pharmaceutical drugs developed to target and combat biological infections, primarily from bacteria but also may include drugs that arrest fungal, viral and protozoan infections. Antibiotics are defined as any chemotherapeutic agent that is capable of inhibiting or killing bacteria (bacteriostatic or bactericidal). While compounds with these properties have been used for centuries, it was not until the late 1920s that the first antibiotic was isolated (Van Epps 2006). Since this initial discovery, the use and production of pharmaceuticals has continued to increase yearly. Wise (2002) estimated that between 1 and 2 x 10⁸ kg of antibiotics are consumed annually worldwide. The estimated percentages vary between countries and availability of information for most of the developing countries is scarce. For instance, for the European Union (EU), Switzerland, and the US, the percentage usage is estimated to be 50% for human and 50% for veterinary medicine (Kümmerer 2009). A more recent estimate for the US released by the FDA in 2010 indicates that 1.3 x 10⁷ kg of antibiotics (~60%) are used for agricultural purposes, including aquaculture, while the remaining 40% is used for clinical use (FDA 2010).

Chemicals with antimicrobial properties may naturally occur in the environment and can produce natural pressures for selection of antimicrobial resistance within microbes. As a result, many microbes have “intrinsic resistance” to certain antibiotics when their normal characteristics render them immune

to the antibiotic's activity. Intrinsic resistance is not affected by misuse of antibiotics. This natural intrinsic resistance is invaluable in determining which antibiotic will be most effective as some bacteria often have conferred intrinsic resistance due to the over-expression of certain genes which makes them relatively impermeable to hydrophobic compounds such as macrolide antibiotics (Rosenblatt-Farrell 2009). In addition, some microbes may temporarily over express or suppress certain genes which allows them to survive in the presence of antibiotics, with expression patterns returning to normal once the threat posed by those particular drugs has passed.

When antibiotics were first introduced Alexander Fleming, who won a Nobel Prize for the discovery of penicillin, warned in 1945 that misuse of the drug could result in selection for resistant bacteria (Rosenblatt-Farrell 2009). Within 10 years of the wide-scale introduction of penicillin, antibiotic resistance to this drug was observed. Although antibiotics have transformed the treatment of biological infections and greatly reduced the duration of infections and associated morbidity and mortality, the over-prescription and use of these drugs in medicine and agriculture have resulted in the development of resistant microbial populations. This problem is considered so significant that many experts suggest the value of existing antibiotic therapies over the next 100 years is now uncertain (Rosenblatt-Farrell 2009).

The reason for some of the recently observed increase in antibiotic resistance is due to the fact that microbes have additional adaptive capacities besides Intrinsic Resistance to further develop resistance. In addition to Intrinsic Resistance, certain microbes may also have "Acquired Resistance" to an antibiotic by taking on new adaptive characteristics either through gene mutation or the transfer of genetic material between bacteria (Rosenblatt-Farrell 2009). Acquired resistance enables microbes to become more resistant to antibiotics and examples may include changes to the bacterial membrane such as over-expression of multidrug resistance (MDRs) proteins that may prevent antibiotics from entering the cell. Microbes may also use enzymes to break down antibiotics, or they may employ "efflux pumps" to remove the antibiotic entirely or reduce its concentration below effective levels (Rosenblatt-Farrell 2009).

The term antimicrobial resistance has been broadly defined as the development of adaptive physiological responses to all pharmaceuticals used to kill or inhibit the growth of pathogenic microorganisms (bacteria, viruses, fungi and protozoa) and include antibiotics (antibacterials), antifungals, antivirals, and antiparasitics drugs. Antibiotic resistance involves physiological, metabolic or molecular adaptation by microbes in response to antibiotic mode of action and may involve several major adaptive responses (Table D.4) including changes in the cell wall, metabolism, proteins or nucleic acids (DNA or RNA). Cell wall inhibition is perhaps the most widespread response observed among microbes and this includes several types of antibiotics including penicillins, cephalosporins, carbapenems, and vancomycin which target the bacterial cell wall and kill bacteria by damaging or inhibiting the cell wall synthesis. Other antibiotics may affect microbes by (1) affecting bacterial metabolism such as trimethoprim and the sulfonamides; (2) by affecting DNA or RNA synthesis such as quinolones and rifampin; or (3) by affecting protein synthesis such as chloramphenicol, the tetracyclines, the aminoglycosides, and the macrolide antibiotics.

Table D.4. Mechanism of action of antibiotics in causing microbial resistance.

Mode of Action	Examples of Antibiotics Causing Effects
Cell Wall Inhibitors	Penicillins, cephalosporins, carbapenems, and vancomycin
Inhibition of Metabolism	Trimethoprim and sulfonamides
Disruption of Protein Synthesis	Aminoglycosides, chloramphenicol, tetracyclines, macrolide antibiotics
Disruption of DNA or RNA	Quinolones and rifampin

The spread of antimicrobial resistance has generally been attributed to the use of antibiotics in: (1) prescriptive drug use by people and animals given therapeutic doses in medical and agricultural practices; (2) environmental release from waste treatment and disposal activities that concentrate animal, medical and human wastes such as wastewater treatment plants (FIWG-PIE 2009), municipal land fields (Wintgens et al. 2003, Barnes et al. 2004, Slack et al. 2005) and confined farm animal practices; and (3) Aquaculture practices that use these drugs directly in aquatic environments (FIWG-PIE 2009, Uyagaura et al. 2009). National monitoring programs have identified detectable levels of select antibiotics in 48% of 139 US surface waters tested (USGS 2002), indicating the widespread nature of the use and discharge of these compounds in the environment. Several regional studies (Kaspar et al. 1990, Parveen et al. 1997, Van Dolah et al. 2000, Webster et al. 2004, Thompson 2004, NOAA 2011) have surveyed wastewater treatments plants and confined animal feeding operations (CAFOs) throughout mid Atlantic and southeastern US and found the rate for detection of multiple antibiotic resistance *E. coli* bacteria ranged from 5 to 22 % in wastewater treatment plants and from 12 to 16 % in farm animal operations (chicken and hog farms). The number of antibiotics to which multiple antibiotic resistance was observed ranged from 1 to 8 antibiotics, averaging 4.6 antibiotics/wastewater treatment plants (Webster et al. 2004, Thompson 2004, NOAA 2011).

In addition once antibiotic resistance develops, the spread and maintenance of antibiotic resistance becomes a secondary issue within the environment, including aquatic environments (FIWG-PIE 2009, Uyaguari et al. 2011). Monitoring of marine surface waters in various regions of the U.S. has indicated rates of antibiotic resistance vary based upon tidal range, which may dilute the microbial source, and land use activity (urban versus rural), with urban areas generally having 2-3 times higher levels of antibiotic resistance observed than rural areas. Levels of antibiotic resistant *E. coli* bacteria ranged from 13 to 25% in FL (microtidal - <1 m), 2.6 to 9% (mesotidal - >1- <2 m) in MD and 1 to 3% in SC (mesotidal - >2 - <3 m) coastal waters. Environmental realistic exposures from these sources generally result in pharmaceutical exposure concentrations that are much lower than therapeutic doses; however, uncertainty exists about the potential for biologically meaningful human and ecological effects from chronic exposures to low concentrations and mixtures of these compounds, especially in the environment and in subpopulations of humans and wildlife that might be particularly sensitive (Pomati et al. 2006, 2008).

Antibiotic resistance can be conferred not only from chemical exposure to antibiotics but from gene mutation associated with plasmids (packets of external DNA) exchange with naïve and antibiotic resistant bacteria. Furthermore, recent investigations have demonstrated that WWTP treatment does not reduce the number of known antibiotic resistance genes (Auerbach et al. 2007, Uyaguari et al. 2011). Thus, WWTPs may play a very important role as a reservoir of pre-existing resistance genes, generator of novel bacterial resistance, or vehicles for the adaptation of microbes. Interestingly, the rate

of antibiotic resistance may be higher in WWTP effluent than in pretreated sewage (Reinthal et al. 2003, Uyaguari et al. 2011), suggesting that the treatment process could be further effective in selecting for more resistant bacteria. Evidence about the effluent discharges containing genetic material (plasmids, free DNA, integrons, bacterial genomes) has been well documented (Tennstedt et al. 2003, Szczepanowski et al. 2004, Auerbach et al. 2007, Szczepanowski et al. 2009, Munir et al. 2011, Pellegrini et al. 2011). Bacterial acquisition of genes involved in these resistance mechanisms is achieved by a variety of promiscuous gene transfer systems or elements such as bacterial conjugative plasmids, transposons, and integrons (Bennett 2008, Garriss et al. 2009). These horizontal gene transfer elements allow genes to move from one genomic system to another and from one microbial cell to another, regardless of the gene donor (Bennett 2008). The horizontal transfer of genes may in part explain why antibiotic resistance phenotypes are widely distributed across geographical regions (Zaneveld et al. 2008). These mechanisms may also play a role in the continued loss of antibiotic effectiveness against a range of microbes. For example, the selection of the antibiotic vancomycin as the first choice to treat Gram-positive bacterial infections has declined due to acquired resistance first observed in enterococci and later documented in the US as a complete resistant strain in *S. aureus* (MIC>16µg/mL) (Ala'Aldeen and Hiramatsu 2004). Among the different elements of antibiotic resistance transfer, integrons are considered the main agents of bacterial evolution that have been often overlooked in their importance in the dissemination of antibiotic resistance genes, as well as their capacity to add larger structures into a bacterial genome (Mazel 2006, Joss et al. 2009).

PIE Report

The major goal of federal interagency collaborations to address antimicrobial resistance is to determine in fact whether environmental release of antimicrobials contributes to the development and/or maintenance of antimicrobial resistance in human and animal pathogens. There are three primary foci of this research.

1. Identifying naturally occurring and other synthetic chemicals that exhibit antimicrobial properties, but have not traditionally been used as antimicrobials,
2. Identifying and characterizing environmental settings that have elevated levels of chemicals that exhibit antimicrobial properties, and
3. Bringing antimicrobial-resistance research specialists into environmental studies in these settings to determine if antimicrobial resistance can be developed or maintained via environmental release pathways and, if so, to identify the controlling processes.

Identifying other chemicals exhibiting antimicrobial properties will require a laboratory approach guided by leads taken from the toxicological literature. Chemicals will be identified for testing based on chemical structure and chemicals found to co-occur in environmental settings where antimicrobials are released to the environment. This knowledge will guide field investigations seeking to determine the relative role of antimicrobials compared to other chemicals (metals, pesticides, and antimicrobial degradation byproducts) found routinely in the environment.

Major areas of needed new research on antibiotics identified by the Federal Interagency Working Group on Pharmaceuticals in the Environment (2009) include:

- Identifying chemicals other than pharmaceutical antimicrobials that may affect development of antimicrobial resistance in the environment.

- Evaluating the potential for development of antimicrobial resistance and release of resistant pathogens at stream sites highly affected by AFO wastes
- Evaluating environmental occurrence and levels of antibiotics and evidence of antimicrobial resistance (including development of antimicrobial resistant fish pathogens) at stream sites adjacent to aquaculture facilities.
- Evaluating the occurrence and levels of chemicals with antimicrobial properties and evidence of resistant microbes in other susceptible environmental settings, including sites with land application of wastewater, biosolids and manure.

Strategies for Prioritizing Antibiotic Resistance in the Environment

Tier 1 - Individual Antibiotic Resistance and Bacterial Toxicity

Tier 1 - Individual Antibiotic Resistance would be determined from chemical contaminant monitoring from STPs and other likely sources for antibiotics using the following approach for ranking hazards:

- (1) Detectable levels of an antibiotics that are measured in the environment that exceed the MIC for a given antibiotic = **Low Potential to Develop ABR**
- (2) Detectable levels of antibiotic that are measured in the environment that are <MIC but >50% of the MIC for a given antibiotic = **Moderate Potential to Develop ABR**
- (3) Detectable levels of antibiotic that are measured in the environment that are >minimum concentration that will induce antibiotic resistance and <lowest concentration causing mortality = **High Potential to Develop ABR.**

For those compounds where these above criteria are unknown, the criteria of <50% of the MIC but >10% of the MIC for a given antibiotic will be used. The rationale for this is given in Figure D.1 below. Note there is a decreased time to the development of ABR with decreased dose.

The longest time for development of ABR at the MIC (24 hours, 72 generations) and the shortest time to development of ABR at a dose 1/10,000 of the MIC (10 µg/L) (6.5 hours; 20 generations). Mutations are relatively rare, occurring in only 1 event per $10^7 - 10^{10}$ bacteria, according to a review by Mulvey and Simor (2009). The combination of lower dose and larger numbers of bacteria result in a greater potential to develop antibiotic resistance.

Induction of *E. coli* Antibiotic Resistance

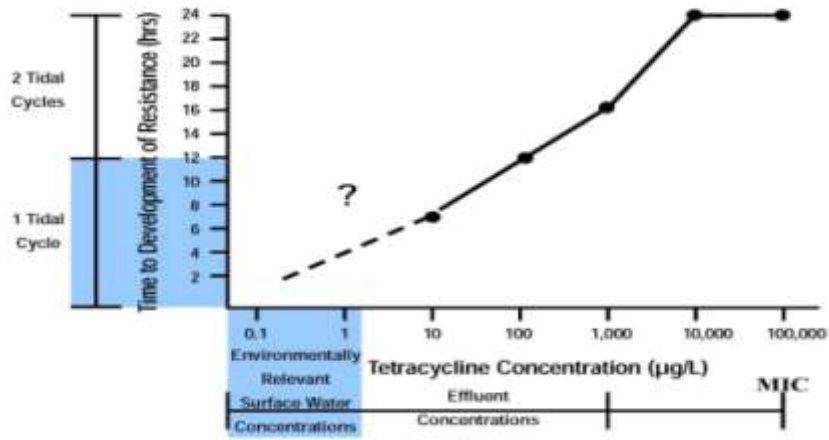


Figure D.1. Development of antibiotic resistance in a naïve strain of *E. coli* bacteria exposed to tetracycline (NOAA 2011).

APPENDIX E - OCCURRENCE DATA

Table E.1. Aqueous concentration values and data sources for occurrence metric and Los Angeles Regional Board (LARB) River Study maximum occurrence values.

Compound	Data Tier	Data Source	Max Value (ng/L)	90% Value (ng/L)	LARB River Study - Max (ng/L)
17-alpha estradiol	1	(Anderson et al. 2010)	ND	ND	<1.2
17-beta estradiol	1	(Anderson et al. 2010)	8.4	8.4	
Acetaminophen	1	(Anderson et al. 2010)	550	550	25.8
AHTN (tonalide)	2	(Trenholm et al. 2008)	250		
Amphetamine	2	Personal Communication	26		
Atenolol	1	(Anderson et al. 2010)	1800	1780	
Atorvastatin	1	(Anderson et al. 2010)	79	79	<20
Atrazine	1	CA Dept. Pesticide Regulation (2007)	83		19.3
Azithromycin	2	(Nelson et al. 2011)		337*	
Beclomethasone	5	(Kugathas and Sumpter 2011)			
Benzophenone	1	(Anderson et al. 2010)	120	120	
Bifenthrin	2	(Delgado-Moreno et al. 2011)	85		ND
Bisphenol A	1	(Anderson et al. 2010)	520	286	691
Butylated hydroxyanisole	2	(Trenholm et al. 2008)	3520		
Butylated hydroxytoluene	2	(Trenholm et al. 2008)	240		
Butylbenzyl phthalate	2	(Loraine and Pettigrove 2006)	1190		
Carbamazepine	1	(Anderson et al. 2010)	480	400	330
Chlorpyrifos	1	(Bailey et al. 2000)	190		
Ciprofloxacin	3	(Kolpin et al. 2002)	30		
Cis-androstenedione	1	(Kolodziej et al. 2003)	4.5		
Clarithromycin	3	(Spongberg and Witter 2008)	611		
DEET	1	(Anderson et al. 2010)	1700	1520	860
Diazepam	4	(Diamond et al. 2011)	660		6.1
Diazinon	2	(Bailey et al. 2000)	1500		NM
Di-n-butylphthalate	3	(Diamond et al. 2011)	900		
Diclofenac	1	(Anderson et al. 2010)	230	203	124
Dilantin	1	(Anderson et al. 2010)	220	217	291
Bis(2-ethylhexyl)phthalate (BEHP)	2	(Loraine and Pettigrove 2006)	5940		

Table E.1. Continued

Compound	Data Tier	Data Source	Max Value (ng/L)	90% Value (ng/L)	LARB River Study - Max (ng/L)
Drospirenone	5	(Zeilinger et al. 2009)			
Erythromycin	2	(Nelson et al. 2011)		110*	29.4
Estrone	1	(Anderson et al. 2010)	73	72	<2.5
Fenofibrate	3	(Rosal et al. 2010)	129	79*	
Fipronil	2	(Delgado-Moreno et al. 2011)	11		13.6
Fipronil desulfinyl	2	(Delgado-Moreno et al. 2011)	8		13.8
Fipronil sulfide	2	(Delgado-Moreno et al. 2011)	2.5		2
Fipronil sulfone	2	(Delgado-Moreno et al. 2011)	17.5		6
Fluorouracil	3	(Yu et al. 2006)	ND		
Fluoxetine (Prozac)	2	(Nelson et al. 2011)		22*	31
Furosemide	1	(Anderson et al. 2010)	63	63	
Galaxolide (HHCB)	2	(Snyder et al. 2007)	2780		
Gemfibrozil	1	(Anderson et al. 2010)	4300	3550	324
Hydrocodone	3	(Bisceglia et al. 2010)	68		
Ibuprofen	1	(Anderson et al. 2010)	1000	500	40.5
Iopromide	1	(Anderson et al. 2010)	2174	2174	
Levonorgestrel	4	(Vulliet et al. 2008)	7		
Meprobamate	1	(Anderson et al. 2010)	430	430	461
Metformin	4	(Scheurer et al. 2009)	1700		
Miconazole	4	(Huang et al. 2010)	3		
Naproxen	1	(Anderson et al. 2010)	860	851	<112
NP1EO	2	(Lavado et al. 2009b)	40		
NP2EO	2	(Lavado et al. 2009b)	240		
Octocrylene	4	(Balmer et al. 2005)	270		
Octylphenol	1	(Anderson et al. 2010)	210	207	
o-Hydroxy atorvastatin	1	(Anderson et al. 2010)	10	10	
Oxybenzone (benzophenone-3)	2	(Trenholm et al. 2008)	13		
PBDE -47	2	(North 2004)		10.5*	
PBDE -99	2	(North 2004)		11.2*	
Permethrin	2	(Weston and Lydy 2010)	45.8		
PFBA	4	(Moller et al. 2010)	335		9
PFDA	2	(Plumlee et al. 2008)	11		<1
PFDoA	2	(Quiñones and Snyder 2009)	1		<1
PFHxS	2	(Plumlee et al. 2008)	24		<1

Table E.1. Continued

Compound	Data Tier	Data Source	Max Value (ng/L)	90% Value (ng/L)	LARB River Study - Max (ng/L)
PFNA	2	(Plumlee et al. 2008)	32		<1
PFOA	1	(Anderson et al. 2010)	28	28	36.5
PFOS	1	(Anderson et al. 2010)	90	90	9.4
PFOSA	2	(Plumlee et al. 2008)	4.8		NM
PFUdA	2	(Quiñones and Snyder 2009)	ND		<1
p-Hydroxy atorvastatin	1	(Anderson et al. 2010)	10	10	
p-nonylphenol	1	(Anderson et al. 2010)	161	161	
Prednisolone	4	(Chang et al. 2007)	0.72	0.56*	<112
Progesterone	1	(Anderson et al. 2010)	18	18	2.3
Propranolol	1	(Anderson et al. 2010)	25	25	
Sertraline	4	(Metcalf et al. 2010)	16		
Sulfamethoxazole	1	(Anderson et al. 2010)	2100	1400	933
TCEP	1	(Anderson et al. 2010)	730	688	785
TCPP	1	(Anderson et al. 2010)	7200	5920	2899
Testosterone	1	(Anderson et al. 2010)	1	1	<0.62
Triamterene	5	No environmental data	NM	NM	NM
Triclocarban	1	(Anderson et al. 2010)	223	223	102
Triclosan	1	(Anderson et al. 2010)	510	485	26.3
Trimethoprim	1	(Anderson et al. 2010)	120	112	180
Ziprasidone	5	No environmental data	NM	NM	NM

Table E.2. Aqueous concentrations (ng/L) utilized in hazard calculations for WERF CEC5R8a (Diamond et al. 2011).

Compound	ng/L	Source of Data
17-alpha estradiol	74	Stream Max – Kolpin, D.W., et al. 2002. EST - Pharmaceuticals, hormones, and other organic waste contaminants in U.S. streams, 1999-2000: A national reconnaissance. Environ. Sci. Technol. 36:1202-1211.
17-beta estradiol	650	Kolodziej, E.P., et al. 2004. Dairy wastewater, aquaculture, and spawning fish as sources of steroid hormones in the aquatic environment. Environ. Sci. Technol. 38:3201-3206 as cited by Campbell, C.G., et al. 2006. Biologically directed environmental monitoring, fate, and transport of estrogenic endocrine disrupting compounds in water: A review. Chemosphere 65:1265-1280.
Acetaminophen	10,000	Stream Max - Kolpin, D.W., et al. 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance. Environ. Sci. Technol. 36:1202-1211.
AHTN (tonalide)	2300	Effluent Max - Sando, S.K., et al. 2005. Occurrence of organic wastewater compounds in wastewater effluent and the Big Sioux River in the Upper Big Sioux River Basin, South Dakota, 2003-2004. USGS Scientific Investigations Report 2005-5249, 108 p.
Amphetamine	NA	
Atenolol	960	Effluent Max - Batt, A.L., et al. 2008. Analysis of ecologically relevant pharmaceuticals in wastewater and surface water using selective solid-phase extraction and UPLC-MS/MS. Anal. Chem. 80:5021-5030.
Atorvastatin	42	Effluent Max - Batt, A.L., et al. 2008. Analysis of ecologically relevant pharmaceuticals in wastewater and surface water using selective solid-phase extraction and UPLC-MS/MS. Anal. Chem. 80:5021-5030.
Atrazine	25000	Water Max - Alvarez, D.A., et al. 2009. Reproductive health of bass in the Potomac, USA, drainage: Part 2. Seasonal occurrence of persistent and emerging organic contaminants. Environ. Toxicol. Chem. 28:1084-1095.
Azithromycin	1650	Stream Max - Loper, C.A., et al. 2007. Concentrations of selected pharmaceuticals and antibiotics in south-central Pennsylvania waters, March through September 2006. USGS Data Series 300, 101 p.
Beclomethasone	NA	
Benzophenone	220	Effluent Ave - Drewes, J.E., et al. 2009. Contributions of Household Chemicals to Sewage and Their Relevance to Municipal Wastewater Systems and the Environment. WERF Report 03-CTS-21UR, 180 p.
Bifenthrin	NA	
Bisphenol A	12000	Stream Max - Kolpin, D.W., et al. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. Streams, 1999-2000: A national reconnaissance. Environ. Sci. Technol. 36:1202-1211.

Table E.2. Continued

Compound	ng/L	Source of Data
Butylated hydroxyanisole	5000	Reporting Limit - Focazio, M.J., et al. 2008. A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States - II) Untreated Drinking Water Sources. <i>Sci. Tot. Environ.</i> 402:201-216.
Butylated hydroxytoluene	100	Stream Max - Kolpin, D.W., et al. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. Streams, 1999-2000: A national reconnaissance. <i>Environ. Sci. Technol.</i> 36:1202-1211.
Butylbenzyl phthalate	2060	Stream Max - King County. 2007. Survey of Endocrine Disruptors in King County Surface Waters. Prepared by Richard Jack and Deb Lester, Water and Land Resources Division. Seattle, Washington.
Carbamazepine	2300	Effluent Max - Metcalfe, C.D., et al. 2003. Occurrence of Neutral and Acidic Drugs in the Effluents of Canadian Sewage Treatment Plants. <i>Environ. Toxicol. Chem.</i> 22:2872-2880.
Chlorpyrifos	310	Stream Max - Kolpin, D.W., et al. 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance. <i>Environ. Sci. Technol.</i> 36:1202-1211.
Ciprofloxacin	182	Stream Max - Loper, C.A., et al. 2007. Concentrations of selected pharmaceuticals and antibiotics in south-central Pennsylvania waters, March through September 2006. <i>USGS Data Series 300</i> , 101 p.
Cis-androstenedione	NA	
Clarithromycin	3	Surface Water - Boyd R.A. and E.T. Furlong. 2002. Human-Health Pharmaceutical Compounds in Lake Mead, Nevada and Arizona, and Las Vegas Wash, Nevada, October 2000-August 2001. <i>USGS Open-File Report 02-385</i> , 24p.
DEET (N,N-diethyl-meta-toluamide)	1500	Effluent Max - Knepper, T.P. 2004. Analysis and fate of insect repellants. <i>Water Sci. Technol.</i> 50:301-308 as cited in Costanzo, S.D. et al. 2007. Is there a risk associated with the insect repellent DEET (N,N-diethyl-m-toluamide) commonly found in aquatic environments? <i>Sci. Tot. Environ.</i> 384:214-220.
Diazepam	660	Effluent - van der Ven, K. 2004. Determination of diazepam in aquatic samples by capillary liquid chromatography-electrospray tandem mass spectrometry. <i>Chemosphere</i> 57:967-973 as cited in Fent, K. et al. 2006. Review: Ecotoxicology of human pharmaceuticals. <i>Aquatic Toxicology</i> 76:122-159.
Diazinon	510	Source Water - Focazio, M.J., et al. 2008. A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States - II) Untreated Drinking Water Sources. <i>Sci. Tot. Environ.</i> 402:201-216.
Di-n-butylphthalate	900	Stream Max - King County. 2007. Survey of Endocrine Disruptors in King County Surface Waters. Prepared by Richard Jack and Deb Lester, Water and Land Resources Division. Seattle, Washington.

Table E.2. Continued

Compound	ng/L	Source of Data
Diclofenac	2500	Effluent - Germany - Heberer, T. 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: A review of recent research data. <i>Toxicol. Lett.</i> 131:5-17 as cited in Fent, K., et al. 2006. Review: Ecotoxicology of human pharmaceuticals. <i>Aquatic Toxicology</i> 76:122-159.
Dilantin	325	Water Max - Guo, Y. C. 2009. Occurrence, Fate and Transport of PPCPs in Three Drinking Water Sources in California. 2009 AWWA Research Symposium Presentation.
Bis(2-ethylhexyl) phthalate (BEHP)	NA	
Drospirenone	NA	
Erythromycin	5700	Effluent Max - Sando, S.K., et al. 2005. Occurrence of organic wastewater compounds in wastewater effluent and the Big Sioux River in the Upper Big Sioux River Basin, South Dakota, 2003-2004. USGS Scientific Investigations Report 2005-5249, 108 p.
Estrone	112	Stream Max - Kolpin, D.W., et al. 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance. <i>Environ. Sci. Technol.</i> 36:1202-1211.
Fenofibrate	400	Effluent Ave (Brazil) - Stumpf, M., et al. 1999. Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. <i>Sci. Total Environ.</i> 225:135-141 as cited in Fent, K. et al. 2006. Review: Ecotoxicology of human pharmaceuticals. <i>Aquatic Toxicology</i> 76:122-159.
Fipronil	NA	
Fipronil desulfinyl	NA	
Fipronil sulfide	NA	
Fipronil sulfone	NA	
Fluorouracil	NA	
Fluoxetine (Prozac)	73	Effluent Max - Batt, A.L., et al. 2008. Analysis of ecologically relevant pharmaceuticals in wastewater and surface water using selective solid-phase extraction and UPLC-MS/MS. <i>Anal. Chem.</i> 80:5021-5030.
Furosemide	930	Effluent Max - Batt, A.L., et al. 2008. Analysis of ecologically relevant pharmaceuticals in wastewater and surface water using selective solid-phase extraction and UPLC-MS/MS. <i>Anal. Chem.</i> 80:5021-5030.

Table E.2. Continued

Compound	ng/L	Source of Data
Galaxolide (HHCb)	970	Source Water - Focazio, M.J., et al. 2008. A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States - II) Untreated Drinking Water Sources. <i>Sci. Tot. Environ.</i> 402:201-216.
Gemfibrozil	1400	Water - Brun, G.L., et al. 2006. Pharmaceutically active compounds in Atlantic Canadian sewage treatment plant effluents and receiving waters, and potential for environmental effects as measured by acute and chronic aquatic toxicity. <i>Environ. Toxicol. Chem.</i> 25:2163-2176.
Hydrocodone	190	Effluent Max - Batt, A.L., et al. 2008. Analysis of ecologically relevant pharmaceuticals in wastewater and surface water using selective solid-phase extraction and UPLC-MS/MS. <i>Anal. Chem.</i> 80:5021-5030.
Ibuprofen	27256	Effluent (UK) - Ashton, D., et al. 2004. Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. <i>Sci. Tot. Environ.</i> 333:167-184.
Iopromide	NA	
Levonorgestrel	NA	
Meprobamate	73	Drinking Source Max - Benotti, M.J., et al. 2009. Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water. <i>Environ. Sci. Technol.</i> 43:597-603.
Metformin	150	Stream Max - Kolpin, D.W., et al. 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance. <i>Environ. Sci. Technol.</i> 36:1202-1211.
Miconazole	ND<18	Effluent Max - Sando, S.K., et al. 2005. Occurrence of organic wastewater compounds in wastewater effluent and the Big Sioux River in the Upper Big Sioux River Basin, South Dakota, 2003-2004. USGS Scientific Investigations Report 2005-5249, 108 p.
Naproxen	33900	Effluent Max - Metcalfe, C.D., et al. 2003. Occurrence of neutral and acidic drugs in the effluents of Canadian Sewage treatment plants. <i>Environ. Toxicol. Chem.</i> 22:2872-2880.
NP1EO	620	Effluent - Murphy, S.F., et al., eds. 2003. Comprehensive Water Quality of the Boulder Creek Watershed, Colorado, During High-Flow and Low-Flow Conditions, 2000. USGS Water-Resources Investigations Report 03-4045.
NP2EO	4900	Effluent - Murphy, S.F., et al., eds. 2003. Comprehensive Water Quality of the Boulder Creek Watershed, Colorado, During High-Flow and Low-Flow Conditions, 2000. USGS Water-Resources Investigations Report 03-4045.
Octocrylene	NA	
Octylphenol	NA	
o-Hydroxy atorvastatin	NA	
Oxybenzone (benzophenone-3)	40	Effluent Ave - Drewes, J.E., et al. 2009. Contributions of Household Chemicals to Sewage and Their Relevance to Municipal Wastewater Systems and the Environment. WERF Report 03-CTS-21UR, 180 p.

Table E.2. Continued

Compound	ng/L	Source of Data
PBDE -47	ND	Lee, K.E, et al. 2008. Occurrence of endocrine active compounds and biological responses in the Mississippi River - study design and data, June through August 2006. USGS Data Series 368, 27 p. with Appendix.
PBDE -99	ND	Lee, K.E, et al. 2008. Occurrence of endocrine active compounds and biological responses in the Mississippi River - study design and data, June through August 2006. USGS Data Series 368, 27 p. with Appendix.
cis-Permethrin	0.27	Water Max - Alvarez, D.A., et al. 2009. Reproductive Health of Bass in the Potomac, USA, Drainage: Part 2. Seasonal Occurrence of Persistent and Emerging Organic Contaminants. Environ. Toxicol. Chem. 28:1084-1095.
trans-Permethrin	NA	
PFBA	NA	
PFDA	NA	
PFDoA	NA	
PFHxS	NA	
PFNA	NA	
PFOA	NA	
PFOS	NA	
PFOSA	NA	
PFUdA	NA	
p-Hydroxy atorvastatin	NA	
p-nonylphenol	5000	Reporting Limit - Focazio, M.J., et al. 2008. A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States - II) Untreated Drinking Water Sources. Sci. Tot. Environ. 402:201-216.
Prednisolone	NA	
Progesterone	199	Stream Max - Kolpin, D.W., et al. 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance. Environ. Sci. Technol. 36:1202-1211.
Propranolol	304000	Effluent Median - Roberts, P.H. and K.V. Thomas. 2005. The occurrence of selected pharmaceuticals in wastewater effluent and surfacewaters of the lower Tyne catchment. Sci. Total Environ. as cited in Fent, K., et al. 2006. Review: Ecotoxicology of human pharmaceuticals. Aquatic Toxicology 76:122-159.
Sertraline	87	Effluent Max - Batt, A.L., et al. 2008. Analysis of ecologically relevant pharmaceuticals in wastewater and surface water using selective solid-phase extraction and UPLC-MS/MS. Anal. Chem. 80:5021-5030.
Sulfamethoxazole	1340	Stream Max - Loper, C.A., et al. 2007. Concentrations of selected pharmaceuticals and antibiotics in south-central Pennsylvania waters, March through September 2006. USGS Data Series 300, 101 p.

Table E.2. Continued

Compound	ng/L	Source of Data
TCEP	530	Drinking Source Max - Benotti, M.J. et al. 2009. Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water. <i>Environ. Sci. Technol.</i> 43:597-603.
TCPP	500	Water Max - Alvarez, D.A. et al. 2009. Reproductive Health of Bass in the Potomac, USA, Drainage: Part 2. Seasonal Occurrence of Persistent and Emerging Organic Contaminants. <i>Environ. Toxicol. Chem.</i> 28:1084-1095.
Testosterone	214	Stream Max - Kolpin, D.W., et al. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. <i>Environ. Sci. Technol.</i> 36:1202-1211.
Triamterene	440	Effluent Max - Batt, A.L., et al. 2008. Analysis of ecologically relevant pharmaceuticals in wastewater and surface water using selective solid-phase extraction and UPLC-MS/MS. <i>Anal. Chem.</i> 80:5021-5030.
Triclocarban	NA	
Triclosan	2300	Stream Max - Kolpin, D.W., et al. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. <i>Environ. Sci. Technol.</i> 36:1202-1211.
Trimethoprim	1288	Effluent (UK) - Ashton, D., et al. 2004. Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. <i>Sci. Tot. Environ.</i> 333:167-184.
Ziprasidone	NA	

Table E.3. Maximum aqueous concentrations (ng/L) in rain and stormwater.

Compound	SCCWRP Stormwater Max (ng/L)	SCCWRP Rainwater Max (ng/L)	Data Tier	Literature Max (ng/L)	Matrix	Reference
17-alpha estradiol			5			
17-beta estradiol			2	3*	Ag Runoff	(Pedersen et al. 2005)
Acetaminophen	153		5			
AHTN (tonalide)	<25		5			
Amphetamine			5			
Atenolol		<1	5			
Atorvastatin		<0.5	5			
Atrazine	10.5	<0.25	3	321800	Ag Runoff	(Southwick et al. 2003)
Azithromycin			5			
Beclomethasone			5			
Benzophenone		150	5			
Bifenthrin			2	29.8	Urban Runoff	(Weston and Lydy 2010)
Bisphenol A	14357	500	3	158	Urban Runoff	(Boyd et al. 2004)
Butylated hydroxyanisole	<1	<1	2	<300	Ag Runoff	(Pedersen et al. 2005).
Butylated hydroxytoluene			2	<2600	Ag Runoff	(Pedersen et al. 2005)
Butylbenzyl phthalate			4	330	Urban Runoff	(Clara et al. 2010)
Carbamazepine	5.6		2	440	Ag Runoff	(Pedersen et al. 2005)
Chlorpyrifos			2	220	Urban Runoff	(Pedersen et al. 2005)
Ciprofloxacin			5			
Cis-androstenedione			5			
Clarithromycin			5			
DEET	74.1	7.4	5			
Diazepam		<0.25	5			
Diazinon			2	37000	Ag Runoff	(Pedersen et al. 2005)

Table E.3. Continued

Compound	SCCWRP Stormwater Max (ng/L)	SCCWRP Rainwater Max (ng/L)	Data Tier	Literature Max (ng/L)	Matrix	Reference
Di-n-butylphthalate			5			
Diclofenac	814	<0.5	4	270	Urban Runoff	(Clara et al. 2010)
Dilantin	20.2		5			
Bis(2-ethylhexyl) phthalate (BEHP)			4	24000	Urban Runoff	(Clara et al. 2010)
Drospirenone			5			
Erythromycin	<5		5			
Estrone			4	52*	Ag Runoff	(Pedersen et al. 2005)
Fenofibrate			2	<730	Ag Runoff	(Pedersen et al. 2005)
Fipronil			2	25	Ag Runoff	(Delgado-Moreno et al. 2011)
Fipronil desulfinyl			2	10	Ag Runoff	(Delgado-Moreno et al. 2011)
Fipronil sulfide			2	7.5	Ag Runoff	(Delgado-Moreno et al. 2011)
Fipronil sulfone			2	18	Ag Runoff	(Delgado-Moreno et al. 2011)
Fluorouracil			5			
Fluoxetine (Prozac)	<2	<0.5	5			
Furosemide			5			
Galaxolide (HHCB)			5			
Gemfibrozil	16.9	<0.25	2	790	Ag Runoff	(Pedersen et al. 2005)
Hydrocodone			5			
Ibuprofen	339	<1	2	11	Ag Runoff	(Pedersen et al. 2005)
Iopromide			5			
Levonorgestrel			5			
Meprobamate	1.9	<0.25	5			
Metformin			5			
Miconazole			5			
Naproxen	24.2	<0.5	2	145	Urban Runoff	(Boyd et al. 2004)
NP1EO			5			
NP2EO			5			

Table E.3. Continued

Compound	SCCWRP Stormwater Max (ng/L)	SCCWRP Rainwater Max (ng/L)	Data Tier	Literature Max (ng/L)	Matrix	Reference
Octocrylene			5			
Octylphenol		<25	4	59	Urban Runoff	(Bressy et al. 2011)
o-Hydroxy atorvastatin			5			
Oxybenzone (benzophenone-3)			5			
Permethrin			1100*	2	Ag Runoff	(Delgado-Moreno et al. 2011)
PFBA	49	NM	1			
PFDA	6	<1	1			
PFDoA	1.4	<1	1			
PFHxS	6.4	<1	1			
PFNA	4.9	<1	1			
PFOA	205	1.1	1			
PFOS	48	<1	1			
PFOSA			5			
PFUdA	1	<1	1			
p-Hydroxy atorvastatin			5			
p-nonylphenol			4	920	Urban Runoff	(Bressy et al. 2011)
Prednisolone			5			
Progesterone			2	3*	Ag Runoff	(Pedersen et al. 2005)
Propranolol			5			
Sertraline			5			
Sulfamethoxazole	304	<0.25	1			
TCEP	160	25	1			
TCPP	440	<100	1			
Testosterone	<2		2	16*	Ag Runoff	(Pedersen et al. 2005)
Triamterene			5			
Triclocarban	<5		5			
Triclosan	110	2.1	3	29	Urban Runoff	(Boyd et al. 2004)
Trimethoprim	5.8	<0.5	1			
Ziprasidone	NM		5			

Table E.4. Maximum aqueous concentrations (ng/L) in treated municipal wastewater effluent discharged to coastal ocean, receiving ocean and San Francisco Bay water and from the literature.

Compound	Ocean Outfall ng/L	Ocean Water ng/L	SF Bay ng/L		Tier	Literature ng/L	Reference
17-alpha estradiol	NM	NM			5	NM	
17-beta estradiol	30	ND <0.5			4	1.8	(Saravanabhavan et al. 2009)
Acetaminophen	11000	11			3	EFF	(Benotti and Brownawell 2007)
AHTN (tonalide)	2700	NM			4	EFF	(Sumner et al. 2010)
Amphetamine		NM	10		5	NM	
Atenolol	3140	11	37		5	NM	
Atorvastatin	150	0.4			5	NM	
Atrazine	20	ND <2.5			3	32.3	(Alegria and Shaw 1999)
Azithromycin		NM			5	NM	
Beclomethasone		NM			5	NM	
Benzophenone	2700	57			5	NM	
Bisphenol A	1600	ND <50			4	47	(Rocha et al. 2011)
Butylated hydroxyanisole	230	ND <25			5	NM	
Butylated hydroxytoluene	840	170			5	NM	
Butylbenzyl phthalate	1500	ND <50			4	0.35	(Xie et al. 2007)
Carbamazepine	360	1	44		3	6.3	(Benotti and Brownawell 2007)
Chlorpyrifos		NM			5	NM	
Ciprofloxacin		NM			5	NM	
Cis-androstenedione		NM			5	NM	
Clarithromycin		NM	18				
DEET	1970	ND <2.5	21		5	NM	
Diazepam		NM	0.5		5	NM	
Diazinon		NM			5	NM	
Diclofenac		ND <2.5					
Dilantin		ND <10					
Bis(2-ethylhexyl) phthalate(BEHP)	1420	85					
Drospirenone		NM					
Erythromycin		NM	12				
Estrone	120	0.3			4	1.5	(Saravanabhavan et al. 2009)
Fenofibrate		NM					
Fipronil		NM					
Fipronil desulfinyl		NM					

Table E.4. Continued

Compound	Ocean Outfall ng/L	Ocean Water ng/L	SF Bay ng/L		Tier	Literature ng/L	Reference
Fipronil sulfide		NM					
Fipronil sulfone		NM					
Fluorouracil		NM					
Fluoxetine (Prozac)		NM					
Furosemide		NM					
Galaxolide (HHCB)		ND <2500					
Gemfibrozil	3800	13	38				
Hydrocodone	110	NM	7				
Ibuprofen	12000	30	38				
Iopromide		ND <500					
Levonorgestrel		NM					
Meprobamate	570	2	36				
Metformin		NM					
Miconazole		NM					
Naproxen	13100	26	8				
NP1EO		NM			4	264	(Rocha et al. 2011)
NP2EO		NM			4	1756	(Rocha et al. 2011)
Octocrylene		NM					
Octylphenol	1550	42			4	20	(Rocha et al. 2011)
o-Hydroxy atorvastatin	170	ND <5					
Oxybenzone (benzophenone-3)	3600	9					
PBDE -47		NM					
PBDE -99		NM					
cis-Permethrin		NM					
trans-Permethrin		NM					
PFBA		NM					
PFDA		NM					
PFDoA		NM					
PFHxS		NM					
PFNA		NM					
PFOA		NM					
PFOS		NM					
PFOSA		NM					
PFUdA		NM					

Table E.4. Continued

Compound	Ocean Outfall ng/L	Ocean Water ng/L	SF Bay ng/L		Tier	Literature ng/L	Reference
p-Hydroxy atorvastatin	190	ND <5					
p-nonylphenol	7200	230	73		4	12	(Rocha et al. 2011)
Prednisolone		NM					
Progesterone	50	ND <0.5					
Propranolol		NM					
Sertraline		NM					
Sulfamethoxazole	2040	3	67				
TCEP	1700	ND <50					
TCCP	2700	56					
Testosterone	90	ND <0.5					
Triamterene		NM	10				
Triclocarban		NM					
Triclosan	1500	6					
Trimethoprim	980	2	4				
Ziprasidone		NM					

APPENDIX F - MONITORING FOR ANTIBIOTIC RESISTANCE

How do we effectively monitor for antibiotic resistance in a phased monitoring program?

Given the uncertainty associated with the MTQ screening levels developed for ABR (e.g., mixture effects) in bacteria and antibiotics (chemical exposure and gene transfer potential), it is recommended that the levels of ABR in *E. coli* or other suitable water quality indicator bacteria be investigated by establishing baseline conditions for effluents and sediments at several WWTP outfalls as an initial starting point for hazard characterizations. The rationale for this approach is that wastewater treatment processes may select for bacteria that are most resistant to antibiotics. ABR determinations should be started in a phased approach, beginning initially with WWTP effluent and then subsequently expanded to sediments at the outfall, based upon results obtained (e.g., high levels of ABR in effluent may warrant additional sediment sampling at the outfall).

Figure F.1 depicts results from Uyaguari et al. (2011) who assessed the levels of bacterial antibiotic resistance genes (bla_{M-1}) and gene "survival" in moving through different stages of a secondary waste treatment plant. In the top figure (A) note there is a >95% reduction in the amount of this antibiotic resistant gene present in the final effluent indicating that the different stages of waste treatment effectively reduced the overall total amount of this gene. However, in examining the lower figure (B) note that the amount of this resistant gene material per ng of DNA is much higher in the final effluent than in the raw effluent or activated sludge. This indicates that while the overall amount of total bacterial DNA for this gene is reduced by waste treatment, the levels of this resistant gene for bacteria surviving waste treatment are much higher in the final effluent and is thus highly enriched in this antibiotic resistant gene. Thus sampling final wastewater effluent may provide the most effective mechanism for testing for antibiotic resistance since bacteria there are potentially enriched in these antibiotic resistant genes. While the results cited by Uyaguari et al. (2011) provide evidence for selecting final effluent as an appropriate initial monitoring focus for ABR, it is noteworthy to mention that the results obtained were for one WWTP and may not necessarily be the same in all WWTPs. In addition, another consideration for selecting final effluent is that the final effluent is routinely tested for indicator bacteria as part of the routine monitoring at waste water treatment plants. Thus adding ABR analysis on the final effluent would seem a logical focus for these efforts.

We propose a method that can take advantage of this routine bacterial monitoring by adding an additional custom panel for antibiotics that can be used to screen for ABR. NOAA has developed a custom panel that analyzes for ABR for 26 different antibiotics using 3 different levels for each antibiotic (10% MIC, 100% MIC and 200% MIC Concentrations) (Figure F.2). This combination of doses provides not only a determination of which antibiotics have resistance but it also provides an overall quantitative assessment of the strength of the resistance for each antibiotic. This panel has been effectively used with *E. coli* isolated from positive fecal coliform samples taken for compliance monitoring purposes, grown on selective media plates for *E. coli*. Random colonies are picked from each plate and analyzed for growth in the presence of each antibiotic. Colonies growing at or >MIC values are considered ABR. This NOAA ABR panel or another similar customized ABR panel [e.g., Siemens - Dade Behring's Microscan System, Becton-Dickinson (Phoenix) and bioMerieux (VITEK)] should be used to screen for ABR in final effluent. Also, since these panels can be custom made, it may be possible to design panels specifically for individual wastewater treatment plants based upon initial monitoring results. We further recommend that SCCWRP work NOAA and other federal and state agencies and academia to develop a standardized ABR Panel Protocol that can be adopted by use by participating WWTPs dischargers. Prior to implementation in monitoring, this new ABR Panel should be tested by on split samples by multiple laboratories to assess reproducibility and consistency of results reported. This will assure the

effectiveness and utility of this method prior to implementation in pilot monitoring. Using this approach, antibiotic compounds with High or Moderate potential for development of ABR would be identified as *Antibiotics of Concern* and further tested through our tiered monitoring approach for analytical chemistry and microbiology. For example, it may also be possible by filtering the final effluent samples (0.22 micron filter) it would be possible to evaluate ABR potential in whole effluent (antibiotics and gene transfer), filtered material washed off the filters (primarily gene transfer potential) and the filtrate (dissolved concentrations of antibiotics). This may allow determination of whether or not a microbial (gene transfer components) or analytical chemistry (dissolved antibiotics) or a combination of microbial and analytical chemistry approach is warranted.

For multiple antibiotic resistance using the *E. coli* ABR Panel approach developed by NOAA for some 26 different antibiotics or an equivalent approach (Uyaguari et al. 2009), the following scheme is proposed based on ABR ranking using the following criteria:

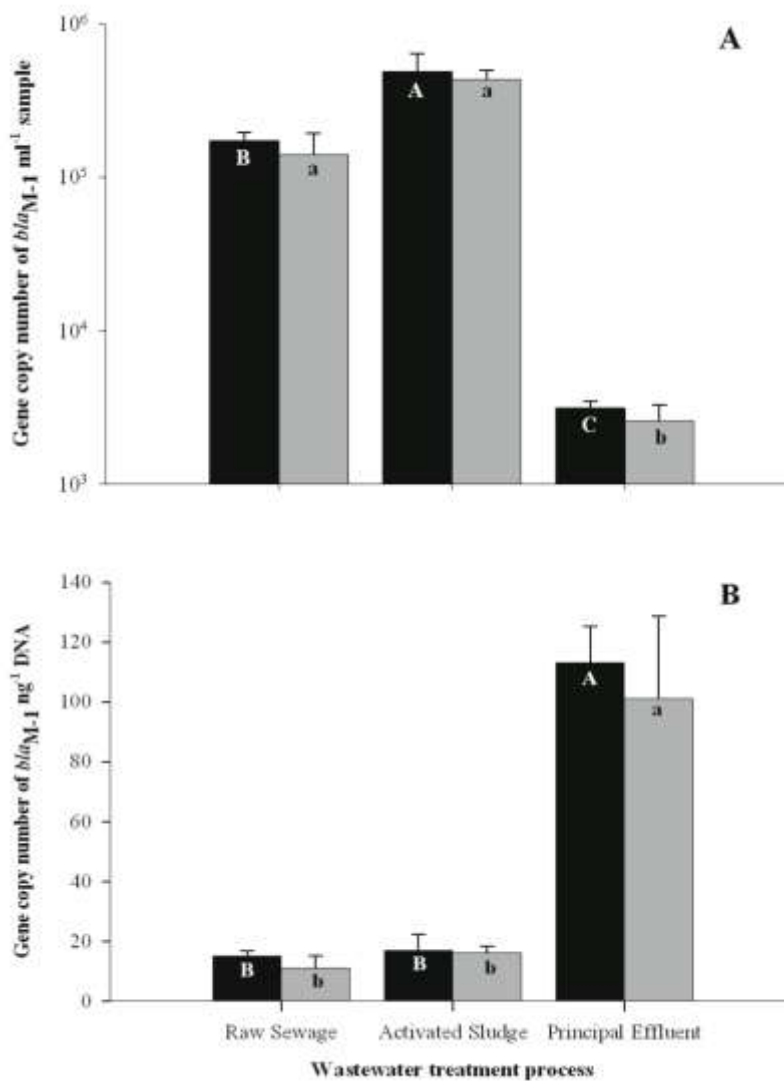


Figure F.1. Differential survival of antibiotic resistant genes (*bla_{M-1}*) in a secondary wastewater treatment plant (Uyaguari et al. 2011).

26 Antibiotics Used in ABR Panel

- Amikacin
- Amoxicillin
- Ampicillin
- Apramycin
- Azithromycin
- Cefoxitin
- Ceftriaxone
- Cephalothin
- Chloramphenicol
- Ciprofloxacin
- Erythromycin
- Gentamicin
- Imipenem
- Meropenem
- Moxifloxacin
- Nalidixic Acid
- Nitrofurantoin
- Ofloxacin
- Oxytetracycline
- Penicillin
- Streptomycin
- Sulfathiazole
- Tetracycline
- Trimethoprim
- Trimethoprim/Sulfamethoxazole



26 different antibiotics—determine MIC value for each

Figure F.2. Custom antibiotic resistance (ABR) panel developed by NOAA.

I. The number of antibiotics to which resistance is measured using the following ranking scheme:

- (1) ABRs detected is ≤ 1 Antibiotic = Low Potential to Develop ABR
- (2) MARs detected is > 1 antibiotic but ≤ 3 antibiotics = Moderate Potential to Develop ABR
- (3) MARs detected is > 3 antibiotics = High Potential to Develop ABR

II. The strength of the resistance for each antibiotic with resistance would be ranked according to the results of the rate of resistance measured for each antibiotic in the bioassay using the following ranking scheme:

- (1) $< 100\%$ of the MIC = Low Rate of Resistance
- (2) $> 100\%$ but $< 200\%$ of the MIC = Moderate Rate of Resistance
- (3) $> 200\%$ of the MIC = High Rate of Resistance.

How do we link up ABR microbial assessment endpoints with analytical chemistry monitoring for antibiotics and other pharmaceuticals to better discern multiple pathways for development of ABR?

Triclosan, one of the CECs listed for monitoring in Table 8.1, is a good indicator chemical for ABR assessments as it has been measured in California waters, sediments and biota. Also it would be possible to add this compound to the custom ABR panels developed for this monitoring program. If indeed hits are obtained for triclosan in most microbial and chemical monitoring programs, this may suggest that antibiotics determined using microbial screens may need to be considered and added to future chemical

monitoring assessments. On the other hand, if no antibiotic levels are determined in chemical monitoring programs, yet ABR is observed in effluent, this may suggest that ABR is being driven not by chemical exposure *per se* but possibly by the ABR gene elements that cause resistance shed by humans. The differential ABR testing of filtered final effluent [filtered material (gene elements), filtrate (dissolved antibiotics and whole unfiltered final effluent (both gene elements and dissolved antibiotics)] describe above provides a potential mechanism to enhance our insight on the need to move from ABR testing to analytical chemistry. This knowledge of ABR characteristics within the final effluent will provide greater insight into the need for analytical chemistry monitoring and may aid in the selection of analytical chemistry methods to best employ. When ABR results suggest that gene elements may be driving ABR levels in the final effluent, additional molecular based methods may need to be employed which will require further research and analysis which is beyond the scope of the current pilot monitoring effort.

What new gene or molecular tools are needed?

For those antibiotics/antibacterial agents identified as causing multiple antibiotic resistance, it will be important to investigate and assess the potential for gene transfer using appropriate molecular methodologies such as the bla_{M-1} gene. The State of California and its collaborating entities are encouraged to continue to work with EPA, NOAA and other federal and state agencies as well as academia in developing a process for developing these molecular tools for future assessments. One example of a current technology that could be explored for ABR potential is the MUTATOX Assay, which utilizes *Vibrio fischeri*, a marine bacterium, to assess the ability of compounds to mutate DNA (e.g. develop resistance). It is an established referenced assay that could be considered for use. This may be very appropriate given the current levels of increased *Vibrio* resistance reported in the literature (Dr. Rita Colwell, 2012. University of Maryland: Personnel Communications; Baker-Austin et al. 2008, 2009).