Draft Final Report

Monitoring Strategies for Constituents of Emerging Concern (CECs) in Recycled Water

Recommendations of a Science Advisory Panel

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Table of Contents

Acknowledgments ..... **v**

Executive Summary ..... **vi**

Acronyms and Symbols ..... **xiii**

List of Figures ..... **xvii**

List of Tables ..... **xviii**

1. Introduction and Panel Charge **1**

1.1 Background 1

1.2 The Science Advisory Panel 1

1.3 Charge to the Science Advisory Panel 2

1.4 Organization of the Report 3

2. Regulatory Practices for Water Recycling in California **4**

2.1 The State Water Board's Recycled Water Policy 4

2.2 Regulatory Developments for Recycled Water Applications 5

2.2.1 CEC monitoring requirements 6

2.3 Improving Regulatory Practices for CEC Monitoring and Assessment 8

3. Assessing Risk to Human Health **14**

3.1 Recycled Water Applications in California 15

3.1.1 Non-potable water reuse 15

3.1.2 Planned potable water reuse 17

3.2 Assessing Relative Risk to Human Health for Non-Potable Reuse Scenarios 19

3.2.1 Exposure pathways and assumptions 20

3.2.2 Summary of exposure pathways for non-potable reuse scenarios 24

3.3 Human Health Considerations for Potable Reuse Scenarios 25

3.3.1 Planned potable reuse criteria for groundwater replenishment 25

3.3.2 Planned potable reuse criteria for surface water augmentation 28

4. Assessment of CEC Monitoring Programs in California following Recommendations of the CEC Expert Panel 2010 ..... **31**

4.1 Summary of Current Status of Monitoring Program 31

4.2 Measured Environmental Concentrations (MECs) 31

4.2.1 Data sources 31

4.2.2 Comparison of MECs in the 2010 and 2018 Panel reports 32

4.3 Monitoring trigger levels (MTLs) 34

4.3.1 Non-potable reuse applications 34

4.3.2 Updated list of Predicted No Effect Concentrations (PNECs) and MTLs 34

4.4 Updated MEC/MTL Analysis to Identify Health-Based Indicator CECs 39
4.5. Summary of 2018 MEC/MTL Ratio Update 40
5. Increasing Efficiency and Relevance of CEC Monitoring in Recycled Water 41
5.1 Introduction 41
5.2 Timely Periodic Update of the Panel's Risk-Based Framework 41
5.3 New Strategies for Broader Screening of Relevant CECs in Recycled Water 44
5.3.1 Consideration of screening studies of CECs reported outside California 44
5.3.2 Bioanalytical monitoring methods 44
5.3.3 Non-target analysis 45
5.4 Revised Risk-Based Framework for CEC Monitoring 45
5.4.1 Screening of unmonitored CECs 45
5.4.2 Revised monitoring of performance-based indicator CECs and surrogate parameters 46
5.5 Relevance of Safety Factors 48
5.6 Summary 48
6. Monitoring of CECs using Analytical Chemistry 50
6.1 Introduction 50
6.2 Extraction Issues 50
6.3 Targeted Analyses 51
6.3.1 Advances in environmental chemical analysis 51
6.3.2. Quality assurance and quality control 52
6.4 Non-Targeted Analyses (NTA) 58
6.4.1 Introduction 58
6.4.2 Recent developments and applications of NTA 59
6.4.3 Conclusions regarding the use of NTA for CEC monitoring 62
7. Role of Bioanalytical Methods to Assess the Relevance of Unknown CECs 64
7.1 What Are Bioanalytical Tools and How Can They Help? 64
7.2 Bioanalytical Methods are Standardized for Applications Worldwide 66
7.3 Interpreting Bioanalytical Results 68
7.3.1 What do in vitro assays measure exactly? 68
7.3.2 How are samples extracted for bioscreening analyses? 69
7.4 Regulatory Concerns 70
7.5 Panel Recommendations for Implementation of Bioanalytical Tools 71
7.5.1 Which in vitro assays are ready for screening of recycled water quality? 72
7.5.2 Recommendations for standardized protocols for in vitro assays 74

7.5.3 Decision making logic for bioanalytical screening results 76
7.5.4 Recommendations for monitoring of potable reuse projects 76
7.5.5 Guidance for implementation of bioanalytical monitoring 77
7.6 Challenges for Applying Bioanalytical Tools and Next Steps 77
7.6.1 Toolbox development and standardization 77
7.6.2 Linkage of in vitro responses to in vivo effects 78
7.6.3 Effect of mixtures on assay performance 78
7.7 Conclusions 78
8. Importance of Antibiotic Resistance in Water Recycling 79
8.1 Introduction 79
8.2 Occurrence and Treatment Efficiencies for Removal of ARB/ARGs in Wastewater 80
8.3 Occurrence, Fate and Transport of ARB/ARGs in the Three Major Categories of Recycled Municipal Wastewater 80
8.3.1 Agricultural irrigation 80
8.3.2 Landscape irrigation 84
8.3.3 Groundwater recharge 86
8.3.4 Fate and transport of ARB/ARGs during advanced wastewater treatment processes 88
8.4 Recommendations from Recent Scientific Conferences on Antibiotic Resistance in the Environment 92
8.5 Summary 93
8.6 Research Recommendations 93
9. Recommendations for CEC Monitoring Programs for Water Reuse Practices in California 96
9.1 Panel Charge 96
9.2 Need for CEC Monitoring for Non-Potable Reuse Practices and Surface Water Augmentation Projects 96
9.3 Relevance of Antibiotic Resistance to Recycled Water 97
9.4 Updated 2018 CEC Monitoring Recommendations for Potable Reuse Practices 97
9.5 Administrative Adjustments to Improve the State Water Board's CEC Monitoring Program 98
10. References 101
Appendix A – Biographies of Panel and Stakeholder Advisory Group Members 110
Appendix B – Monitoring Program and Suggested Response(s) for Indirect Potable Reuse Projects (adopted from Anderson et al., 2010) 119
Appendix C – Water Reuse Practices and Public Health Considerations 123
Appendix D – Updates to the Master List of CECs Considered by the 2018 Expert Panel . 126

Appendix E – Importance of Antibiotic Resistance in Water Recycling 147
Appendix F – Decision Tree for Bioanalytical Measurements 150

Acknowledgments

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Executive Summary

With its large population and regionally arid climate, the State of California has a long history of water reclamation and reuse. Now faced with an ever-increasing demand for water as well as diminishing new sources, water reclamation, recycling, and reuse are integral components of water resource planning and management. As evidenced by adoption of the Policy for Water Quality Control for Recycled Water (Recycled Water Policy) in 2009, recycled water is and will continue to be an important water resource across the State. Maintaining a water quality that is protective of both human health and the environment is paramount to the success of the Policy. The current report addresses public health protection, which requires that microbiological pathogens and some chemicals in municipal wastewater (the "source" of recycled water) be attenuated before discharge to the environment. The chemical universe is evolving at a rate that is challenging for traditional risk assessment paradigms, particularly evaluating chemical interactions between complex mixtures of CECs and transformation products formed during treatment and environmental processes. In order to remain vigilant in comprehensive evaluation of CECs, more modern water quality characterization tools -- both analytical and bioanalytical -- that may not yet be fully standardized or validated will be needed. *Thus, water recycling practices require appropriate treatment barriers and monitoring strategies to minimize exposure to a wide range of constituents of emerging concern (CECs) that may be harmful to human health*.

Expanding the Charge to the Science Advisory Panel

In their Policy, the California State Water Resources Control Board (State Water Board) sought to incorporate the most current scientific knowledge on CECs. In response, a Science Advisory Panel was formed in 2009 to address a series of questions.

- What are the appropriate constituents to be monitored in recycled water and what are the applicable monitoring methods and detection limits?
- What human-relevant toxicological information is available for these constituents?
- Would the constituent list change based on the level of treatment? If so, how?
- What are the possible indicators (i.e. surrogates) that represent a suite of CECs?
- What levels of CEC should trigger enhanced monitoring in recycled water, groundwater, or surface water?

The 2010 Panel produced several products to guide the State Water Board's approach to managing CECs in recycled water. First, the Panel developed a risk-based framework for prioritizing and selecting CECs for recycled water monitoring programs (Anderson et al., 2010). The framework was then used to develop a list of monitoring parameters, including four health-relevant and four performance-based ("indicator") CECs to demonstrate a consistent capacity for reduction of CECs by recycled water treatment processes. This initial list of eight CECs, representing multiple source classes (e.g., pharmaceuticals, personal care products, food additives, and hormones), were identified for groundwater recharge (GWR) potable reuse applications. In contrast, surrogate parameters (i.e., turbidity, chlorine residual, and total coliform bacteria) were deemed sufficient for monitoring of non-potable recycled water quality used for landscape irrigation. In addition, the Panel highlighted the need for new monitoring methods, including bioanalytical tools, and developed guidance for interpreting and responding to monitoring results.

As also specified in the Policy, periodic updates to CEC monitoring recommendations are needed to keep the data collected relevant and to incorporate new scientific information. The 2018 Panel was thus charged to update their recommendations from 2010, and to expand their recommendations to include surface water augmentation (SWA) and all non-potable reuse applications in the State of California allowed under Title 22. The Panel was further instructed to evaluate potential risks for all routes of exposure, except potential exposures associated with consumption of crops irrigated with recycled water, but to limit their deliberations to impacts on human (and not ecological) health. Lastly, the Panel was asked to comment on the state-of-the-science regarding the likelihood of human health impacts posed by antibiotic resistant bacteria/antibiotic resistance genes (ARB/ARGs) in recycled water.

Updating the List of CECs and other Monitoring Parameters

For indirect potable water reuse practices (i.e. groundwater recharge, GWR and surface water augmentation, SWA)[1], the Panel updated monitoring trigger levels (MTLs) based on toxicological information gathered from several new sources, including state, federal, industry and international organizations, as well as based on the Panel's own professional judgment. Regarding the selection of specific MTLs, the Panel made minor modifications to the process developed by the 2010 Panel. Greatest priority continues to be assigned to drinking water thresholds developed by the State of California followed by USEPA. *The result of this update was a revised set of MTLs, some higher and some lower than MTLs used in 2010, and others included for the first time.*

In response to the expanded charge to evaluate all non-potable use Title 22 scenarios, the 2018 Panel developed an approach that relies on comparing the exposure to CECs in recycled water for non-potable Title 22 reuse scenarios to exposure to CECs in water produced for potable reuse; considered a conservative assumption because treatment levels at the point of application are similar to those for most non-potable uses. In addition to ingestion of potable recycled water, incidental (i.e. non-intentional) exposure via several other pathways (e.g., absorption through skin, inhalation) was considered for all non-potable Title 22 applications. *This comparison revealed that potential exposures and potential human health risks associated with CECs in non-potable use scenarios are expected to be 10% or lower than exposure to CECs in water intentionally consumed in the potable reuse scenario*.

The Panel also updated measured environmental concentrations (MECs) based on more recent data collected by water reuse facilities in California. The Panel retained its conservative

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[1] On October 6, 2017 the Governor of California approved an act to amend Sections 13560 and 13561 of, to amend the heading of Chapter 7.3 (commencing with Section 13560) of Division 7 of, and to add Sections 13560.5 and 13561.2 to, the Water Code, relating to water. As noted below, the amended Section 13561 in part modifies the following definitions related to indirect potable reuse type projects. However, for the purpose of the CEC 2018 Panel update and consistency with the 2010 CEC Panel report the Panel elected to rely on the previous Water Code definitions.

> *(c) "Indirect potable reuse for groundwater recharge" means the planned use of recycled water for replenishment of a groundwater basin or an aquifer that has been designated as a source of water supply for a public water system, as defined in Section 116275 of the Health and Safety Code.*
>
> *(d) "Reservoir water augmentation" means the planned placement of recycled water into a raw surface water reservoir used as a source of domestic drinking water supply for a public water system, as defined in Section 116275 of the Health and Safety Code, or into a constructed system conveying water to such a reservoir.*

assumption of considering MECs for CECs measured in secondary/tertiary effluent as feed water for recycled water facilities. In addition, the Panel reviewed available monitoring data for individual treatment processes and product water for GWR applications as well as some select CEC monitoring studies outside of California. Because of wide variation in analytes reported, frequency of monitoring, and time period and duration of monitoring, the 2018 Panel compiled and reported 90th percentile concentration values to retain the conservatism established by the 2010 Panel.

The updated MECs and MTLs were employed to screen a total of 489 CECs (increased from 418 in 2010) using the same screening framework used by the 2010 Panel to identify candidate compounds for monitoring (Figure ES.1). This exercise indicated that regular monitoring of three of four 2010 health-based indicator CECs (17β-estradiol, triclosan and caffeine) is no longer necessary, as the monitoring data set collected over the past several years (2008-2017) indicate that concentrations are consistently below MTLs (i.e., the MEC/MTL ratio is less than 1). In contrast, the collected monitoring data indicated that concentrations of NDMA were eight times higher than the MTL and, therefore, *NDMA should be retained as a human health-based indicator*. Of the remaining CECs screened, the 90th percentile MECs for two compounds, *N-Nitrosomorpholine (NMOR) and 1,4-dioxane*, exceed their respective MTLs by factors of 9 and 7, respectively, thus warranting their addition as human health indicators. Table ES.1 summarizes the updated 2018 health-based and performance-based indicators for CECs and performance surrogates.

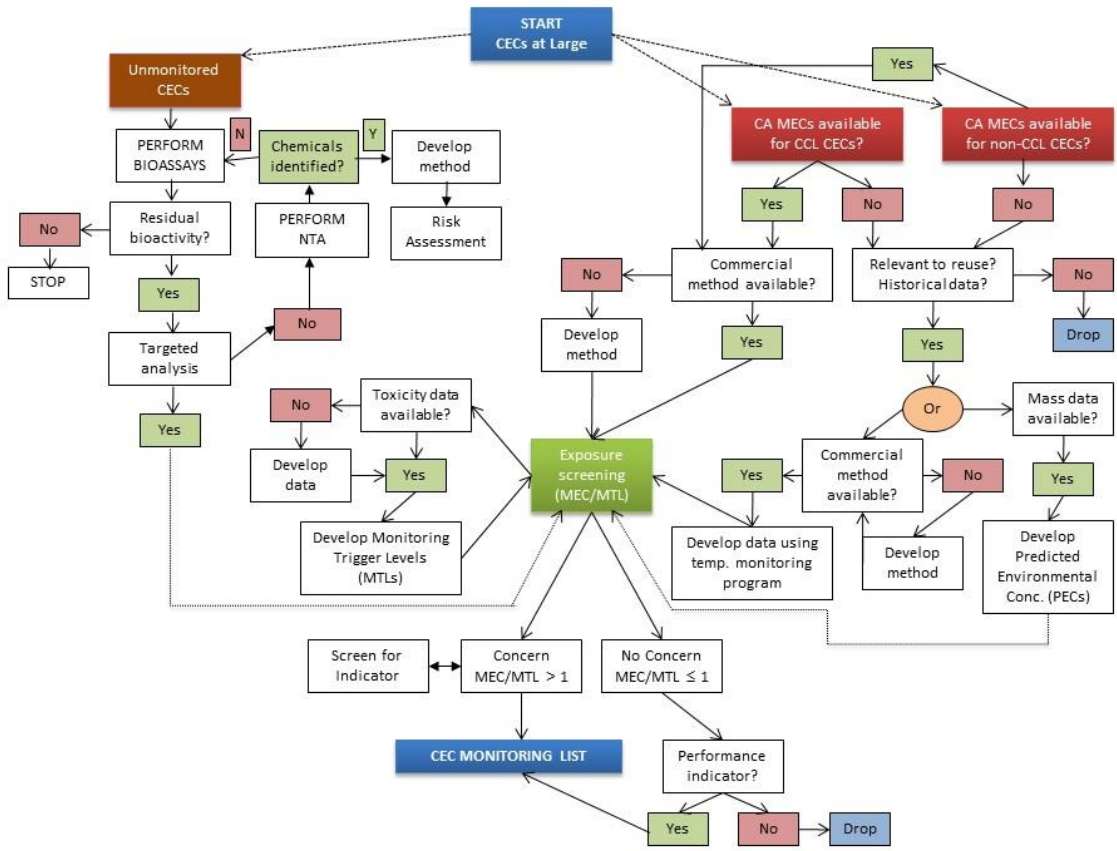

Figure ES.1. Revised risk-based CEC selection framework.

The Panel reiterates that the MEC/MTL ratio employed in the risk-based, screening framework is operationally defined, and should not be compared to (or confused with) regulatory criteria (i.e. enforceable maximum contaminant levels or MCLs). Furthermore, a large margin of safety is incorporated into this framework. Therefore, a MEC/MTL ratio of greater than 1 does not represent an immediate threat to public health. *With this in mind, the very small percentage of CECs that are recommended for health-based monitoring (3 of 489 or < 1%) reinforces the inherent low potential risk of CECs in recycled water to human health currently attributable to water reuse applications that include most Title 22 uses and potable reuse surface water augmentation under current regulatory practices*.

Improving the State Water Board's CEC Monitoring Program

Bioanalytical screening tools and non-targeted analysis. While the Panel's risk-based framework is clearly effective in identifying CECs for which pertinent data are available, the framework cannot capture all possible new compounds that may be entering the market, nor does it adequately address their transformation products. To help identify such compounds that may occur in recycled water and their potential, if any, to affect human health, the Panel believes that bioanalytical screening methods are a critically important tool whose value and applicability needs to be explored over the next few years in a series of special studies (see Figure ES.1). *The Panel recommends that the Estrogen Receptor alpha (ER-α) and the Aryl hydrocarbon Receptor (AhR) bioassays be used to respectively assess estrogenic and dioxin-like biological activities in recycled water*. These two *in vitro* bioassays were selected because each have clear adverse outcome pathways that allow specific molecular responses to be adequately standardized for screening recycled water quality at potable reuse projects.

Relevance of antibiotic resistance to recycled water. While antibiotic resistance is still a major challenge and potentially an issue for any wastewater discharge into the environment, information to date is not complete and seems to indicate that the causes for antibiotic resistance are still not well known and the current studies do not show that antibiotic resistance transmission is a consequence of water reuse practices considered in this report. The lack of standardized methods for investigating the occurrence and removal of, and risks associated with, ARB and ARGs hinders the assessment of the severity of ARB and ARGs as an issue for potable water reuse applications in California. Focused investigations are needed to better understand the occurrence, fate and risks associated with ARB and ARGs in recycled water applications across California. The State Water Board should encourage the collection of data in reclaimed water and sites within California while keeping abreast of scientific advances related to methods and risk assessment.

Increasing communication, efficiency and responsiveness. While the key recommendations from the 2010 Panel report were clearly captured in the Policy (amended in 2013), implementation of these recommendations was not conducted as thoroughly as presented in the Policy update. The Panel herein notes that all recommendations represent important steps in assisting the State Water Board to be proactive in their approach to managing CECs in recycled water. Due to the uncertainty that is inherently associated with the universe of chemicals that might occur in recycled water now and in the future, *the need to establish a formal CEC monitoring and assessment program for recycled water that is responsive to rapidly changing CEC issues is critical*. Identifying and incorporating new information on occurrence and toxicity provides the basis for adding new CECs to the framework (i.e., an on-ramp) as well as for removing CECs that do not pose a risk to human health (i.e., an off-ramp).

New knowledge might also point to direct evidence for health relevance justifying the need for a continuous updating process that cannot be provided by convening a review panel only every five (or more) years. Instead, these programmatic upgrades should be reviewed internally as well as by independent experts on a relatively frequent (e.g. triennial) schedule.

Final Recommendations Provided by the 2018 Panel

The Panel cannot stress strongly enough that the outcome of the 2018 application of the risk-based framework clearly points to the safety of potable and non-potable reuse practices in California. It is essential that all stakeholders and the public realize that the Panel's findings and recommendations include a very large margin of safety. That large margin of safety arises from conservative assumptions that are built into each step of the overall human health CEC screening process. In addition, the Panel offers the following additional recommendations:

- The risk-based screening framework established by the Panel in 2010 was successful in incorporating current information leading to the addition of new and removal of existing CECs from the monitoring list (i.e., in providing on- and off-ramps) and should continue to be applied to update the CEC monitoring list into the future.
- The Panel recommends implementation of the estrogen and aryl hydrocarbon receptor (ER and AhR) assays for screening of unmonitored CECs in potable reuse projects. These assays are now sufficiently standardized and robust for screening level data collection and assessment.
- Additional investment in research and training is needed to provide an expanded, robust "bioscreening toolbox", and to increase capacity for bioanalytical measurement.
- Non-targeted (chemical) analysis (NTA) holds promise as a powerful tool for identifying previously unidentified chemicals in recycled water samples. However, at this time, unlike some bioanalytical tools, NTA remains highly complex, labor and capital cost intensive. The Panel recommends these be attempted and/or applied with clear goals (e.g. as guided by the responses from bioanalytical tools) as part of investigative type studies.
- The Panel recommends that the State Water Board consider taking several procedural steps to clarify roles and responsibilities for the State and Regional Water Boards (as described in Section 2.3) for permitting of potable water reuse projects, to improve the management of potable water facility monitoring data (i.e., CEC, bioanalytical, and high-frequency operation data), and the reporting of potable water operations to the public.
- A more flexible and responsive program should be developed to update CEC monitoring recommendations in response to rapidly emerging science, technology advances and monitoring (screening) data collected. In this context, the State Water Board might want to take a more active role in procuring, managing and assessing CEC monitoring data and associated toxicological thresholds, that are subject to rapid/continual evolution.
- The Panel recommends that the State Water Board consider the results of more definitive research showing an actual relationship of antibiotic resistance to recycled water before changing its current policy.

- The Panel recommends that the State Water Board reconvene an independent Panel to review proposed changes to CEC monitoring recommendations every three years.

Table ES.1. Revised monitoring requirements for health-based and performance-based indicator CECs and performance surrogates for potable and non-potable reuse practices.

Reuse Practice	Health-based indicator	MRL (ng/L)	Bioanalytical methods	MRL (ng/L)	Performance-based Indicator	Expected Removal[6]	MRL (ng/L)	Surrogate	Method	Expected Removal[6]
Surface Spreading Application (SAT)	NDMA[2]	2	ER	0.5	ΔGemfibrozil[3]	>90%	10	ΔAmmonia	SM	>90%
	NMOR[1]	2	AhR	0.5	ΔSulfamethoxazole[4]	>80%	10	ΔNitrate	SM	>30%
	1,4-Dioxane[1]	100			ΔIohexol[3]	>90%	50	ΔDOC	SM	>30%
					ΔSucralose[5]	>25%	100	ΔUVA	SM	>30%
								ΔTotal fluorescence		>30%
Subsurface Application (Direct Injection) and Surface Water Augmentation	NDMA[2]	2	ER	0.5	ΔSulfamethoxazole	>90%	10	ΔConductivity	SM	>90%
	NMOR[1]	2	AhR	0.5	ΔSucralose	>90%	100	ΔDOC	SM	>90%
	1,4-Dioxane[1]	100			ΔNDMA	25-50%	2	ΔUVA	SM	>50%
Non-potable reuse practices					None			Turbidity	SM	
								Cl_2 residual or operational UV dose	SM	
								Total coliform	SM	

[1]Industrial chemical; [2]Disinfection byproduct; [3]Pharmaceutical residue; [4]Antibiotic; [5]Food additive; [6]travel time in subsurface two weeks and no dilution, see details in Drewes *et al*., 2008; SM – Standard Methods; MRL – Method Reporting Limit.

Acronyms and Symbols

ADI	Acceptable Daily Intake
AFY	Acre-Feet per Year
AhR	Aryl Hydrocarbon Receptor
AL	Action Level
AMR	Antimicrobial Resistance
AOP	Advanced Oxidation Process
ARB	Antibiotic Resistant Bacteria
ARG	Antibiotic Resistance Gene
AS	Activated Sludge
AWT/AWTF	Advanced Water Treatment Facility
BAF	Bioaccumulation Factor
BEQ	Bioanalytical Equivalent Concentration
CCL3	USEPA Candidate Contaminant List 3
CCL4	USEPA Candidate Contaminant List 4
CCR	California Code of Regulations
CDPH	California Department of Public Health (the CDPH drinking water group is now DDW which is a division of the State Water Board)
CECs	Constituents of Emerging Concern
CEQA	California Environmental Quality Act
CFUs	Colony Forming Units
CIWQS	California Integrated Water Quality System
CWA	Clean Water Act
CWC	California Water Code
DDT	Dichlorodiphenyltrichloroethane
DDW	California Division of Drinking Water
DEET	N,N-Diethyl-meta-Toluamide
DI	Direct Injection
DMSO	Dimethylsulfoxide
DOC	Dissolved Organic Carbon
DPR	Direct Potable Reuse
DWTF	Drinking Water Treatment Facility
E2	17β-Estradiol
EC50	Half Maximal Effective Concentration
EDCs	Endocrine Disrupting Compounds

EDSP	Endocrine Disruptor Screening Program
EE2	17α-Ethinylestradiol
EFSA	European Food Safety Authority
EI	Electronic Ionization
ELAP	Environmental Laboratory Accreditation Program
ER	Estrogen Receptor
ESI	Electrospray Ionization
EU	European Union
GAC	Granular Activated Carbon
GC-MS	Gas Chromatography-Mass Spectrometry
GR	Glucocorticoid Receptor
GRRP	Groundwater Replenishment Reuse Project
GWR	Groundwater Recharge
H_2O_2	Hydrogen Peroxide
HPLC	High Performance Liquid Chromatography
HPV	High Production Volume
HRMS	High Resolution Mass Spectrometry
IPR	Indirect Potable Reuse
IPR-GWR	Indirect Potable Reuse via Groundwater Recharge
JWPCP	Joint Water Pollution Control Plant
K_{ow}	Octanol-water partition coefficient
LACSD	Sanitation Districts of Los Angeles County
LC-MS	Liquid Chromatography-Mass Spectrometry
LC-QQQ	Liquid Chromatography-Triple Quadrupole Mass Spectometry
LC-QTOF	Liquid Chromatography-Quadrupole Time of Flight
LLE	Liquid Liquid Extraction
LOD	Limit of Detection
LOEC	Lowest Observed Effect Concentration
LOQ	Limit of Quantification
LRV	Log10 Reduction Value
MCLs	Maximum Contaminant Levels
MDH	Minnesota Department of Health
MDL	Method Detection Limit
MEC	Measured Environmental Concentration
MF	Microfiltration

MGE	Mobile Genetic Element
MPN	Most Probable Number
MRL	Method Reporting Limit
MTL	Monitoring Trigger Level
NDMA	N-nitrosodimethylamine
NGS	Next Generation Sequencing
NIST	National Institute of Standards and Technology
NMOR	N-nitrosomorpholine
NOEC	No Observed Effect Concentration
NPDES	National Pollutant Discharge Elimination System
NRC	National Research Council
NTA	Non-Targeted Analyses
NTU	Nephelometric Turbidity Unit
NWRI	National Water Research Institute
OECD	Organisation for Economic Cooperation and Development
PAHs	Polycylic Aromatic Hydrocarbons
PCA	Principal Component Analysis
PCBs	Polychlorinated Biphenyls
PCCL	Preliminary Candidate Contaminant List
PEC	Predicted Environmental Concentration
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctanoic Sulfonate
PNEC	Predicted No-Effect Concentration
POE	Point of Exposure
POM	Point of Monitoring
POTWs	Publicly Owned Treatment Works
PPCPs	Pharmaceuticals and Personal Care Products
QA/QC	Quality Assurance/Quality Control
QMRA	Quantitative Microbial Risk Assessment
QTOF	Quadrupole-Time-of Flight
REF	Relative Enrichment Factor
RO	Reverse Osmosis
RSC	Relative Source Contribution
RSD	Relative Standard Deviation
RSL	Regional Screening Level

RW	Recycled Water
RWC	Recycled Water Contribution
Regional Water Boards	Regional Water Quality Control Boards
SA	Surface Spreading Application
SAG	Stakeholder Advisory Group
SAT	Soil-Aquifer Treatment
SEF	Sample Enrichment Fold
SOP	Standard Operating Procedure
SPE	Solid Phase Extraction
SCCWRP	Southern California Coastal Water Research Project Authority
SDWA	Safe Drinking Water Act
SDWIS	Safe Drinking Water Information System
SFEI	San Francisco Estuary Institute
SWA	Surface Water Augmentation
SWPP	Source Water Protection Program
State Water Board	State Water Resources Control Board
SWTP	Surface Water Treatment Plant
TIC	Tentatively Identified Compounds
TIE	Toxicity Identification Evaluation
TN	Total Nitrogen
TOC	Total Organic Carbon
TOrCs	Trace Organic Chemicals
Tr	Theoretical Residence Time
TTC	Threshold of Toxicological Concern
UCM	Unregulated Contaminant Monitoring
UCMR	Unregulated Contaminant Monitoring Regulation
US	United States
USEPA	United States Environmental Protection Agency
WE&RF	Water Environment and Reuse Foundation
WET	Whole Effluent Testing
WHO	World Health Organization
WRP	Water Reclamation Plant
WWTP	Wastewater Treatment Plant

List of Figures

Page

Figure ES.1. Revised risk-based CEC selection framework..... viii

Figure 3.1. Types of wastewater reuse in California as a percentage of annual use (2015)..... 14

Figure 3.2. Schematics of (a,b) indirect potable reuse in California using groundwater replenishment and (c) surface water reservoir augmentation (per revised CWC, SWRCB 2017b)..... 18

Figure 4.1. Comparison of 90th percentile measured environmental concentrations (MECs) reported in secondary/tertiary treated effluents compiled during the 2010 and 2018 Panel reviews (concentrations in ng/L)..... 33

Figure 5.1. Revised risk-based CEC selection framework..... 45

Figure 7.1. Screening approach for unmonitored CECs using bioanalytical tools and non-targeted chemical analysis (NTA) in recycled water..... 65

Figure 7.2. Application of bioanalytical tools complements chemical analyses in monitoring and assessment of recycled water quality and treatment performance..... 73

Figure F.1. Decision tree for bioanalytical measurements (from Leusch and Snyder, 2015)..... 150

List of Tables

Page

Table ES.1. Revised monitoring requirements for health-based and performance-based indicator CECs and performance surrogates for potable and non-potable reuse practices..... xii

Table 2.1. Health-based and performance based indicator CECs and performance surrogates for potable and non-potable reuse practices adopted in 2013 in Attachment A of the Recycled Water Policy..... 7

Table 2.2. Summary list of 2010 Panel recommendations to the State Water Board, status and need for future follow-up implementation by the State Water Board.... 8

Table 3.1. Summary of California Department of Public Health non-potable water reuse treatment requirements..... 16

Table 3.2. Estimated level of human exposure for several high exposure non-potable water recycling uses..... 20

Table 3.3. Summary of key groundwater recharge regulation criteria (2014)..... 26

Table 3.4. Summary of key surface water augmentation (SWA) criteria..... 30

Table 4.1. CEC monitoring data requested by the 2018 Panel..... 32

Table 4.2. Updated list of monitoring trigger levels (MTLs) (in ng/L) for CECs in recycled water that were lower than the 2010 report..... 35

Table 4.3. Updated list of monitoring trigger levels (MTLs) (in ng/L) for CECs in recycled water that were higher than the 2010 report..... 37

Table 4.4. 2010 Exposure screening for CCL3 and non-CCL3 CECs in recycled water (from Anderson et al., 2010)..... 39

Table 4.5. 2018 Exposure screening for CECs in recycled water..... 39

Table 5.1. Updated monitoring requirements for performance based indicator CECs and performance surrogates for potable and non-potable reuse practices..... 47

Table 6.1. Target analytes of different standardized methods..... 54

Table 6.2. Target analytes of the Unregulated Contaminant Monitoring Rule (UCMR4)..... 57

Table 6.3. Comparison of the detectability of CECs using targeted LC-QQQ vs. non-targeted LC-QTOF on identical wastewater samples. 62

Table 7.1. Commercially available EDSP Tier I Bioassays with adequate quality assurance guidelines..... 64

Table 7.2. I*n vitro* assays that screen for CECs by mode of biological action..... 72

Table 7.3. Quality assurance/quality control (QA/QC) guidelines for the GeneBLAzer® *in vitro* transactivation bioassays adapted and standardized for water quality screening (adapted from Mehinto et al., 2015)..... 74

Table 8.1. Title 22 treatment requirements for agricultural irrigation (uses 37% of municipal recycled water). 82

Table 8.2. Title 22 treatment requirements for landscape irrigation (uses 24% of municipal recycled water). 85

Table 8.3. The quantity of *blaSHV/TEM, sul1* and bacterial 16S genes in full-scale water reclamation plant (WRP) treatment processes: solids fraction (based on LACSD data)..... 91

Table 8.4. Log reductions through treatment (based on LACSD data)..... 91

Table 9.1 Revised monitoring requirements for health-based and performance-based indicator CECs and performance surrogates for potable and non-potable reuse practices. 100

Table C.1. Recycled water uses allowed in California..... 123

Table D.1. CEC added to the CEC master list during the 2018 Panel's review process..... 126

Table D.2. CEC removed from the CEC master list during the 2018 Panel's review process..... 129

Table D.3. Master list of CECs considered by the 2018 Panel..... 130

Table E.1. Reported antibiotic resistance genes (ARGs) removal by wastewater treatment processes from DPR Final report (Olivieri et al., 2016)..... 147

Table E.2. Reported antibiotic resistant bacteria (ARB) removal by wastewater treatment processes from DPR Final report (Olivieri et al., 2016)..... 148

Table E.3. Log reduction of antibiotic resistant bacteria and antibiotic resistance genes (ARB and ARGs) in water by disinfection and barrier processes from DPR Final report (Olivieri et al., 2016)..... 149

1. Introduction and Panel Charge

1.1 Background

Enhanced population demands coupled with changes in climate are causing recycled water to become an increasingly important part of California's water supply. California presently recycles approximately 714,000 acre-feet of water per year (AFY), an amount that has doubled in the last twenty years (SWRCB, 2017a). The California State Water Resources Control Board (State Water Board) established goals of increasing recycled water use over 2002 levels by >1 million AFY by 2020 and >2 million AFY by 2030.

The State Water Board adopted the Recycled Water Policy in 2009 (adopted under Resolution No. 2009-0011, SWRCB 2009) to support sustainable local water supplies and promote the use of recycled water in a manner that is protective of human health and the environment. The Recycled Water Policy ("Policy") adopted in 2009 recognized the challenge of addressing the potential risks of unregulated chemicals referred to as constituents of emerging concern (CECs) and required the State Water Board to convene a Science Advisory Panel to make CEC monitoring recommendations for recycled water. In 2013, the Recycled Water Policy was amended (adopted under Resolution No. 2013-003) to include monitoring requirements for CECs based on the recommendations of the Science Advisory Panel. The Recycled Water Policy also states that the Science Advisory Panel should update their recommendations every five years, and this report is the first update following the initial recommendations.

1.2 The Science Advisory Panel

Recognizing that consideration of CEC effects on human health and aquatic life is a rapidly evolving field and that regulatory requirements need to be based on best available science, the State Water Board included a provision in the Recycled Water Policy to establish a Science Advisory Panel ("Panel") that would provide guidance in developing monitoring programs that assess the potential health threat of CECs from various water recycling practices. A six-member Panel was first formed in 2010 and delivered their initial recommendations in 2012 (Anderson et al., 2010). Because of the rapid evolution of CEC science and measurement technology, the Policy also required that a Science Advisory Panel revisit and update CEC monitoring recommendations, as needed, every five years. Hence, in July 2017, a Panel of seven national experts in the fields of chemistry, biochemistry, toxicology, environmental microbiology, epidemiology, risk assessment, and engineering with more than 150 years of combined experience investigating CEC issues, was convened to update and expand upon the original Panel recommendations currently specified in the Policy (amended in 2013). A brief biography of each Panel member is provided in Appendix A:

- Dr. Paul Anderson, Arcadis and Boston University
- Dr. Nancy Denslow, University of Florida
- Dr. Jörg E. Drewes, Technical University of Munich (*Panel Chair*)
- Dr. Adam Olivieri, EOA, Inc.
- Dr. Daniel Schlenk, University of California-Riverside
- Mr. Walter Jakubowski, WaltJay Consulting
- Dr. Shane Snyder, University of Arizona.

The Panel was assisted by a Stakeholder Advisory Group (SAG), consisting of nine members representing public interest groups, municipalities, wastewater and potable water utilities, and the recycled water advocacy and research communities that are active in California. The role of SAG was to serve as a conduit of communication for their respective constituencies statewide and as a data and information resource for the Panel. The Panel held initial public meetings on July 19 and 21, 2017 in Costa Mesa, CA to review the Panel's charge and solicit feedback from the SAG and the general public. Over the next few months, the Panel deliberated and then held a public meeting on December 15, 2017 in Sacramento, CA to report their preliminary findings and recommendations. Each of the public meetings was structured to allow for ample interaction by the Panel with stakeholders and members of the public, e.g. to provide input to the Panel, request clarifications, to exchange information, and to engage in dialog with the Panel. The draft report was released for a 30-day public comment period on January 31, 2018. This report provides the results from the Panel's deliberations.

Charge to the Science Advisory Panel

- What are the appropriate constituents to be monitored in recycled water, including analytical methods and method detection limits?
- What is the known toxicological information of the above constituents?
- Would the above list change based on level of treatment and uses as specified in Title 22 and for surface water augmentation? If so, how?
- What indicators or surrogates can be used to represent a suite of CECs?
- What concentrations of CECs should trigger enhanced monitoring?
- The evaluation of surface water augmentation (SWA) using recycled water should consider potential human health risks associated with ingestion of water originating from a reservoir used as a source of drinking water (this evaluation will not consider potential ecosystem risks in reservoirs augmented with recycled water).
- Evaluate the use of recycled water for irrigation of crops as allowed under Title 22 regarding potential human health risks except potential human health risks associated with ingestion of crops irrigated with recycled water. For all other uses of recycled water allowed under Title 22, the evaluation shall include potential human health risks for all routes of exposure. The Panel shall evaluate potential exposure from groundwater potentially impacted by recycled water as allowed under Title 22.
- Provide recommendations for additional research regarding antibiotic resistant bacteria and antibiotic resistance genes related to the use of recycled water for SWA and other uses allowed under Title 22 (indirect potable reuse; landscape irrigation; crop irrigation; etc.) to further understand potential human exposure and potential impacts to human health.
- Recommend actions that should be taken to improve the understanding of CECs and, as appropriate, to protect public health and the environment. These recommendations will focus on potential changes (updates) to the list of performance and health-based CECs that were recommended for monitoring in the 2010 Panel report.

1.3 Charge to the Science Advisory Panel

Using the conceptual framework developed by the original Panel in 2010, the 2018 Science Advisory Panel was asked to conduct a review of scientific literature and develop recommended actions to provide updates to our original findings. The review would focus on literature published since 2009 and monitoring data gathered following our previous recommendations. In particular, the Panel was asked to consider each of the uses of recycled water allowed under Title 22 (e.g., indirect potable reuse via groundwater recharge; landscape irrigation; crop irrigation; dust control) and use of recycled water for augmentation of surface water reservoirs. The Panel was asked not to consider potential health risks associated with ingestion of crops irrigated with recycled water.

The Panel was provided with nine specific charge questions (see accompanying box). The Panel was instructed to focus its recommendations on toxicological relevance of CECs to human health. The Panel did not address other practices that could result in discharge of recycled water to surface water, estuaries, and the ocean and subsequent exposure to ecological receptors.

In considering the charge, the Panel defined CECs to represent unregulated chemicals including personal care products, pharmaceuticals, transformation products, industrial, agricultural and household chemicals, including those produced in high production volumes, natural hormones, food additives, inorganic constituents, nanomaterials and microplastics. In addition, the Panel addressed non-chemical constituents such as antibiotic resistant bacteria and antibiotic resistance genes (ARBs/ARGS).

The Panel also chose not to consider the occurrence of waterborne microbial pathogens. Given the multiple barrier concept and water treatment process redundancy requirements in place, the Panel believes that the potential public health risk associated with exposure to pathogens in recycled water used for non-potable reuse and potable reuse practices[2] is rather small and well managed. However, the Panel acknowledges that some uncertainties exist regarding the occurrence of emerging waterborne microbial contaminants, such as ARBs and ARGs, and encourages additional research into their fate in water reuse systems.

1.4 Organization of the Report

This report contains nine chapters and six appendices. Chapters 2 and 3 provide material on the California Reuse Regulatory Practices and public health considerations. Chapters 4 and 5 update measured environmental concentrations and toxicological data for CECs to be considered for monitoring as well as the need for revisions to monitoring requirements. In addition, modifications to the Panel's original risk-based framework for the selection of relevant CECs are also discussed. Chapter 6 provides updated methods for targeted and non-target chemical analyses and insights into sample collection, handling and extraction challenges. Chapter 7 discusses updates and recommendations for bioanalytical methods for recycled water quality assessment. Chapter 8 describes the issues associated with the assessment of recycled water for ARBs/ARGs. Chapter 9 summarizes the 2018 Panel's updated recommendations.

[2] Multiple barriers for groundwater recharge or surface water augmentation projects include source control and consideration of the treatment processes at the water recycling plant, attenuation during passage through an environmental buffer including detention time, dilution, and die-off, and various potable water treatment processes associated with the production of finished potable water.

2. Regulatory Practices for Water Recycling in California

The State of California has a long history of water reclamation and reuse and in 1918 developed the first reuse regulations in the United States to address the use of reclaimed water for agricultural irrigation. The regulations have been modified over the years and additional information on California history is provided in Crook et al. (1994), Harris-Lovett and Sedlak (2015), and Olivieri et al. (2016).

In California, as well as in many water-scarce areas, water reclamation, recycling, and reuse are integral components of water resource planning and management. Historically, the driving motivation for water recycling was to supplement scarce resources and to provide alternative options for effluent disposal into surface waters. With increasing water demand due to periodic drought and a growing population, recycled water is now considered an important water resource. Engaging in non-potable and potable water reuse can enable communities to maximize and extend the use of limited freshwater resources.

The purpose of this chapter is to provide a brief summary of the following topics:

- Current water recycling regulations in the State of California
- Recycled water practices across California (non-potable and planned potable reuse)
- State Water Resources Control Board policy addressing CEC monitoring
- Assessment of and recommendations to improve the State Water Board's CEC monitoring program

2.1 The State Water Board's Recycled Water Policy

The California State Water Resource Control Boards are composed of the State Water Board, along with the nine Regional Water Quality Control Boards (Regional Water Boards). The State Water Board mission is

"To preserve, enhance, and restore the quality of California's water resources and drinking water for the protection of the environment, public health, and all beneficial uses, and to ensure proper water resource allocation and efficient use, for the benefit of present and future generations."

The State Water Board develops statewide policy and regulations for water quality control and allocates water rights. The Regional Water Boards provide local implementation of policy and regulations, develop long-range plans for their areas, issue waste discharge permits and take enforcement actions against violators. The State Water Board establishes general policies governing the permitting of recycled water projects consistent with its role of protecting water quality and sustaining water supplies. The State Water Board exercises general oversight over recycled water projects, including review of Regional Water Board permitting practices, and leads the effort to meet the State Water Board's recycled water use goals. Since July 1, 2014, when the California Department of Public Health Drinking Water Program was transferred to the State Water Board, the State Water Board has been charged with the development and adoption of uniform water recycling criteria appropriate for specific uses of recycled water. The State Water Board also is charged with the responsibility

to enforce the Clean Water and the Safe Drinking Water Acts, thus requiring the melding of state and federal processes together[3].

In 2009, the State Water Board developed a Recycled Water Policy ("Policy") (adopted under Resolution No. 2009-0011, SWRCB 2009). In 2013, the Recycled Water Policy was amended (adopted under Resolution No. 2013-003, SWRCB 2013a) to include monitoring requiremens for CECs based on the recommendations of the Science Advisory Panel.

The Policy was adopted to promote the use of recycled water in a manner that is protective of public health and water quality by providing streamlined permitting criteria for recycled water projects. The Policy also includes goals and mandates for recycled water use and guidance for the collaborative development of salt and nutrient management plans for groundwater basins or sub-basins in California.

In addition to the above topics, the expansion of the Policy to address new potable water sources (both raw and finished drinking water sources), and the approach for permitting and enforcement of the new sources needs to be clarified and made consistent with the current State Water Board findings and regulations regarding such new potable water sources. There are several State and Federal regulations that have bearing on planned potable water reuse projects. For example:

- The Clean Water Act (CWA) with regard to water quality for discharge to receiving waters
- The CWA relative to the regulation of discharges to publicly owned treatment works (POTWs) (e.g., source control and pretreatment regulations)
- The Safe Drinking Water Act (SDWA) relative to the protection of water supply sources [e.g., source assessments and risk reduction barriers as part of the source water protection program (SWPP)]
- The SDWA relative to drinking water treatment requirements for different source waters (e.g., the Long-Term 2 Enhanced Surface Water Treatment Rule)

Treatment technologies (i.e., advanced water treatment (AWT)) capable of producing high-quality potable water from wastewater for supplementing drinking water supplies have been demonstrated in a number of full-scale AWT facilities (AWTFs). In California, water recycling *"constitutes the development of new basic water supplies"*. California maintains primacy relative to permitting POTWs, drinking water sources, and associated water treatment facilities. Consideration should be given to integrating regulatory programs that implement the provisions of the CWA and SDWA as they relate to potable reuse to allow for more efficient and effective management of the growing demand for potable reuse.

2.2 Regulatory Developments for Recycled Water Applications

There are two main water reuse types, non-potable and planned potable reuse.

Non-potable reuse: The planned use of recycled water for non-potable reuse

[3]All suppliers of domestic water to the public are subject to regulations adopted by the USEPA under the U.S. Safe Drinking Water Act (SDWA) of 1974, as amended (42 U.S.C. §300f et seq.), as well as by the State Water Board under the California SDWA (Health & Saf. Code, div. 104, pt. 12, ch. 4, §116270 et seq.). Pursuant to section 116270 of the Health and Safety Code, et al., it is the objective of the California SDWA that public water systems (PWS) deliver drinking water to consumers that is, at all times, pure, wholesome, and potable.

applications[4] has been practiced for many decades in California, several other areas of the United States, and in other countries. The reuse guidelines and regulations that existed in the 1960s and early 1970s, which addressed only non-potable reuse, reflected the state-of-the-art at that time and the conservative approach taken by public health officials. California Water Recycling Criteria governing the production and use of recycled water are contained in Title 22, Division 4, of the California Code of Regulations (State of California, 2000).

Planned indirect potable reuse (IPR)[5]: Planned IPR involves the introduction of recycled water either into an environmental buffer such as a groundwater aquifer or a reservoir before the blended water is subject to post-treatment and introduced into a water supply system. The relevant forms of IPR covered as part of this report include:

- Indirect potable reuse for groundwater recharge (IPR-GWR): planned use of recycled water for replenishment of a groundwater basin or an aquifer that has been designated as a source water supply for a public water system (CWC section 13561c)[6].
- Surface water augmentation (SWA)[7]: planned placement of recycled water into a surface water reservoir used as a source of domestic drinking water supply for a public water system (CWC section 13561d).

Section 2.3 includes a more detailed discussion of the State Water Board regulations governing the various types and categories of water reuse as well as the public health considerations associated with CECs.

2.2.1 CEC monitoring requirements

The Recycled Water Policy in both its original form (SWRCB 2009) and as updated in 2013 (SWRCB, 2013a,b) sought to incorporate the most current scientific knowledge on CECs into regulatory policies for use by California state agencies. A Science Advisory Panel was formed in 2009 to address the following questions:

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[4]In non-potable reuse, recycled water is used for purposes other than drinking, such as providing water for agricultural and landscape irrigation, as well as water for power plants and oil refineries, industrial processes, toilet flushing, construction, artificial lakes, and other non-drinking applications (USEPA, 2016).

[5] On October 6, 2017 the Governor of California approved an act to amend Sections 13560 and 13561 of, to amend the heading of Chapter 7.3 (commencing with Section 13560) of Division 7 of, and to add Sections 13560.5 and 13561.2 to, the Water Code, relating to water. As noted below, the amended Section 13561 in part modifies the following definitions related to indirect potable reuse type projects. However, for the purpose of the CEC 2018 Panel update and consistency with the 2010 CEC Panel report the Panel elected to rely on the previous Water Code definitions.

> *(c) "Indirect potable reuse for groundwater recharge" means the planned use of recycled water for replenishment of a groundwater basin or an aquifer that has been designated as a source of water supply for a public water system, as defined in Section 116275 of the Health and Safety Code.*
>
> *(d) "Reservoir water augmentation" means the planned placement of recycled water into a raw surface water reservoir used as a source of domestic drinking water supply for a public water system, as defined in Section 116275 of the Health and Safety Code, or into a constructed system conveying water to such a reservoir.*

[6] Public water systems are defined per Health and Safety code section 116275.

[7] On October 6, 2017 amendments to Sections 13560 and 13561 of Chapter 7.3 (commencing with Section 13560) of Division 7 of, and to add Sections 13560.5 and 13561.2 to, the Water Code, relating to potable reuse that modify terminology. For example, Surface Water Augmentation (SWA) is now titled Reservoir Water Augmentation (RWA). However, for the purpose of this report the term SWA is utilized for this practice.

- What are the appropriate constituents to be monitored in recycled water, and what are the applicable monitoring methods and detection limits?
- What toxicological information is available for these constituents?
- Would the constituent list change based on the level of treatment? If so, how?
- What are the possible indicators (i.e., surrogates) that represent a suite of CECs?
- What levels of CEC should trigger enhanced monitoring in recycled water, groundwater, or surface water?

The 2010 Panel produced several products to guide the State Water Board's update of their recycled water management approaches relative to CECs. First, the Panel developed a risk-based framework for prioritizing and selecting CECs for recycled water monitoring programs (Anderson et al., 2010). The framework was then used to develop a short list of recommended monitoring parameters, including both health-based (i.e., toxicologically relevant CECs) and performance-based indicators (i.e., CECs with representative physicochemical properties and structures tested to demonstrate a capacity for reduction by a particular water treatment process). The list also incorporated CECs from multiple source classes (e.g., pharmaceuticals, personal care products, food additives, and hormones). Four health-based and five performance-based indicators were identified for recycled water used for groundwater recharge, whereas only three surrogate parameters (i.e., turbidity, chlorine residual, and total coliform bacteria) were recommended for monitoring water used for landscape irrigation (Table 2.1). In addition, the Panel developed guidance for interpreting and responding to monitoring results. The State Water Board considered the Panel's report and public comments before adopting an amendment to the Recycled Water Policy to establish monitoring requirements for CECs in recycled water (Drewes et al., 2013; SWRCB, 2013b).

Table 2.1. Health-based and performance based indicator CECs and performance surrogates for planned potable and non-potable reuse practices adopted in 2013 in Attachment A of the Recycled Water Policy.

Reuse Practice	Health-based Indicator	MRL (ng/L)	Performance-based Indicator	Expected Removal[8]	MRL (ng/L)	Surrogate	Method	Expected Removal[8]
Groundwater Recharge	17β-estradiol[1]	1	Δgemfibrozil[5]	>90%	10	Δammonia	SM	>90%
SAT	Triclosan[2]	50	ΔDEET[6]	>90%	10	Δnitrate	SM	>30%
	Caffeine[3]	50	ΔCaffeine[3]	>90%	50	ΔDOC	SM	>30%
	NDMA[4]	2	Δiopromide[5]	>90%	50	ΔUVA	SM	>30%
			ΔSucralose[7]	<25%	100			
Direct Injection	17β-estradiol[1]	1	ΔDEET	>90%	10	Δconductivity	SM	>90%
	Triclosan[2]	50	ΔSucralose	>90%	100	ΔDOC	SM	>90%
	Caffeine[3]	50	ΔNDMA	25-50%	2			
	NDMA[4]	2	ΔCaffeine	>90%	50			
Landscape Irrigation	None		None			Turbidity	SM	
						Cl_2 Residual	SM	
						Total Coliform	SM	

[1]Steroid hormones; [2]Antimicrobial; [3]Stimulant; [4]Disinfection byproduct; [5]Pharmaceutical residue; [6]Personal care product; [7]Food additive; [8]travel time in subsurface two weeks and no dilution, see details in Drewes *et al.*, 2008; SM – Standard Method; MRL – Method Reporting Limit

Results of the Panel's 2018 review on the assessment of the current CEC monitoring programs in California and new toxicological information are discussed in Chapters 4 and 5.

2.3 Improving Regulatory Practices for CEC Monitoring and Assessment

To carry out the monitoring program for the indicator CECs listed in Table 2.1[8], the Panel recommended a multi-tiered approach for implementing and interpreting results from CEC monitoring programs for non-potable and groundwater recharge water reuse projects. The Panel also noted that differences in recycled water quality and facility operations will occur by region and that investigation of chronic exceedances will need to be tailored on a region-by-region or case-by-case basis.

In addition, the Panel recommended that the State Water Board develop a process to rapidly compile, summarize, and evaluate monitoring data as they become available. The Panel further recommended that the State Water Board establish an independent review panel that can provide periodic review of the proposed selection approach, reuse practices, and environmental concentrations of ongoing CEC monitoring efforts, particularly as data from the monitoring programs recommended here become available.

While the key recommendations from the 2010 Panel report were clearly captured in the 2013 Recycled Water Policy update, implementation of the recommendations was not conducted as thoroughly as described in the Policy update (Table 2.2). This, in part, was due to the reorganization and structuring of the recycled water and drinking water programs of the Department of Public Health as a new Division of Drinking Water (DDW) under the State Water Board. In addition to addressing the regular recycled water and drinking water regulatory functions, DDW staff were also tasked with addressing two key potable reuse regulatory tasks: 1) creating new surface water augmentation criteria and regulations; and 2) conducting a technical feasibility analysis for developing planned direct potable water reuse criteria and regulations. Table 2.2 summarizes the list of recommendations developed by the 2010 Expert Panel and an assessment regarding their implementation based on the 2018 review.

Table 2.2. Summary list of 2010 Panel Recommendations to the State Water Board, status and need for future follow-up implementation by the State Water Board.

Recommendations of the 2010 Expert Panel	Implementation by State Water Board and Need for a Follow-Up based on 2018 Panel Assessment
Panel recommended using the process described in Snyder et al. (2010) to develop screening level ADIs.	Risk-based framework for CEC selection has been endorsed. This framework should be followed in the future and will be subject to review by an expert panel reconstituted on a regular basis.
Panel recommended that the next Panel review the development of relative source contributions (RSCs)	Has not occurred. However, considering the MEC and MTL data reviewed, the Panel is

[8]The Panel noted that the guidance provided in the 2010 Panel report regarding a start-up and baseline monitoring program did not address all situations that the regulator and regulated entity needed to address. Under these circumstances, the Panel recommended that the affected stakeholders consult experts to recommend a plant or regional-specific solution.

and recommend values to use in the development of MTLs.	deemphasizing the need to derive RSCs because their effect on MTLs is relatively small.
Panel recommended that the State Water Board conduct a more thorough review of CECs likely to occur in recycled water using MEC and PEC data from peer-reviewed literature and occurrence studies outside California. The Panel recommended that the State Water Board charge the next Panel with evaluating a production volume-based system to prioritize known unknown CECs for a monitoring program	This review has not happened. However, the Panel recognizes that such a review should be targeted at relevant potable reuse applications in CA. Has not occurred. The Panel recommends taking advantage of existing databases in the public domain that have compiled high-production volume chemicals.
The Panel recommended that the State Water Board charge the next Panel with developing a pilot program that documents the efficacy of bioanalytical tools for screening of CECs, assuming robust methods are commercially available, and compares their predictions to those of a chemical-by-chemical monitoring program.	Several programs have been initiated since 2010 to further develop the efficacy of bioanalytical tools to screen for CECs by the State Water Board. Matching this information to chemical-by-chemical monitoring efforts has only partially been done.
The Panel strongly recommended that once monitoring of the initial priority list of CECs was implemented by the State Water Board, commercial laboratories again be surveyed to determine their capability to analyze CECs on the initial list.	This survey of laboratories has occurred. The State and the Regional Boards have procedures in place to require that QA/QC requirements by laboratories are met (i.e., the Environmental Laboratory Accreditation Program).
Once every five years, conduct one additional round of CEC monitoring to confirm monitoring results and screen for occurrence of a broader list of CECs for planned potable reuse projects.	The intent of conducting this special study was to screen for occurrence of a broader list of CECs. Some recycled water purveyors routinely analyze for a broader list of CECs than required by their permits. However, this study was not conducted under the guidance of the State Water Board.
The Panel recommended that the State Water Board review and update the list of indicator CECs and surrogate parameters at least triennially as well as new toxicity data to update MTLs. ▪ Collect and review readily available toxicity data and update MTLs. ▪ Collect and review California advanced treatment plant effluent data including IPR monitoring data collected as part of CDPH (now DDW) permitted projects and update MECs. ▪ Update list of indicator CECs to include newly identified CECs where the MEC/MTL>1 and remove CECs where updated data indicate that the current MEC/MTL ≤1. ▪ Review CECs that have been removed from the monitoring list to see if use patterns have changed and whether such change warrants their re-listing for monitoring. ▪ Review and update guidance on sampling frequency and locations.	This review should have happened three years after adopting the recommendations by an Independent Expert Panel.

DRAFT

▪ Review and update conclusions regarding performance of laboratory analytical methods. ▪ Review and update biological and chemical screening methods, as discussed in Chapter 6, and provide guidance on potential new monitoring methods/tools that would significantly enhance conventional chemical monitoring methods. ▪ Develop guidance for the State Water Board to update the monitoring requirements in groundwater recharge project permits. ▪ Review and update Panel guidance on selecting viable surrogate parameters and performance indicator CECs.	
The Panel recommended that the State Water Board convene and charge a Science Advisory Panel to scope out an investigative, short-term monitoring study (e.g. quarterly sampling over a one-year period) for CECs with relatively low MTLs (e.g. < 500 ng/L), but for which no or little MEC or PEC information is available for secondary/tertiary effluents used for the water reuse practices of interest. Encourage development of bioanalytical screening techniques that include CECs currently not identified but potentially present in recycled water ("unknown unknown" chemicals). Develop appropriate trigger levels for these bioanalytical screening techniques that correspond to a response posing a concern from a human health standpoint.	This investigative study has not been performed. Some utilities and research organizations have conducted special studies in CA to identify relevant CECs. The 2018 Panel recommends a modified approach to acquire data of CECs that are relevant to potable reuse applications (see Chapter 5). The State Water Board has taken action regarding the development of bioanalytical screening techniques.

At the present time, State Water Board staff are updating the Policy to address the results of the 2018 Panel review of new and relevant CEC monitoring data collected since 2010 as well as expanding the Policy to address additional non-potable reuse categories and planned potable reuse categories covering surface water augmentation.

The following summary provides the Panel's recommended next steps (in addition to those described in the 2010 report) regarding the permitting of potable water reuse projects, the management of potable water facility monitoring data (i.e., CEC, bioanalytical, and high-frequency operation data), the need to update CEC monitoring data, the external review of CEC data, and the reporting of potable water operations to the public.

1) Permit potable reuse projects – DDW regulates domestic water supplies and thus should issue drinking water permits to all potable reuse projects rather than Regional Water Boards, taking into account both site-specific conditions and the CEC and bioanalytical monitoring recommendations[9], under existing drinking water regulations and water reuse regulations. Potable reuse projects include all facilities that produce a raw water source and finished water source for potable use. The

[9] The development of drinking water permits should be consistent relative to the inclusion of CEC and bioanalytical monitoring requirements).

production of all raw and finished potable water should be regulated (i.e., permitting and enforcement) by the State Water Board through DDW consistent with California drinking water, IPR-GWR and SWA programs and regulations, as appropriate to the potable reuse project. The regulation of concentrate streams from potable reuse facilities should continue to be regulated by Regional Water Boards. Enhanced source control programs (a planned DDW project intends to investigate/define enhanced source control program criteria) developed for all potable reuse projects should be regulated through DDW as part of the drinking water permits.

2) Manage potable water facility monitoring data – several types of data (e.g., CEC chemical specific data, bioanalytical data, and high-frequency process operational data) will be generated as part of the operations and monitoring of potable reuse projects. Current State Water Board data management systems/practices need to be updated to manage (i.e., collect, store, review, and report) the new data. A key piece of this data management and accessibility is having the data submitted in a machine-readable format (e.g., Microsoft Excel™) and uploaded into a database so the data can be easily accessed for review and analysis as described below.

   - CEC and Bioanalytical data – The Safe Drinking Water Information System (SDWIS) is the appropriate database for reporting exceedances of CECs that are included in drinking water permits. However, this database is primarily for drinking water data. To improve accessibility, the State Water Board should assess the best data repository for the CEC data (e.g., California Integrated Water Quality System (CIWQS) and Geotracker) and then establish a protocol to review the data once they have been submitted.
   - High-Frequency data – As the State Water Board develops potable reuse regulations, the Panel anticipates these facilities will generate and submit high-frequency data. The State Water Board should evaluate how to manage these large volumes of data and the best database to which the data should be submitted (see discussion under QMRA).
   - Source Control data – A planned DDW project intends to investigate/define enhanced source control program criteria. At the present time, the intent is that data collected, as part of the enhanced source control program, would be submitted as part of an annual report. However, given the potential nature of further source control monitoring criteria there is a significant likelihood that data submission will become electronic.
   - Non-targeted analysis (NTA) data – As the current state of NTA data is largely qualitative, the intent is for these analyses to be submitted as a special report as a PDF file.

3) Develop internal protocols for DDW staff review and response to CEC and bioanalytical data – Section 8.4 of the Panel 2010 report (reproduced in Appendix B) and Chapters 4, 5, 6 and 7 of this report contain guidance on monitoring programs, review of data, and suggested responses to data collected. Based on the information provided, DDW staff should develop internal protocols for reviewing potable reuse raw source water and finished water data (e.g., define the terms of non-compliance) and develop protocols for appropriate responses. In addition to the overall data management noted above, DDW staff should also consider including in internal protocols a process for managing the review of and response to the potable reuse data, and utility actions.

4) Develop internal protocols for DDW staff review and response to source control data – DDW staff have currently scoped a project to define potential enhancements to conventional POTW based source control programs. To effectively review and respond to the new data generated from implementation of enhanced source control program requirements an internal protocol is needed.

5) Develop internal protocols for DDW staff review and response to high-frequency operational monitoring data – The State Water Board staff are currently scoping a grant to address several of the DPR Expert Panel recommendations. One of the grant projects will develop quantitative microbial risk assessment (QMRA) tools for DDW staff to review potable reuse projects (both at the permit application stage and at the operational stage). An element of the QMRA project includes developing tools to manage (i.e., storage and analysis) high-frequency data.

6) Develop consistent permittee electronic reporting requirements – Based on the outcome of recommendations 2 and 4 above, State Water Board staff should develop protocols for potable water reuse utilities (i.e., permittee) to manage the reporting and transmitting of all data (i.e., type and frequency) in a machine-readable electronic format.

7) Develop communication protocols – The State and Regional Water Boards should develop a protocol outlining the roles and responsibilities for reviewing and communicating CEC data from potable reuse projects. These protocols should include a process for communicating with the utilities.

8) External Panel review of reported data on potable reuse program implementation – Section 8.4.3 of the 2010 Panel report included recommended items for external review of the CEC monitoring data (also summarized in Table 2.2). The following is an updated list of those recommendations that provides additional detail to the recommendations presented in Chapter 9. The intent is for State Water Board staff to conduct these tasks over the next three years and then reconstitute the Panel to review State Water Board staff efforts and provide Panel guidance/updates on the application and structure of the risk-based framework.

   - Collect and review readily available toxicity data and update MTLs;
   - Collect and review California advanced treatment plant effluent data including IPR monitoring data collected as part of DDW permitted projects and update MECs;
   - Update list of priority CECs to include newly identified CECs where the MEC/MTL>1 and remove CECs where updated data indicate that the current MEC/MTL≤1;
   - 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;
   - Review and update guidance on sampling frequency and location;
   - Review and update conclusions regarding laboratory analytical methods;
   - Review and update guidance on selecting viable surrogate parameters and performance indicator CECs.

9) The State Water Board should develop a protocol for providing the public an annual report summarizing performance of potable reuse projects. Public transparency is a

key element to public acceptability. The intent is to be able to have a web portal for potable reuse projects and post annual utility reports and any State Water Board staff reports on the operations of the State Water Board program.

DRAFT

3. Assessing Risk to Human Health

For nearly a century, recycled water has been used intentionally as a non-potable water supply source in California. The implementation of reclamation projects has increased significantly over the years, even in the face of regulatory, economic, and social constraints. In 1989, the reuse of municipal wastewater in California was estimated at 325,000 acre-feet per year (AFY)[10]. In 2002, the State Water Board conducted a comprehensive statewide survey of municipal facilities that focused on documenting the current levels of non-potable reuse of treated municipal wastewater. The results of the 2002 survey indicated that, as of the end of 2001, approximately 525,460 AFY of recycled water was used in California (SWRCB, 2011). State Water Board data indicate that during 2009 approximately 669,000 AFY of recycled water was used (State Water Resources Control Board, 2012). The most recent State Water Board survey data, collected in 2015, indicates that approximately 713,000 AFY of recycled water was used (SWRCB, 2017a).

A summary of the 2015 statewide survey is shown in Figure 3.1, suggesting that the top three uses of recycled water are for agricultural irrigation (30 percent), landscape irrigation (18 percent), and groundwater recharge and seawater intrusion barrier uses (24 percent). At present, estimates indicate that about 8 to 10 percent of municipal wastewater generated in California is recycled in planned reuse projects. Estimates regarding future recycling indicate that California has the potential to recycle an additional 1.4 to 1.6 million AFY of water by the year 2030 (Smith, 2010).

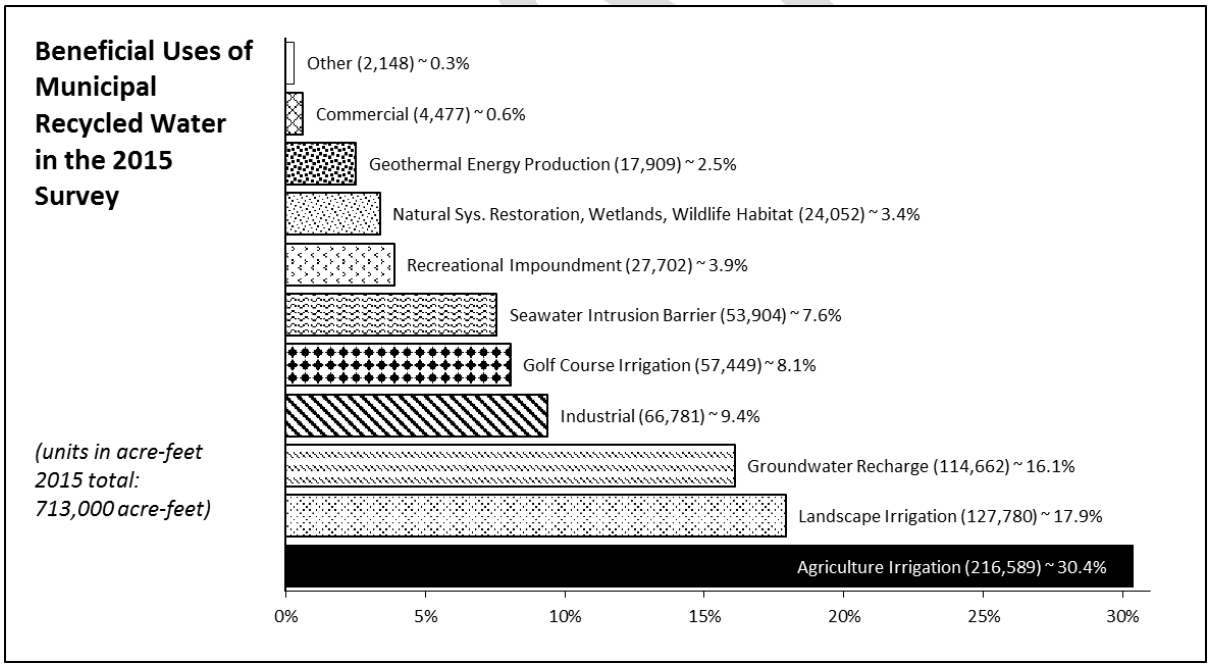

Figure 3.1. Types of wastewater reuse in California as a percentage of annual use (2015) (Source: State Water Resources Control Board, 2017a).

The estimated total percentage of agricultural reuse in California (roughly 30%) can be further divided (based on 2000 estimates) into six main categories (USEPA, 2004):

- Mixed (approx. 44 percent of total agricultural reuse);

[10]One acre-foot is equivalent to approximately 325,851 gallons of water.

- Harvested feed, fiber, and seed (approx. 37 percent);
- Pasture (approx. 12 percent);
- Orchards and vineyards (approx. 3 percent);
- Food crops (approx. 2 percent); and
- Nursery and sod (approx. 2 percent).

Estimated future demand, as noted above, could increase agricultural reuse by a factor of 3.2 to 3.5 times current reuse levels by 2030.[11]

The purpose of this chapter is to provide a summary of the following topics:

- Overview of non-potable reuse
- Public health considerations for non-potable reuse
- Overview of planned potable reuse
- Public health considerations for planned potable reuse

3.1 Recycled Water Applications in California

3.1.1 Non-potable water reuse

The planned use of recycled water for non-potable reuse applications[12] has been practiced for many decades in California, several other areas of the United States, and in other countries. The guidelines and regulations that existed in the 1960s and early 1970s for non-potable reuse, reflected the state-of-the-art at that time and the conservative approach taken by public health officials. As the need grew for more water, additional reclaimed water applications (for both non-potable and potable reuse) were proposed. Over the last 30 years, a significant increase has occurred in both the types of reclaimed water applications now available and quantities of water being reused. This increase resulted (in part) from an intense era of research and demonstration studies – beginning in the late 1960s – that provided valuable information and confidence to California regulatory agencies involved with adopting water reuse regulations (Crook, 1998).

The most common concern associated with non-potable reuse is the potential transmission of infectious disease from ***microbial pathogens*** by (1) inadvertent ingestion of recycled water, (2) skin contact, (3) consumption of food crops irrigated with recycled water, and (4) inhalation of aerosols, although it is recognized that certain ***chemicals*** also can be a concern (e.g., heavy metals taken up by food crops could present potential health risks to consumers). Consequently, California regulations for non-potable reuse focus mainly on mitigating health risks from microbial pathogens by reducing or eliminating them in recycled water and/or by imposing use area controls (e.g., fencing, signage, buffer zones, color-coded pipes and appurtenances) or other controls to prevent human contact with recycled water.

California Water Recycling Criteria governing the production and use of recycled water are contained in Title 22, Division 4, of the California Code of Regulations (State of California, 2000). A summary of the criteria is presented in Table 3.1 and a complete list of allowable uses is contained in Appendix C, Table C.1.

[11] Current estimates indicate that approximately 2 percent of edible food crops are irrigated with reclaimed water and, based on a linear extrapolation, estimated food crop use could increase to 8 percent.
[12] In non-potable reuse, recycled water is used for agricultural and landscape irrigation, as well as water for power plants and oil refineries, industrial processes, toilet flushing, construction, artificial lakes, and other non-drinking applications (USEPA, 2016).

As noted in Table 3.1, specific treatment processes have been relied on in California to significantly reduce the numbers of viruses and parasites (i.e., applying a process or performance standard rather than a strict pathogen standard). Specifically, the regulations include process standards for crop irrigation (unrestricted) to ensure that the recycled water has a total coliform concentration of less than or equal to 2.2 MPN (most probable number) per 100 milliliters (mL). Water quality meeting these criteria is considered "safe" for human contact. This is further supported by past experiences of health professionals and on a lack of detectable health problems associated with agricultural irrigation (NRC, 1996).

Table 3.1. Summary of California Department of Public Health non-potable water reuse treatment requirements.

Purpose of Use	Treatment Requirement
Orchards and vineyards (no contact with edible crops), nonfood-bearing trees, fodder or fiber crops, seed crops (not eaten by humans), food crops (with additional pathogen treatment for crop), and flushing sanitary sewers.	Undisinfected Secondary[a]
Cemeteries, freeway landscaping, golf courses (restricted access), ornamental nursery stock, sod farms, pasture (milk animals), non-edible vegetation (controlled access), commercial/industrial cooling towers (with drift reduction), landscape impoundments (no decorative fountains), industrial boiler feed, soil compaction, mixing concrete, dust control (roads), cleaning roads, nonstructural firefighting.	Disinfected Secondary, 23 MPN/100 mL[b]
Food crops (edible portion of crop above ground – no contact), restricted recreational impoundments.	Disinfected Secondary, 2.2 MPN/100 mL[c]
Food crops (including edible root crops) where recycled water comes into contact with edible portions of the crop, parks and playgrounds, school yards, residential landscaping, golf courses (unrestricted), commercial/industrial cooling towers (mist devices), unrestricted recreational impoundments (with specific pathogen monitoring), flushing toilet and urinals, structural firefighting, decorative fountains, artificial snow making, commercial car washes, groundwater recharge (with additional treatment –see State Water Board groundwater regulations).	Disinfected Tertiary[d]

Notes:

a) **Undisinfected secondary treatment**: means oxidized wastewater (oxidized wastewater: wastewater in which the organic matter has been stabilized, is non-putrescible, and contains dissolved oxygen).

b) **Disinfected secondary – 23 MPN per 100 mL recycled water**: oxidized and disinfected so that the median concentration of total coliform bacteria does not exceed a most probable number of 23 MPN per 100 mL, and the MPN does not exceed 240/100 mL in more than one sample in any 30-day period.

c) **Disinfected secondary – 2.2 MPN per 100 mL recycled water**: oxidized and disinfected so that the median concentration of total coliform bacteria does not exceed a most probable number of 2.2/100 mL, and the MPN does not exceed 23/100 mL in more than one sample in any 30-day period.

d) **Disinfected tertiary recycled water**: a filtered and disinfected wastewater (see definition below) that meets a CT (product of total chlorine residual and modal contact time measured at the same point) value of not less than 450 mg-min/L at all times, with a modal contact time of 90 minutes (min.) (based on peak dry weather design flow) or provides a 5-log removal/reduction of MS2 F-specific phage or poliovirus or similar virus.

Filtered wastewater: an oxidized, coagulated, clarified wastewater that has been passed through natural undisturbed soils of filter media, such as sand or diatomaceous earth, so that the turbidity, as determined by an approved laboratory method, does not exceed 5 NTU more than 5 percent of the time during any 24-hour period, an average of 2 NTU during a 24-hour period, and does not exceed a 10 NTU at any time; in addition, the filter may not exceed 5 gallons per min. per square foot (traveling bridge automatic backwash filters cannot exceed 2 gallons per min.).

Source: Summary adapted from the State of California, 2000.

3.1.2 Planned potable water reuse

Planned potable water reuse can occur directly or indirectly via an environmental buffer. Several categories of planned potable reuse are defined in CWC section 13560. The relevant forms of potable reuse covered as part of this report include:

- **Indirect potable reuse for groundwater recharge (IPR-GWR):** planned use of recycled water for replenishment of a groundwater basin or an aquifer that has been designated as a source water supply for a public water system (CWC section 13561 c)[4].
- **Surface water augmentation (SWA)**: planned placement of recycled water into a surface water reservoir used as a source of domestic drinking water supply for a public water system (CWC section 13561 d).

In California, the practice of planned potable reuse has occurred in the form of IPR-GWR for over 50 years (Crook, 2010; Drewes and Khan, 2011; Drewes and Horstmeyer, 2016). A key element of an IPR system is its reliance on an environmental buffer. While some environmental buffers might offer opportunities for further treatment (e.g., groundwater basins), ***the main functions of the environmental buffer*** are to provide – through storage – some level of water quality equalization and time to respond to any process failures or out-of-compliance water quality monitoring results (Drewes and Khan, 2011).

The schematics of IPR schemes in California (as defined by the California Water Code) are shown in Figure 3.2, which depicts advanced treated recycled water being introduced into an environmental buffer as part of the water supply upstream of a drinking water treatment facility (DWTF). In Figure 3.2 (a, b), the environmental buffer is a groundwater aquifer, therefore, the project must meet regulations for groundwater replenishment (CCR, 2015). For such a project, advanced treated water is required for subsurface application (direct injection), whereas tertiary effluent can be applied prior to surface application (surface spreading) to take advantage of soil-aquifer treatment. In Figure 3.2 (c), the environmental buffer is a surface water reservoir, so the project must meet the draft criteria for SWA (i.e., the reservoir has a theoretical minimum hydraulic retention time of ≥2 to 6 months)[13] (SWRCB 2017b, NWRI, 2015a, b, c).

Because a key element of an IPR-GWR or a SWA project is its reliance on a regulatory defined environmental buffer with specified retention times, by default, all potable reuse projects that do not meet California regulations for groundwater replenishment or the draft criteria for SWA are considered a DPR practice.

[13] Per Sections 13560-13569 of the California Water Code, the State Water Resources Control Board is required by December 31, 2016, to adopt regulations for Surface Water Augmentation Using Recycled Water. The Expert Panel on Direct Potable Reuse reviewed the proposed regulations and provided recommendations to the State Water Board in 2015 (NWRI, 2015a,b,c). The SWRCB is currently conducting a public review of the draft SWA criteria. More information is available at: http://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/RecycledWater.shtml.

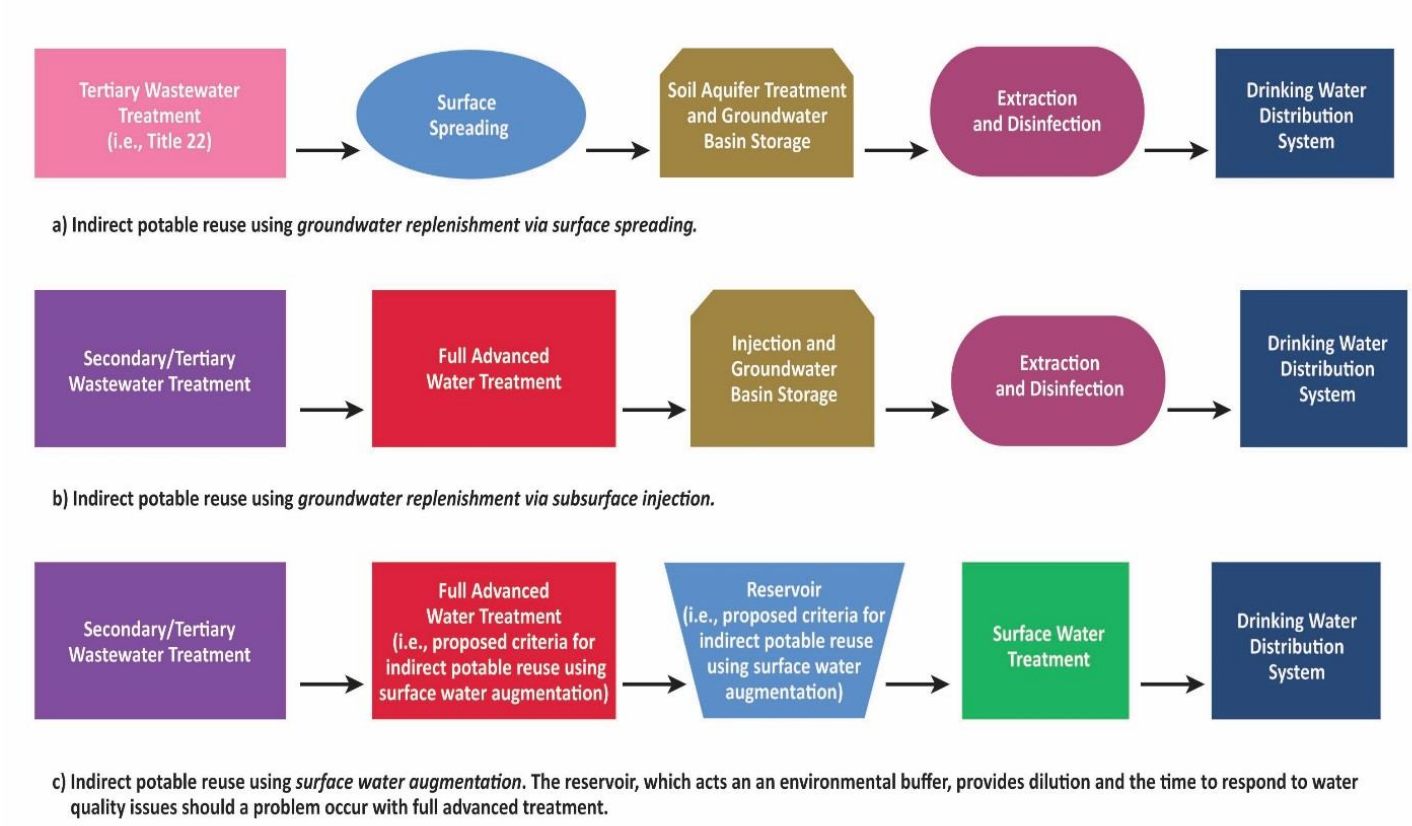

Figure 3.2. Schematics of (a,b) indirect potable reuse in California using groundwater replenishment and (c) surface water reservoir augmentation (per revised CWC, SWRCB 2017b). The environmental buffer is represented by a groundwater aquifer in (a) and (b), and by a reservoir in (c). Wastewater treatment could include either secondary or tertiary treatment. Tertiary treated wastewater per Title 22 involves well-oxidized, filtered, and disinfected wastewater. Soil-aquifer treatment involves the percolation of water through the vadose zone, which provides soil treatment. In California, full advanced treatment per Title 22 requires reverse osmosis and an oxidation treatment process. Drinking water treatment for surface water meets California drinking water standards (Olivieri et al., 2016).

3.2 Assessing Relative Risk to Human Health for Non-Potable Reuse Scenarios

The 2018 Panel was charged with evaluating the potential human health risks associated with exposure to CECs in non-potable reuse applications allowed under Title 22. Such reuse applications include 20 exposure scenarios where recycled water is used for irrigation, three scenarios associated with recycled water in impoundments, two scenarios associated with cooling and air conditioning, and 18 other scenarios classified as "other uses" (Appendix C). The Panel was asked to evaluate potential risks for all routes of exposure, excepting the potential exposures associated with consumption of crops irrigated with recycled water. For most of these exposure scenarios the most likely exposure routes are incidental ingestion of or dermal contact with recycled water containing CECs.

Given that quantifying the potential exposure and risk for all possible CECs in over 40 exposure scenarios is beyond the scope of what the Panel could complete in the time and resources available, the Panel developed an alternate approach to the evaluation of the human health risks associated with various recycled water uses allowed in California. That approach relies on comparing the exposure to recycled water in the non-potable reuse scenarios to exposure to water produced for groundwater recharge via surface spreading application (GWR-SA), a potable reuse scenario.

In addition, the relative exposure analysis is based on a comparison of water quality at the point of application for the GWR-SA scenario. This approach is consistent with that relied upon for the Panel's 2010 CEC analysis (Anderson et al., 2010). Specifically, the Panel noted that

> *"…these reuse practices engage conventional and advanced water treatment processes that result in very different water qualities, the Panel chose a conservative approach in comparing MECs to MTLs for the exposure screening that was proposed to select priority CECs for monitoring programs. This conservative measure considered a water quality that represents a secondary or tertiary treated effluent quality meeting California's Title 22 requirements for urban irrigation. These MECs were also chosen as a representative wastewater effluent quality for groundwater recharge practices using surface spreading or direct injection (DI) into a potable aquifer."* (Anderson et al., 2010).

GWR through surface spreading application was selected as a conservative basis for representing potable reuse because treatment levels at the point of application are similar to those for most non-potable uses[14]. Other potable reuse approaches would typically utilize more multiple barriers and thus would not allow for a similar basis of comparison.

Further, typical potable use scenarios assume people ingest between 2 and 2.4 liters of water per day for a lifetime and are, thus, exposed to CECs in 2 to 2.4 liters of ingested water. The relative risk associated with non-potable use scenarios can be estimated by comparing the

[14] The need to evaluate the potential exposure from groundwater potentially impacted by recycled water as allowed under Title 22 was included in the Panel charge. Clarification offered by State Water Board staff indicates that irrigation with reclaimed water meeting Title 22 (i.e., secondary/filtered and disinfected) occurs during the winter season and may co-mingle with groundwater and could be extracted through local shallow wells. The Panel discussed the charge and notes that the State IPR-Ground Water Recharge regulations currently address the criteria necessary for surface application of Title 22 water that will reach local groundwater and criteria relevant to eventual extraction. In addition, the Panel notes that the State also has criteria/guidance for the construction of wells to protect local groundwater. Thus, the Panel believes that the alternative described by the State Water Board staff is addressed by current State regulation and criteria.

amount of recycled water to which a person might be exposed in a non-potable reuse scenario to the amount of water a person is exposed to in a potable use scenario.

3.2.1 Exposure pathways and assumptions

The comparison of relative exposure is straightforward for the ingestion pathway. The exposure routes are the same in both scenarios. Therefore, the amount of recycled water assumed to be ingested in a non-potable reuse scenario can be compared directly to the amount assumed to be ingested in a potable reuse scenario (i.e., 2-2.4 liters per day). Comparison of potential dermal exposures in non-potable use scenarios to ingestion exposure in potable scenarios is more complicated as it involves estimating and comparing relative CEC doses associated with the dermal and ingestion pathways. What follows is a summary of ingestion and dermal exposure assumptions for non-potable reuse scenarios.

3.2.1.1 Ingestion

Review of the non-potable water reuse scenarios allowed in California (Appendix C), reveals no scenarios in which ingestion of recycled water would even approach a daily ingestion rate of 2 liters per day for 350 days per year for 30 years, the exposure assumptions used by California when establishing drinking water criteria consistent with the federal SDWA. Review of Table 3.2 indicates that ingestion exposure associated with potentially high exposure non-potable scenarios is most likely incidental, comprised of a few mL per day, and only on the days that a person engages in the activity when recycled water is present.

Table 3.2. Estimated level of human exposure for several high exposure non-potable water recycling uses.

Reuse Type	Exposure Activity	Volume Consumed per event	Duration of event	Frequency per year	Consumption Rate (mL/day)
Recreation – Impoundment	Swimming	35 (mL/hr)[1]	1.8 to 3.1 (hrs/month)[2]	6 (mo/yr)	1 to 1.8
Landscape Irrigation	Golf/Parks	0.12 to 12 (mL/event); median 6 (mL/event)[3]		25 events/yr	0.01 to 0.8

1 – average of child and adult (USEPA, 2011)
2 – average to 95th% (USEPA, 2011)
3 – Tanaka et al. (1998)

Thus, ingestion exposures associated with non-potable use of recycled water are likely to be much smaller than potable use exposures (i.e., more than three orders of magnitude lower than potable use scenarios or less than 0.1% of potable water consumption).

3.2.1.2 Dermal

Comparing potential dermal exposures in non-potable use scenarios to potable use ingestion exposures is more complicated. The amount of water that one is exposed to dermally is not as easily estimated and compared to the amount ingested. Additionally, even if the amounts of water that a person is exposed to via ingestion and dermal exposure are equal, the amount of CEC that is absorbed will differ because the exposure routes differ. CECs in ingested water are absorbed via the gastrointestinal tract lining (a membrane that has evolved with the purpose of facilitating absorption) while CECs in water contacting the skin are absorbed across the skin (a membrane that has evolved to limit absorption). Further, the rate of absorption of

CECs across the skin can be greatly affected by the physiochemical properties of each CEC. Fortunately, USEPA has estimated the relative magnitude of the dermal and ingestion doses for 200 chemicals (USEPA, 2004).

The USEPA (2004) comparison of ingested to dermal exposure to chemicals in water is based on default assumptions about ingestion and dermal exposure for an adult. The ingestion exposure estimates use typical default assumptions, including a drinking water ingestion rate of 2 liters per day, for 350 days per year, for 30 years. The dermal exposure assumptions reflect a showering exposure. Those assumptions assume an adult showers 35 minutes a day, for 350 days a year, for 30 years and that while showering a person's entire skin surface area (18,000 cm^2) is exposed to water containing chemicals. For the majority of organic chemicals, USEPA finds that estimated dose from dermal exposure is less than 10% of the ingestion exposure and dermal doses are smallest for organic chemicals that have either a low octanol-water partitioning coefficient (K_{ow}) or that are ionized. For non-ionized chemicals that have a high K_{ow}, estimated dermal exposures can exceed estimated ingestion exposures; for some chemicals, such as PCBs, the exceedance can be substantial, more than 10-fold, though USEPA cautions that all of the compounds with such large exceedances are either outside of the effective prediction domain of the dataset used to estimate skin permeability coefficients based on K_{ow} or are halogenated compounds for which the equation may underestimate the skin permeability coefficient (USEPA, 2004; Exhibit B-3). The greatest contribution of dermal exposure is 2.7 times the ingestion exposure for a compound that USEPA has not noted with one of the above two caveats (methylene bis(N,N'-dimethyl)aniline, 4,4'-).

Thus, based on the USEPA evaluation, dermal exposures for the majority of chemicals are substantially smaller than potable use ingestion exposures. Further, when using the information presented in the USEPA (2004) report to evaluate the magnitude of potential dermal exposures associated with non-potable use of recycled water, several considerations must be kept in mind. All of these suggest that the relative magnitude of non-potable use dermal exposures will be even lower relative to potable use ingestion exposures than the above-described estimates of dermal exposure associated with the showering scenario.

Frequency of exposure

First, USEPA's scenario assumes dermal exposure to water 350 days a year, for 30 years. While is it is possible that some people may be engaged in the activities listed as non-potable uses allowed in California for 30 years, daily exposure for 350 days a year is unlikely for most non-potable use scenarios. Most, if not all, recreational uses will have substantially lower exposures, including potentially high exposure non-potable use scenarios, such as swimming in an impoundment. While swimming in an impoundment will result in a person's entire surface area being exposed to water, just as in USEPA's showering scenario, as noted in the table above, people are expected to swim in an impoundment less than four hours per month. USEPA's showering scenario assumes more than five times as many hours spent showering (about 17 hours per month). Even most workers at locations where recycled water is used daily, are likely to be exposed no more than 250 days a year (5 days a week times 50 weeks a year), the default number of work days assumed by USEPA in commercial/industrial scenarios (USEPA, 2014a). Thus, exposure frequency will be lower for most non-potable use scenarios. That in turn will lead to a lower relative contribution of potential dermal exposures than suggested by USEPA (2004).

Presence of recycled water

Second, USEPA's showering scenario assumes, as it should, that every time a person showers, he or she will contact water. That is not likely to be the case for many of the non-potable use scenarios. Landscapers, farm workers, recreational users, construction workers, may engage in their respective activities at locations where recycled water is used, but it may not be used at the particular time that the person is at that location. For example, golfers may be on the course at a time when the course is dry and not be exposed to any recycled water even though the course is irrigated with recycled water. Thus, the possibility that contact with recycled water will not occur every time a person engages in an activity that has the potential to bring them into contact with recycled water, will also lead to a lower relative contribution of potential dermal exposures than suggested by USEPA (2004).

Surface area exposed

Third, USEPA's showering scenario assumes that the entire skin surface area of a person's body is covered with water. That is unlikely to be the case for virtually all of the non-potable uses of recycled water, with the possible exception of exposures at impoundments, if such exposure includes swimming in the impoundment. Most of the non-potable uses are likely to have only the hands, and perhaps the arms, of a person exposed to recycled water. Hands comprise approximately 5% of an adult's entire surface area (USEPA, 2011). Arms comprise about 14% of an adult's entire surface area (USEPA, 2011). Note as well that the preceding estimates of percent of surface area are for both sides of the hands and all sides of the arms. Most non-potable use scenarios involve touching of objects such as vegetation, tools, sporting equipment and playground structures with just one side of the fingers and the palm of a person's hand. Thus, exposed skin in most non-potable use scenarios is more likely to be 2 - 3% of total surface area. That means that for the majority of compounds, if the difference in surface area between the showering scenario used by USEPA and dermal exposures that are likely in the non-potable reuse scenarios is accounted for, dermal exposures are likely to be less than 0.2% - 0.3% of the drinking water ingestion exposures (dermal exposures for the majority of compounds was less than 10% of ingestion exposures assuming entire body surface area is exposed; if only 2 - 3% of surface area is exposed, dermal exposures will be 0.2%-0.3% of ingestion exposures). For compounds where showering contributed nearly three times the exposure of drinking water (e.g., methylene bis(N,N'-dimethyl)aniline, 4,4'-), the dermal exposure from exposure via the hands would be 6% - 9% of the ingestion dose (300% x 2% - 3% = 6% - 9%). Thus, accounting for only a portion of a person's surface area contacting recycled water in non-potable use scenarios also leads to a lower relative contribution of potential dermal exposures than suggested by USEPA (2004).

Length of exposure event

Finally, USEPA's showering scenario assumes that for the entire 35 minutes of the exposure period (i.e., while the person is showering), water is continuously contacting a person's skin. That is unlikely to be the case for many of the non-potable use scenarios. Contact with recycled water is more likely to be intermittent; only while the person is touching vegetation, sporting equipment, construction equipment, etc. that is wet because of recycled water. Such intermittent exposure will also lead to a lower relative contribution of potential dermal exposures than suggested by USEPA (2004). Taken together, all of these factors suggest that dermal exposures associated with non-potable use of recycled water are likely to be less than

10% of potable use ingestion exposures (upon which the potable use MTLs presented in Section 4 are based) for all CECs, and are likely to be less than 1% for most CECs.

3.2.1.3 Other pathways

The above evaluation of the relative exposure associated with non-potable uses of recycled water compared to potable use exposures focused on ingestion of and dermal contact with recycled water. These two exposure pathways are assumed to be possible for all four of the categories of recycled water reuse allowed in California (i.e., Irrigation, Impoundments, Cooling or Air Conditioning, and Other Uses (Table 3.1). These two exposure pathways are also assumed to be the only pathways for the non-potable uses listed under irrigation and other uses. Additional exposure pathways are possible for the scenarios listed under Impoundments and Cooling or Air Conditioning. Potential consumption of fish living in impoundments has the potential to lead to CEC exposure. Inhalation of airborne mist as part of industrial or commercial cooling or air conditioning is also possible. Each of these pathways is discussed in more detail below.

Consumption of fish from impoundments

If impoundments containing recycled water are used for recreational fishing, it is possible for fish to take up CECs from impoundment water and, if those fish are consumed, for people to be exposed to the CECs in fish. The magnitude of exposure will depend upon the bioaccumulation of CECs by fish and the amount of fish consumed by people. Bioaccumulation is highly dependent upon the physiochemical characteristics of the CEC. CECs with low K_{ow} values that are ionized or that are metabolized by fish generally have low bioaccumulation factors (BAF). Fish consumption exposures for such CECs are low relative to exposures associated with potable water use. CECs that have high K_{ow} values, are not ionized, and are not metabolized, generally have high BAFs. For such CECs, exposure via fish consumption can be relatively high compared to exposures associated with potable water use (i.e., consumption of drinking water).

BAFs can be viewed as the number of liters of water of containing CECs in each kilogram of fish tissue. Thus, a BAF of 1 (liter of water per kilogram of fish (L/kg)) indicates that a kilogram of fish tissue contains the same amount of a CEC as found in one liter of water in which the fish lives. A BAF of 3,000 L/kg indicates that every kilogram of fish contains as much CEC as is found in 3,000 liters of water in which the fish lives. Knowing the amount of fish that a person is assumed to consume from an impoundment and assuming potable use criteria/standards are based on a drinking water consumption rate allows one to determine the BAF at which fish consumption exposures would be the same as drinking water exposures. That information can then be used to identify compounds for which fish consumption exposures may equal or exceed drinking water exposures. When setting national ambient surface water quality criteria, USEPA (USEPA, 2015) assumes that U.S. residents consume 0.022 kilograms of freshwater and estuarine fish and shellfish per day. Given that one liter of water weighs one kilogram, one can estimate that CECs with a BAF equal to about 91 L/kg (2 kilograms of water divided by 0.022 kilograms of fish) will have fish consumption exposures equal to drinking water exposures. Thus, assuming that people are catching and eating fish from an impoundment, and doing so at a frequency that results in a daily fish consumption rate of 0.022 kilograms of fish per day (equal to about 3 meals of fish a month, assuming a fish meal is about 0.2 kilograms), it is possible for fish consumption exposures to exceed drinking water exposures for CECs that have BAFs that exceed about 90 L/kg. The impoundment scenario is

likely to apply to few CECs (for example PFOS) and also appears to be limited to a few impoundments in California. The evaluation of exposure to fish associated with the scenario should be conducted on a case-by-case basis, most likely through the CEQA process.

Inhalation

Non-potable use of recycled water is also allowed for two scenarios classified as cooling and air conditioning. The relative magnitude of potential exposures associated with repair of cooling and air conditioning units would be similar to those discussed above. However, such scenarios also include creating a mist that, hypothetically, could result in inhalation of recycled water. As a conservative estimate of such exposure, the Panel assumed that the amount of mist in the air from an evaporator that a person might inhale could be the same as the amount of water in a cubic meter of fog. Information from the web (http://wxguys.ssec.wisc.edu/2011/09/12/how-much-condensed-liquid-water-is-in-a-cubic-mile-of-fog/) indicates that a cubic mile (mi^3) of fog contains 56,000 gallons (gal) of water. That estimated volume of water corresponds to 5.085×10^{-5} liters of water in 1 cubic meter of air (56,000 gal/1 mi^3 x (3.785 L/1 gal) x (1 mi^3/4.168×10^9 m^3 = 5.085×10^{-5} L/m^3). Assuming a person breathes 20 m^3/day of air means such a person would breathe in 1.02×10^{-3} liters of mist per day, assuming the mist from the evaporator was as dense as occurs in a fog. Such an exposure seems unlikely even for upset conditions, never mind normal operating conditions for a cooling or air conditioning unit. Even in such conditions the potential exposure is more than 1,000 times lower than potable use exposures.

3.2.2 Summary of exposure pathways for non-potable reuse scenarios

The Panel was charged with evaluating the potential human health risks associated with CECs in non-potable reuse applications allowed under Title 22 including: 20 exposure scenarios where recycled water is used for irrigation; three scenarios associated with recycled water in impoundments; two scenarios associated with cooling and air conditioning; and 18 other scenarios classified as "Other Uses" (Appendix C). The Panel was asked to evaluate potential risks for all routes of exposure, excepting the potential exposures associated with consumption of crops irrigated with recycled water. The Panel developed an approach (previously described in section 3.3 above) that relies on comparing the exposure to CECs in recycled water in the non-potable reuse scenarios to exposure to CECs in water produced for groundwater recharge via surface water application, a conservative potable use scenario for the comparison. That comparison revealed that potential exposures and risks associated with CECs in non-potable use scenarios allowed under Title 22 are expected to be lower than exposure to CECs in water in a conservative potable use scenario.

The comparison revealed that total exposures (i.e., ingestion, dermal and inhalation pathways combined) associated with non-potable use scenarios are less than 10% of potable use ingestion exposures (upon which the potable use MTLs presented in Section 4 are based) for all CECs, and are likely to be less than 1% for most CECs. The possible exception to that conclusion are CECs that have the potential to bioaccumulate in fish living in impoundments that are used for fishing and are supplied by recycled water. The overall finding by the Panel of low potential exposure and risk associated with non-potable use scenarios is consistent with the earlier findings of Kennedy et al. (2012) for select CECs and a subset of the non-potable reuse scenarios allowed under Title 22.

3.3 Human Health Considerations for Potable Reuse Scenarios

Public health protection requires that microbiological pathogens and chemicals in wastewater be removed before discharge to the environment (as commonly practiced throughout the world) or for other uses (e.g., non-potable and potable reuse). Generally, low concentrations of non-pathogenic microorganisms and chemicals are not harmful; therefore, a public health goal is not to eliminate all chemicals and microorganisms, but rather ***to limit human exposure to concentrations of chemicals and pathogens that may be harmful to human health***. Such maximum allowable concentrations of potentially harmful agents are established as standards. In the United States, these standards for drinking water are known as "maximum contaminant levels" (MCLs) for chemicals and as "$\log_{10}$ reduction values" (LRVs) for pathogenic microorganisms.

Microbial contaminants, including bacteria, viruses, and protozoan parasites, are the most critical constituents to control in reclaimed water due to the potential human health impacts resulting from short-term exposure. Most effects arise shortly after exposure, although chronic sequelae of acute infection are known to occur. Among the large number of chemical constituents that can be present in reclaimed water, some are of concern due to their potential adverse health effects associated with both short- and long-term exposures (NRC, 2012).

Microbial and chemical contaminants in water produced for reuse may have adverse effects on human health depending on the concentration (a function of the effectiveness and reliability of the treatment system), route (i.e., skin, inhalation and consumption), frequency and duration of exposure. In addition, wastewater used as a source of drinking water raises aesthetic issues related to taste and odor, which can impact public acceptance of potable reuse projects (Agus et al., 2011). While conventional wastewater treatment in California provides a wastewater effluent quality that is suitable for discharge to surface water and subsequent use, treated wastewater effluents still contain a wide range of naturally occurring and anthropogenic trace organic and inorganic contaminants, residual nutrients, total dissolved solids (TDS), residual heavy metals, and pathogens mixed in with those that occur in receiving waters (Drewes and Khan, 2011).

It is important to regulate important constituents that may result in adverse human health impacts. Determining which constituents to regulate can be challenging, but has been done for non-potable reuse, unplanned potable reuse, planned DPR through SWA, and IPR-GWR. Both SWA and IPR-GWR as defined by the State of California, maintain conventional public health practices by utilizing a physical separation (i.e., environmental buffer) between wastewater treatment and water supply. The following sections provide a summary of the key criteria contained in the IPR-GWR regulations (CCR, 2015) and the draft SWA criteria (SWRCB, 2017b).

3.3.1 Planned potable reuse criteria for groundwater replenishment

The GWR regulations address the supplementing of groundwater through surface or subsurface application of treated municipal wastewater prior to later extraction via drinking water wells for potable use as previously shown in Figure 3.1. The California criteria for groundwater recharge reflect a cautious approach toward potential short- and long-term health concerns. The criteria rely on a combination of controls intended to maintain a microbiologically and chemically public health protective groundwater recharge operation and protect current and future potable groundwater supplies. The criteria specify source

control, wastewater treatment processes, water quality, recharge methods (i.e., surface spreading versus DI), dilution, extraction well location, and monitoring frequencies and locations. The State Water Board requires monitoring of additional constituents for unregulated chemicals (e.g., chromium-6, diazinon, 1,4-dioxane, N-nitrosodimethylamine (NDMA), and 1,2,3-trichloropropane) using approved drinking water analytical methods, where available and practicable, and will specify other methods where necessary (e.g., for certain endocrine disrupting chemicals, pharmaceuticals, personal care products). DDW notes that monitoring for these chemicals—or categories of chemicals—is a diligent way of assessing and verifying recycled water quality characteristics, which can be useful in addressing issues of public perception about the safety of recharge projects.

For GWR projects, four indicator compounds based on their toxicological relevance (i.e., N-nitrosodimethylamine, 17β-estradiol, caffeine, and triclosan) were included in the State Water Board Recycled Water Policy (SWRCB, 2013b) based on the 2010 Panel report (Anderson et al., 2010; Drewes et al., 2013). In addition, four additional CECs (N,N-diethyl-meta-toluamide (DEET), gemfibrozil, iopromide and sucralose) were identified for surface spreading and DI operations as viable performance indicator compounds along with certain surrogate parameters (e.g., ammonia, dissolved organic carbon, conductivity), which differ by the type of reuse practice. The Panel emphasized that the compounds identified represented an initial list based on the limited data that were available at that time and several qualifying assumptions. Additional information on the Panel's recommended phased and performance-based approach for implementing CEC reclaimed water monitoring programs and the recommended multi-tiered framework for interpreting the resulting data is available in the following references (Anderson et al., 2010; Drewes et al., 2013; and SWRCB, 2013b)[15]. A summary of the key criteria contained in the State Water Board IPR-GWR regulations is presented in Table 3.3.

Table 3.3. Summary of key groundwater recharge regulation criteria (2014).

	Groundwater Recharge Requirements	
Criteria	**Surface Spreading Application (SA)**	**Subsurface Application (Direct Injection)**
Pathogenic Microorganisms Secondary treatment Filtration Disinfection	Secondary (oxidized), filtered and disinfected recycled water[1] ≤2 NTU (avg. in any 24-hour period) ≥5-log virus inactivation, ≤ 2.2 total coliform per 100 mL	Secondary (oxidized), reverse osmosis[3], and an advanced oxidation process[3]
Downgradient Monitoring	One location at least no less than 2 weeks or more than 6 months of travel through saturated zone and at least 30 days upgradient from nearest drinking water well. Additional well required between groundwater replenishment reuse project (GRRP) and nearest downgradient drinking water well.	One location no less than 2 weeks nor more than 6 months of travel from the GRRP and at least 30 days upgradient from nearest drinking water well. Additional well required between GRRP and nearest downgradient drinking water well.

[15] On February 3, 2009, the State Water Board adopted Resolution 2009-0011, Adoption of a Policy for Water Quality Control for Recycled Water (Recycled Water Policy) (Revised January 22, 2013, effective April 25, 2013)

Alternatives Clause	State Water Board-DDW consider approval of alternative treatment and/or TOC monitoring for proposals providing same level of public health protection (regulations identify specific approach)	Same as for SA projects
Pathogen reductions at compliance point (finished potable water)[2] SA - After Soil Aquifer Treatment DI - At point of injection	12,10,10 – log reductions of viruses, *Giardia,* and *Cryptosporidium*, respectively	12,10,10 – log reductions of viruses, *Giardia,* and *Cryptosporidium*, respectively
Environmental Buffer – Allowable Reduction Credits	1-log virus reduction credit for each month retained underground 10-log reduction credit for *Giardia* and *Cryptosporidium* if the municipal wastewater is retained underground for at least 6 months	1-log virus reduction credit for each month retained underground
Control nitrogen compounds	TN ≤ 10 mg/L in recharge water (recycled water or combination of recycled water and credited diluent water used for recharge)	Same as for SA projects
Regulated contaminants	Meet all drinking water MCLs (except nitrogen), action levels for lead and copper, notification levels, priority pollutants, and any other chemicals specified by State Water Board-DDW	Same as for SA projects
Retention Time Underground Documentation Time Underground	Tracer[3] study – retention time set at T_2 of initial tracer concentration or T_{10} of peak tracer at the downgradient monitoring well Minimum of 2 months	Same as for SA projects

Table 3.3. (Continued) Summary of Key Groundwater Recharge Regulation Criteria.

Criteria	Surface Spreading Application (SA)	Subsurface Application (Direct Injection)
Recycled Water Contribution (RWC)[4] Initial Operation Maximum RWC	≤20% Up to 100% (see note 2) plus TOC performance over 20 weeks meets TOC_{max} ≤ 0.5 mg/L / RWC preceding SAT (with State Water Board-DDW approval)	No initial maximum recycled water contribution (injecting 100% recycled water may be approved)
TOC & SAT Process	TOC performance over 20 weeks meets TOC_{max} ≤ 0.5 mg/L / RWC in - Undiluted recycled water - Diluted percolated recycled water with the value amended to negate effect of dilution, or - Undiluted recycled water adjusted by SAT factor	NA
Advanced Treatment Criteria	NA	Oxidized wastewater (secondary treatment) with RO and oxidation treatment process (e.g., AOP) (RO and oxidation process require meeting specified performance requirements)
Diluent Water	Implement monitoring program, quality not to exceed primary MCLs or a secondary MCL upper limit, meet nitrogen controls and	Same as for SA projects

	notification levels, determine volume for credit. (Initial RWC ≤20%)	
Source Control and Outreach	Industrial monitoring and investigation	Same as for SA projects
Unregulated Contaminants	Data collection for pharmaceuticals, endocrine disruptors and other State Water Board Policy CEC indicators/surrogates (see Table 4.1 "CECs to be Monitored" in State Water Board Recycled Water Policy, April 25, 2013 and Table 2.1 of this report)	Same as for SA projects
Response to Off-Spec Water	Prior to operation of a GRRP, approval of a plan describing steps that will be taken to provide an alternative source of drinking water, or an approved treatment mechanism a project sponsor will provide all owners of a producing water well, that as a result of the GRRP operation: (1) violates a California or federal drinking water standard; (2) has been degraded to a degree that it is no longer safe for drinking: or (3) receives water that fails to meet pathogen reduction levels specified in the recycling criteria.	Same as for SA projects

[1]See Title 22 requirements for disinfected filtered (section 60301.320) and tertiary (section 60302.230) recycled water.
[2] The treatment train consists of 3 separate processes, maximum credit of 6 – $\log_{10}$ red per process and minimum of 1-$\log_{10}$ reduction per process
[3] Log_{10} reductions vary based on tracer approach and method used estimate retention time contained in June 18, 2014 updated regulations (refer to Title 22 CCR, Division 4)
[4] Increasing RWC requires meeting a number of criteria. For example, a health effects study must be conducted including and exposure assessment, review of available epidemiology studies, and evaluation of individual and cumulative effects of regulated contaminants.
[5] NA = not applicable; NTU = nephelometric turbidity unit; RWC = the percent recycled water contribution in groundwater extracted by drinking-water wells; SAT = soil aquifer treatment; TOC = total organic carbon.

3.3.2 Planned potable reuse criteria for surface water augmentation

On February 14, 2017 the State Water Board released a Public Notice (BDDW-16-12 SWA) for the consideration of adopting surface water augmentation regulations a part of CCR Title 22. The SWA regulations establish *minimum* uniform water recycling criteria for the purpose of adequately protecting public health with respect to the planned placement of recycled water into a surface water reservoir that is used as a source of domestic drinking water supply. Existing law required the State Water Board to adopt uniform water recycling criteria for SWA by December 31, 2016; subject to the condition that a statutorily mandated DPR/SWA Expert Panel has made a finding that such criteria would adequately protect public health, which has occurred[16] (SWRCB, 2017b).

The State Water Board has indicated that portions of the existing IPR-GWR regulations and the proposed SWA regulations are comparable and that SWA regulations would not be inconsistent or incompatible with existing State Water Board IPR-GWR regulations. A

[16] On October 31, 2016, the DPR/SWA Expert Panel found: *"The Expert Panel finds, in its expert opinion, that the State Board's proposed uniform water recycling criteria for surface water augmentation titled, 'Surface Water Augmentation Using Recycled Water,' as provided in Appendix C (October 12, 2016), adequately protects public health. This finding, submitted by the Expert Panel on October 31, 2016, represents the collective expert opinion of all members of the DPR/SWA Panel." The DPR/SWA Panel reviewed revised criteria dated October 31, 2016 and found that the criteria adequately protect public health (SWRCB, 2017b). On November 13, 2017 the DPR/SWA Panel re-affirmed its opinion on the SWRCB staff revised criteria developed to respond to public comments.*

summary of the key criteria where differences between the two regulations occurs is contained in Table 3.3.

An advanced water treatment facility, see Figure 3.2c, is required to meet a minimum of the 8-$\log_{10}$ enteric virus, 7-$\log_{10}$ *Giardia* cyst, and 8-$\log_{10}$ *Cryptosporidium* oocyst reduction criteria and is intended to produce a source of drinking water as treatable as an existing source to a surface water reservoir prior to augmentation with treated recycled water. As described in Table 3.3, compliance with these reductions requires a number of multiple barriers including secondary treatment, filtration, disinfection, reverse osmosis (RO), and advanced oxidation processes (AOP).

For SWA, the benefits of the reservoir as an environmental buffer lie primarily in the form of contaminant attenuation to mitigate the potential consequences of a AWTF treatment failure. As a result, the attenuation is not considered part of the treatment train and may not be used as credit to meet the other proposed regulatory requirements associated with contaminant control and removal for SWA projects. To ensure the reservoir provides a meaningful environmental buffer, two types of requirements associated with the robustness of a reservoir are proposed in subsections, the first, the theoretical residence time is an operational requirement, and the second, dilution is a performance-based criterion.

- Theoretical Residence Time (Tr) – Operational Criteria: for a reservoir to be used as part of a SWA project, the reservoir must initially be able to provide a Tr of at least 180 days (monthly basis); the proposed criteria allow the operating agency the option of submitting an application for a reduced minimum Tr of no less than 60 days. Such applications are considered on a case-by-case basis. The minimum Tr requirement establishes a simple operational criterion to ensure that the reservoir is of sufficient size to be able to provide greater opportunity for responding to and potentially mitigating significant treatment failures. Thus, a Tr of less than two months is not considered a DPR project under the current SWA proposed criteria.
- Dilution – Performance-Based Criteria: The proposed SWA criteria require a 100:1 dilution in the reservoir with the minimum pathogen reduction of 8, 7 and 8 $\log_{10}$ reductions for enteric virus, *Giardia* and *Cryptosporidium*, respectively, and an allowance for 10:1 dilution but requiring an additional $\log_{10}$ reduction for all three categories of pathogens.

In addition to the AWTF treatment, additional treatment by a surface water treatment plant (SWTP), as shown in Figure 3.2 and Table 3.4, is required to comply with California SDWA requirements for treatment of the drinking water supplied by the reservoir. The SWTP includes an additional set of barriers that are designed to provide 4-$\log_{10}$ enteric virus, 7-$\log_{10}$ *Giardia* cyst, and 8-$\log_{10}$ *Cryptosporidium* oocyst reduction prior to distributing the potable water for consumption.

Table 3.4. Summary of key surface water augmentation (SWA) criteria[17].

Surface Water Augmentation (SWA) Requirements	
Criterion	**Requirement**
Advanced Treatment Oxidation Reverse Osmosis Advanced oxidation process	See Title 22 requirements for advanced treatment criteria (section 60320.650) Oxidized wastewater (primary and secondary treatment) with RO and oxidation treatment process (i.e., AOP) (RO and oxidation process require meeting specified performance requirements)
Alternatives Clause	State Water Board-DDW will consider approval of alternative treatment proposals providing same level of public health protection (regulations identify specific approach, see Section 60320.330)
Pathogen reductions at compliance points [2] Finished Potable Water	Minimum - 12,10,10 – $\log_{10}$ reductions of viruses (V), *Giardia (G),* and *Cryptosporidium (C)*, respectively
Advanced Water Treatment Facility (AWTF)	Minimum – 8,7,8 – $\log_{10}$ reductions of V, G, C. based on 100:1 dilution additional log reductions for all organisms with 10:1 dilution
Surface Water Treatment Plant (SWTP)	Minimum 4, 3, 2 – $\log_{10}$ reductions of V, G, C.
Environmental Buffer – Allowable Reduction Credits	No treatment credit demonstrated
Reservoir Theoretical Retention Time (Tr, months)	Tr requires hydrodynamic modeling and tracer study
Documentation	6 months (checked monthly)
Initial (Tr months) Alternative Tr	Minimum 2 months (additional pathogen treatment will need to be evaluated and may be required)
Regulated contaminants	Meet all drinking water MCLs
TOC process requirement	No TOC requirement; used as high-frequency monitoring surrogate for process performance
Alternative supply (or additional treatment)	Ensure capability to provide reliably, safe and wholesome supply of drinking water
Source Control and Outreach	Industrial monitoring and investigation
Unregulated Contaminants	Data collection for pharmaceuticals, endocrine disruptors and other State Water Board Policy CEC indicators/surrogates (see Table 1 "CECs to be Monitored" in State Water Board Recycled Water Policy, April 25, 2013)
Monitoring and Response to Off-Spec Water	High-Frequency AWTF process monitoring and response in 24hrs to off-spec production and potential release to reservoir Additional surrogate monitoring for pathogen $\log_{10}$ reductions and threshold criteria to address operational issues
Distribution System Monitoring	Assess and address potential impacts resulting from the introduction of advanced treated water into distribution system

[1] See Title 22 requirements for disinfected filtered (section 60301.320) and tertiary (section 60302.230) recycled water.
[2] The treatment train consists of 3 separate processes, maximum credit of 6 – $\log_{10}$ reduction per process and minimum of 1-$\log_{10}$ reduction per process.

[17] Criteria listed are based on State Water Board 15-day Public Notice dated November 30, 2017 and Proposed Surface Water Augmentation Recycled Water criteria dated October 31, 2017. (Public Notice period closed December 18, 2017).

4. Assessment of CEC Monitoring Programs in California following Recommendations of the CEC Expert Panel 2010

4.1 Summary of Current Status of Monitoring Program

As a result of the original Panel's final report in December 2010 that considered the state-of-the-science regarding CEC monitoring in recycled water applications at that time, the State Water Board adopted in 2013 as an important concept the Panel's recommendation of a risk-based framework to identify relevant CECs for potential inclusion in monitoring programs as specified in Attachment A of the Recycled Water Policy. Considering this adoption into the Recycled Water Policy and other activities of the State Water Board until 2017, the 2018 Panel concludes that the State Water Board has followed through on some, including perhaps the most important, but not all recommendations the Panel provided in the 2010 report.

While the inability to adopt all of the 2010 Panel's recommendations may have been due to limited resources or other priorities (see Table 2.2 and discussion in Chapter 2), the Panel would like to stress that all of its recommendations represent important steps in assisting the State Water Board to continuously stay abreast and ahead of rapid changes regarding CEC production, fate, transport, treatment and toxicological relevance. Due to the uncertainty that is inherently associated with the universe of chemicals that might occur in recycled water, the need to establish a more responsive review and updating process that addresses rapidly developing CEC issues is critical. Identifying and incorporating new information on occurrence and toxicity provides the basis for adding new CECs to the framework (i.e., an on-ramp) as well as for removing CECs that do not (or no longer) pose a risk to human health (i.e., an off-ramp). New knowledge might also point to direct evidence for health relevance justifying the need for a nimble response by the State Water Board that cannot be provided by convening a review panel only every five years or longer.

Applying the risk-based framework recommended by the 2010 Panel requires structure and consistent protocols yet no formal review/update of the selected CECs recommended for recycled water monitoring occurred until 2018 (see Table 2.2). This update was provided by the 2018 Panel and evaluated measured environmental concentrations (MECs) reported by California utilities in secondary/tertiary treated effluents as feed water for potable reuse projects. In addition, new toxicological information was gathered to identify changes to previously defined monitoring trigger levels (MTLs). Considering these updated MEC data and MTLs for the 2010 CEC database, MEC/MTL ratios were compiled to identify relevant chemicals for recycled water monitoring.

In addition, previously suggested performance-based indicators and surrogates were evaluated to determine their suitability for assessing the performance of indirect potable reuse treatment processes and practices. The outcome of this evaluation is documented in Chapter 5.

4.2 Measured Environmental Concentrations (MECs)

4.2.1 Data sources

In preparation of this review, the 2018 Panel requested CEC monitoring data from recycled water facilities across California to assess the relevance and utility of health- and/or performance-based indicators recommended in the 2010 report and additional CECs for which data may also be available. The Panel created a standard data template that identified

water quality for, as a minimum, the eight Panel-recommended CECs measured at various locations (Table 4.1) that was circulated to entities engaged in recycled water monitoring.

Table 4.1. CEC monitoring data requested by the 2018 Panel.

2010 Panel Recommended CECs
17β-estradiol
Caffeine
NDMA
Triclosan
Gemfibrozil
Iopromide
DEET
Sucralose

The Panel received responses from eight water reuse facilities operating in California and one facility outside California. The breadth of data submitted varied widely, in both frequency of monitoring (e.g. weekly to annually), time period and duration of monitoring (i.e., single or multiple years; 2008 to 2017) and target analytes reported. The data were parsed to highlight data for the eight Panel-recommended CECs (Table 4.1) and additional CECs, which were mostly reported for secondary effluent as feed water for facilities that provide recycled water for potable reuse and Title 22 non-potable reuse applications addressed in the Recycled Water Policy. In addition, the Panel reviewed available monitoring data for individual treatment processes, IPR product water and groundwater monitoring wells.

4.2.2 Comparison of MECs in the 2010 and 2018 Panel reports

Because monitoring data were relatively scarce and, in many cases, highly variable for individual CECs in 2010, the Panel at that time selected the 90th percentile of the distribution of CEC concentrations reported in California as a conservative MEC screening value. The 2018 Panel compared MECs for individual CECs from the 2010 report to utility data for the period 2008 to 2017. In 2018, based on the information provided by utilities the availability of MEC data remains highly variable across individual CECs, however, available datasets for selected CECs are more extensive than available in 2010 with some target analytes having hundreds of data points collected over multiple years. MECs reported in secondary/tertiary effluents are generally less variable, which is likely due to occurrence levels significantly above the method reporting level and more consistent and sensitive analytical methods and use of standardized QA/QC procedures.

To populate the updated database, the 2018 Panel compiled and reported 90th percentile concentration values where rich (i.e., n>30) databases were available for extended time periods. The comparison of 90th percentile MECs for 51 CECs reported in secondary/tertiary treated effluents in 2010 and in 2018 is summarized in Figure 4.1. The observed change in concentration ranges from relatively large decreases (e.g., 8.4 to 0.5 ng/L for E2) to moderate increases (e.g., 26,000 to 40,000 ng/L for sucralose) to essentially no change (217 to 220 ng/L for dilantin (also known as phenytoin). The updated MEC values of CECs for 2018 were also used to screen for suitable performance indicators as documented in Chapter 5.

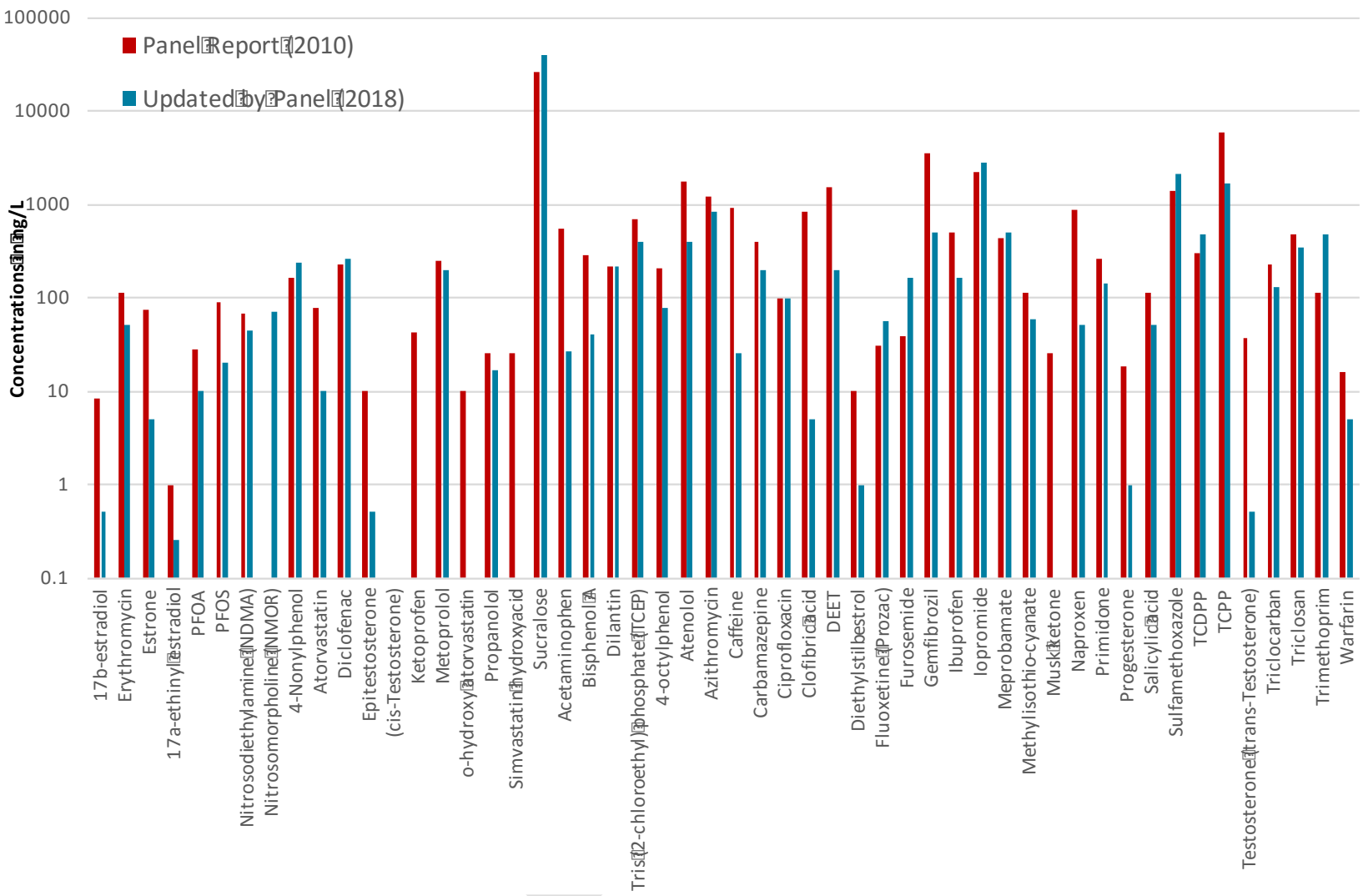

Figure 4.1. Comparison of 90th percentile measured environmental concentrations (MECs) reported in secondary/tertiary treated effluents compiled during the 2010 and 2018 Panel reviews (concentrations in ng/L).

4.3 Monitoring trigger levels (MTLs)

4.3.1 Non-potable reuse applications

In 2010, the Panel developed two unique sets of MTLs corresponding to different degrees of exposure to recycled water assumed for the two water reuse practices evaluated, i.e., potable water reuse (via groundwater recharge) and non-potable landscape irrigation. The 2010 Panel reviewed the relative exposure associated with the potable reuse and non-potable landscape irrigation practices and determined that landscape irrigation exposures were substantially smaller than potable reuse exposures. Based on that finding, landscape irrigation MTLs were set at 100 times the concentration of potable reuse MTLs.

As described in Chapter 3, the charge to the 2018 Panel was expanded to include all of the non-potable reuse practices allowed under Title 22 except for exposures associated with consumption of food crops and surface water augmentation[18]. The 2018 Panel recommends that MTLs for potable reuse applications including SWA be derived using the same methods as described in the 2010 Expert Panel report. Similar to the 2010 Panel, this Panel compared the potential exposure associated with the various Title 22 non-potable to potable reuse practices. With the exception of the non-potable reuse application associated with potential fish consumption from an impoundment fed with recycled water where a higher risk might exist due to the presence of bioaccumulative and persistent CECs (such as perfluorinated chemicals), exposures associated with the other Title 22 non-potable reuse practices were estimated to be at least 10 times lower than exposures associated with the potable reuse applications for all CECs and likely to be more than 100 times lower for most CECs (see discussions in Chapter 3). However, because of the expanded number of non-potable reuse practices (i.e., 45 different applications instead of a single landscape irrigation practice) and the inclusion of the dermal and inhalation pathways in addition to ingestion of recycled water, the Panel concluded that MTLs for non-potable water reuse practices should be derived by increasing the potable use MTLs by a factor of 10 (instead of the factor of 100 recommended by the 2010 Panel).

For the special application of a non-potable reuse practice that includes consumption of fish from an impoundment receiving treated water, the Panel recommends that the State Water Board identify unrestricted recreational impoundments where Title 22 water is a significant source of water and evaluate the level and types of uses (including estimated level of fish consumption) to establish a database for potential consideration of developing a future pilot study. The need for a future pilot study might be triggered based on future Panel review of CEC data[19].

4.3.2 Updated list of Predicted No Effect Concentrations (PNECs) and MTLs

In the 2010 report, the Panel summarized PNECs for 418 CECs from seven sources (see Appendix J in Anderson et al., 2010). Those PNECs served as the basis for the development of interim MTLs used by the 2010 Panel to identify CECs to include in a preliminary statewide monitoring program. As part of the update of the evaluation of toxicological relevance of

[18] Consistent with its charge from the SWRCB, the Panel did not evaluate potential exposures associated with ingestion of crops irrigated with recycled water.

[19] A proposed study, if needed, should collect fish tissue samples from a recreational impoundment and also from a reference water body that does not receive recycled water. The concentrations of bioaccumulative and persistent CECs (such as PFOS and PFOA) in the respective waters can then be compared to determine if: (1) the fish tissue concentrations in the impoundment receiving recycled water are higher than those in the reference water; and (2) if the concentrations are higher, whether they pose a potential risk to fish consumers.

CECs, the 2018 Panel identified several new sources that have compiled PNECs or health advisory data for CECs, namely the USEPA Tapwater Regional Screening Levels or RSLs (USEPA, 2017), the Minnesota Department of Health (2015), the WRRF-15-01 final report (WE&RF, 2016), the German Environment Agency (2016), and the best professional judgement of the Panel itself. This expanded review resulted in additional CECs (see Table D.1, Appendix D) which were added to the CEC master list. In addition, revisions to the USEPA CCL3 to create the CCL4 list resulted in the inclusion of two new compounds (manganese and 4-nonylphenol). The 2018 Panel also removed compounds from the 2010 CEC list that have MCLs, and thus by definition are not CECs (see Table D.2, Appendix D). These updates increased the number of CECs considered by the Panel from 418 to 489 (see Appendix D, Table D.3).

MTLs were based on the PNECs reported by the sources listed above. The selection of a specific MTL followed a process that gives different weight to PNECs developed by the different sources. The greatest weight is given to PNECs developed by the State of California to derive drinking water MCLs and notification levels. If California has developed a PNEC for a CEC, the MTL is set equal to that PNEC for that CEC. If California has not developed a PNEC for a CEC, the lowest of either the USEPA Tapwater Regional Screening Level (RSL) or CCL concentration is used as the MTL for that CEC. If neither California nor USEPA has developed a PNEC for a CEC, the lowest of PNECs available from the other sources, not including PNECs developed by the German Environment Agency, is used as the MTL for that PNEC. If the only available PNEC for a CEC is from the German Environmental Agency, that PNEC is used as the MTL for that CEC.

This review exercise resulted in the identification of MTLs for CECs that were lower (Table 4.2) or higher (Table 4.3) than those developed by the 2010 Panel based on the information presented in the 2010 Expert Report for the same CECs.

Table 4.2. Updated list of monitoring trigger levels (MTLs) (in ng/L) for CECs in recycled water that were lower than the 2010 report.

CEC	2010		2018	
	MTL (ng/L)	Reference	MTL (ng/L)	Reference
1,1,1,2-Tetrachloroethane	1.0E+03	CCL[b]	5.7E+02	USEPA (2017)[i]
1,1-Dichloroethane	6.1E+03	CCL	2.8E+03	USEPA (2017)
2,4,6-Trichlorophenol	1.8E+04	Cotruvo[f]	4.1E+03	USEPA (2017)
2,6-Dinitrotoluene	6.0E+03	Cotruvo	4.9E+01	USEPA (2017)
4,4'-DDE	2.0E+04	Australia (2008)[d]	4.6E+01	USEPA (2017)
4,4'-DDT	2.0E+04	Australia (2008)	2.3E+02	USEPA (2017)
4-Nonylphenol (4NP)	5.0E+05	Australia (2008)	1.1E+05	CCL
Acetaldehyde	2.3E+04	CCL	2.6E+03	USEPA (2017)
Acrolein	3.5E+03	CCL	4.2E+01	USEPA (2017)
Albuterol	4.1E+04	Schwab (2005)[c]	2.0E+04	MDH[g,h]
Atenolol	7.0E+04	AwwaRF (2008)[e]	4.0E+03	WE&RF (2016)
Atorvastatin	5.0E+03	Australia (2008)	1.0E+03	MDH
Atrazine	2.0E+03	Cotruvo	3.0E+02	USEPA (2017)
Azobenzene	3.0E+03	Cotruvo	1.2E+02	USEPA (2017)
Benzyl alcohol	3.0E+06	Cotruvo	2.0E+05	USEPA (2017)

Betaxolol	1.0E+04	Australia (2008)	4.0E+03	MDH
Butylated hydroxytoluene (2,6-Di-tert-Butyl-p-Cresol)	1.0E+06	Australia (2008)	3.4E+03	USEPA (2017)
Butylbenzyl phthalate	1.2E+06	Cotruvo	1.6E+04	USEPA (2017)
Chlordane (gamma-chlordane)	1.0E+03	Australia (2008)	2.0E+01	USEPA (2017)
Chlorpyrifos	1.0E+04	Australia (2008)	8.4E+03	USEPA (2017)
Clarithromycin	2.5E+05	Australia (2008)	6.0E+04	MDH
Clindamycin	3.0E+05	Australia (2008)	7.0E+04	MDH
Cobalt	7.0E+04	CCL	6.0E+03	USEPA (2017)
Codeine	2.9E+04	Schwab (2005)	5.0E+03	MDH
Cotinine	1.0E+04	Australia (2008)	1.0E+03	WE&RF (2016)
Demeclocycline	3.0E+05	Australia (2008)	6.0E+03	MDH
Diazinon	1.4E+03	CCL	1.2E+03	CA[a]
Dibromochloromethane	8.0E+04	Cotruvo	8.7E+02	USEPA (2017)
Dichloroacetic acid	7.0E+03	Cotruvo	1.5E+03	USEPA (2017)
Dichlorodiphenyldicloroethane (DDD)	1.0E+03	Cotruvo	3.2E+01	USEPA (2017)
Dichlorvos	1.0E+03	Australia (2008)	2.6E+02	USEPA (2017)
Diethylhexyl phthalate	4.2E+05	AwwaRF (2008)	5.6E+03	USEPA (2017)
Digoxin	1.0E+03	Schwab (2005)	4.0E+00	MDH
Diltiazem	6.0E+04	Australia (2008)	4.0E+04	MDH
Disulfoton	9.1E+02	CCL	5.0E+02	USEPA (2017)
Doxycycline	1.1E+04	Australia (2008)	8.0E+02	MDH
Ethylene oxide	1.1E+02	CCL	6.7E-01	USEPA (2017)
Fenoprofen	4.5E+05	Australia (2008)	2.0E+04	MDH
Fluoxetine (Prozac)	1.0E+04	Australia (2008)	2.0E+03	MDH
Fyrol FR 2 (tri(dichlorisopropyl phosphate)	1.0E+06	Australia (2008)	3.6E+05	USEPA (2017)
Hydrazine	1.0E+01	CCL	1.1E+00	USEPA (2017)
Isophorone	4.0E+05	Cotruvo	7.8E+04	USEPA (2017)
Malathion	9.0E+05	Australia (2008)	3.9E+05	USEPA (2017)
Meprobramate	2.6E+05	AwwaRF (2008)	1.0E+05	MDH
Metformin	2.5E+05	Australia (2008)	4.0E+04	MDH
Methamidophos	2.1E+03	CCL	1.0E+03	USEPA (2017)
Methyl tert-butyl ether (MTBE)	1.9E+04	CCL	1.4E+04	USEPA (2017)
Mirex	4.8E+03	Cotruvo	8.8E-01	USEPA (2017)
Naproxen	2.2E+05	Australia (2008)	2.0E+05	MDH
Nitrobenzene	1.4E+04	CCL	1.4E+02	USEPA (2017)
Norfloxacin	4.0E+05	Australia (2008)	1.0E+05	MDH
Oxytetracycline	1.1E+05	Australia (2008)	6.0E+03	MDH
Parathion-methyl (methyl parathion)	1.0E+05	Australia (2008)	4.5E+03	USEPA (2017)
PCB 169	1.6E-02	Australia (2008)	4.0E-03	USEPA (2017)
Perfluoroctane sulfonate (PFOS)	2.0E+02	CCL	7.0E+01	CA

Perfluorooctanoic acid (PFOA)	1.1E+03	CCL	7.0E+01	CA
Phenytoin (Dilantin)	6.8E+03	AwwaRF (2008)	2.0E+03	WE&RF 2015
Propranolol	4.0E+04	Australia (2008)	4.0E+03	MDH
Pyrene	1.5E+05	Australia (2008)	1.2E+05	USEPA (2017)
Risperidone	4.9E+02	AwwaRF (2008)	7.1E+01	MDH
Silver	1.0E+05	Australia (2008)	9.4E+04	USEPA (2017)
Simvastatin	1.9E+04	AwwaRF (2008)	2.0E+02	MDH
Temazepam	5.0E+03	Australia (2008)	8.0E+02	MDH
Tetracycline	1.1E+05	Australia (2008)	2.0E+04	MDH
Toluene	4.8E+05	Cotruvo	1.1E+05	USEPA (2017)
Toluene diisocyanate	9.0E+02	CCL	1.7E+01	USEPA (2017)
Trifluralin	5.0E+04	Australia (2008)	2.6E+03	USEPA (2017)
Trimethoprim	6.1E+04	Schwab (2005)	4.0E+04	MDH
Xylenes (total)	5.0E+05	Cotruvo	1.9E+05	USEPA (2017)

Notes: a. From CA Department of Public Health (2007). Drinking Water Notification Levels and Response Levels: An Overview. Drinking Water Program.

b. From USEPA CCL 3 and CA PCC Dossier of Chemicals

c. From Table 6 in Schwab et al. (2005). Human pharmaceuticals in US surface waters: a human health risk assessment. Regulatory Toxicology and Pharmacology 42: 296-312.

d. From Tables 4.4, A1, A2, A8a, and A8b in Environment Protection and Heritage Council et al. (2008). Australian Guidelines for Water Recycling. Augmentation of Drinking Water Supplies. May 2008.

e. From Tables 9.1 and 9.2 in Snyder et al. (2008). Toxicological Relevance of EDCs and Pharmaceuticals in Drinking Water. Awwa Research Foundation. 484 pp.

f. From Table 3.2 in Cotruvo et al. (2010). Identifying Health Effects Concerns of the Water Reuse Industry and Prioritizing Research Needs for Nomination of Chemicals for Research to Appropriate National and International Agencies

g. From Pharmaceuticals Screening Water Values 2015 and Supporting Information Excel file, "All Data and Values" tab. Pharmaceutical Water Screening Values Report. Minnesota Department of Health (MDH). August 2015.

h. MTLs shown as derived by the Minnesota Department of Health (MDH) are 10 times higher than those shown in the original MDH tables. The screening values in the original MDH tables are based on infant exposure assumptions that assume daily water ingestion is about 10 times greater for infants than adults on a kilogram bodyweight basis.

i. From USEPA November 2017 RSL table for tapwater.

j. WE&RF (2016), final report WRRF-15-01.

Table 4.3. Updated list of monitoring trigger levels (MTLs) (in ng/L) for CECs in recycled water that were higher than the 2010 report.

CEC	2010		2018	
	MTL (ng/L)	Reference	MTL (ng/L)	Reference[g]
2,4,5-Trichlorophenol	1.8E+04	Cotruvo[a]	1.2E+06	USEPA (2017)
2,4-Dichlorophenol	1.8E+04	Cotruvo	4.6E+04	USEPA (2017)
2,4-Dimethylphenol	1.0E+05	Cotruvo	3.6E+05	USEPA (2017)
2-Phenylphenol	1.0E+03	Australia (2008)[b]	3.0E+04	USEPA (2017)
Acetone	5.4E+06	Cotruvo	1.4E+07	USEPA (2017)
Acetophenone	4.0E+05	Australia (2008)	1.9E+06	USEPA (2017)
Aluminum	2.0E+05	Australia (2008)	2.0E+07	USEPA (2017)
Anthracene	1.5E+05	Australia (2008)	1.8E+06	USEPA (2017)
Azinphos-methyl	3.0E+03	Australia (2008)	5.6E+04	USEPA (2017)

Benzoic acid	2.4E+07	Cotruvo	7.5E+07	USEPA (2017)
Bromomethane	6.0E+03	Cotruvo	7.5E+03	USEPA (2017)
Chloral hydrate	6.0E+05	Cotruvo	2.0E+06	USEPA (2017)
Chlorfenvinphos	4.2E+03	Cotruvo	1.1E+04	USEPA (2017)
Chlorpropham	1.2E+06	Cotruvo	7.0E+07	USEPA (2017)
Chlorpyrifos-methyl	1.0E+04	Australia (2008)	1.2E+05	USEPA (2017)
Cypermethrin	5.0E+02	Australia (2008)	1.2E+06	USEPA (2017)
Dalapon	1.8E+05	Cotruvo	6.0E+05	USEPA (2017)
Demeton-S	1.5E+02	Australia (2008)	4.2E+02	USEPA (2017)
Dibutyl phthalate	3.1E+05	Cotruvo	9.0E+05	USEPA (2017)
Dibutyltin (DBT)	2.0E+03	Australia (2008)	6.0E+03	USEPA (2017)
Di-n-butyl phthalate	1.4E+04	Australia (2008)	9.0E+05	USEPA (2017)
Endosulfan	3.6E+04	Cotruvo	1.0E+05	USEPA (2017)
Endrin	1.8E+03	Cotruvo	2.3E+03	USEPA (2017)
Fluorene	2.4E+05	Cotruvo	2.9E+05	USEPA (2017)
Methomyl	1.5E+05	Cotruvo	5.0E+05	USEPA (2017)
Methoxychlor	7.0E+02	AwwaRF (2008)[d]	3.7E+04	USEPA (2017)
N-nitrosomorpholine (NMOR)	1.0E+00	Australia (2008)	1.2E+01	USEPA (2017)
Oxamyl	6.0E+03	Cotruvo	5.0E+05	USEPA (2017)
Parathion (ethyl parathion)	1.0E+04	Australia (2008)	8.6E+04	USEPA (2017)
PCB 105	1.6E-02	Australia (2008)	4.0E+00	USEPA (2017)
PCB 118	1.6E-02	Australia (2008)	4.0E+00	USEPA (2017)
PCB 156	1.6E-02	Australia (2008)	4.0E+00	USEPA (2017)
PCB 167	1.6E-02	Australia (2008)	4.0E+00	USEPA (2017)
PCB 77	1.6E-02	Australia (2008)	6.0E+00	USEPA (2017)
Phenol	1.5E+05	Australia (2008)	5.8E+06	USEPA (2017)
Prometon	9.0E+04	Cotruvo	2.5E+05	USEPA (2017)
Pyridine	6.0E+03	Cotruvo	2.0E+04	USEPA (2017)
Tributyl phosphate	5.0E+02	Australia (2008)	5.2E+03	USEPA (2017)
Tributyltin (TBT)	1.0E+03	Australia (2008)	6.0E+03	USEPA (2017)
Tributyltin Oxide	9.0E+00	Cotruvo	5.7E+03	USEPA (2017)
Triphenylphosphine oxide (TPPO)	2.8E+04	Schriks et al. (2009)[e]	3.6E+05	USEPA (2017)

Notes: a. From Table 3.2 in Cotruvo et al. (2010). Identifying Health Effects Concerns of the Water Reuse Industry and Prioritizing Research Needs for Nomination of Chemicals for Research to Appropriate National and International Agencies

b. From Tables 4.4, A1, A2, A8a, and A8b in Environment Protection and Heritage Council et al. (2008). Australian Guidelines for Water Recycling. Augmentation of Drinking Water Supplies. May 2008.

c. From CA Dept of Public Health (2007). Drinking Water Notification Levels and Response Levels: An Overview. Drinking Water Program.

d. From Tables 9.1 and 9.2 in Snyder et al. (2008). Toxicological Relevance of EDCs and Pharmaceuticals in Drinking Water. Awwa Research Foundation. 484 pp.

e. From Table 2 in Schriks et al. (2009). Toxicological relevance of emerging contaminants for drinking water quality. Water Research, doi: 10.1016/j.wateres.2009.08.023.

g. All 2018 MTLs are equal to USEPA November 2017 tapwater RSLs.

4.4 Updated MEC/MTL Analysis to Identify Health-Based Indicator CECs

The updated MECs and MTLs (described above in Sections 4.2 and 4.3, respectively) were employed in the human health screening process used by the 2010 Panel to update the list of CECs to monitor for protection of human health. Note that the screening level process allows CECs to be added or removed (on- and off-ramping) from the list as new information becomes available. The basis for this decision is the MEC/MTL ratio. This ratio is operationally defined and while informed by human health toxicological information, is not comparable to a primary maximum contaminant level (MCL), and a ratio of greater than 1.0 does not represent immediate threat to public health. Further, as discussed in Section 5.6, a significant margin of safety is incorporated into the selection of appropriate MEC and MTL values. Table 4.4 presents the human health-based indicator CECs from 2010. Table 4.5 presents the updated 2018 human health-based indicator CECs.

Table 4.4. 2010 Exposure screening for CCL3 and non-CCL3 CECs in recycled water (from Anderson et al., 2010)[20].

	Secondary/Tertiary Treated MEC 90th (ng/L)	Initial MTLs		MEC/MTLs	
		Potable Reuse	Irrigation	Potable Reuse	Irrigation
CCL3 CECs					
17β-estradiol	8.40	9.0E-01	9.0E+01	9.3	0.09
NDMA	68	1.0E+01	1.0E+03	6.8	0.07
Non-CCL3 CECs					
Caffeine	900	350	35,000	2.6	0.03
Triclosan	490	350	35,000	1.4	0.01

Table 4.5. 2018 Exposure screening for CECs in recycled water.

	Secondary/Tertiary Treated MEC 90th (ng/L)	Initial MTLs		MEC/MTLs	
		Potable Reuse	Title 22 Non-potable	Potable Reuse	Title 22 Non-potable
17β-estradiol	0.50	9.0E-01	9.0E+00	0.6	0.06
NDMA[a]	77	1.0E+01	1.0E+02	7.7	0.77
NMOR	107	1.2E+01	1.2E+02	9.2	0.92
1,4-Dioxane[a]	7,200	1.0E+03	1.0E+04	7.2	0.72
Caffeine	25	3.5E+02	3.5E+03	0.07	<0.01
Triclosan	340	3.5E+02	3.5E+03	0.97	0.1

[a] Monitoring of NDMA and 1,4-Dioxane are part of the State Water Board monitoring requirements for potable reuse.

[20] Please note that the MTLs for irrigation in the 2010 Panel Report were incorrectly stated with a factor of 10x instead of 100x higher than for potable reuse applications. The correct values are reported here.

Comparison of the two tables reveals that as a result of the updated MEC and MTL information, three of four 2010 health-based indicator CECs (17β-estradiol, triclosan and caffeine) are no longer included in the 2018 health-based indicator list. All three of those compounds were removed from the list because the updated large monitoring data sets collected by California utilities over the past seven years indicate that concentrations are consistently below MTLs (i.e., the MEC/MTL ratio is equal to or less than 1) and that continued monitoring based upon potential human health concerns is no longer necessary.

For secondary/tertiary treated effluents, the 90th percentile concentration of NDMA is about eight times higher than the MTL and, therefore, NDMA is retained as a human health-based indicator. In addition, the MEC data collected since 2010 indicate that 90th percentile concentrations of N-nitrosomorpholine (NMOR) and 1,4-dioxane exceed the MTL by about 9-fold and 7-fold, respectively and thus warrant addition as human health indicators.

4.5. Summary of 2018 MEC/MTL Ratio Update

In summary, this Panel recommends that MTLs for potable reuse be derived using the same approach as described in the 2010 Expert Panel report. With the exception of the non-potable reuse practice of consumption of fish from an impoundment, the Panel recommends deriving MTLs for non-potable reuse by multiplying the potable reuse MTLs by a factor of 10.

The low MEC/MTL ratios derived for secondary/tertiary treated effluents based on the updated MTL and MEC data for almost all CECs provide further confirmation of the safety of potable and non-potable water reuse in California. Coupled with the large margin of safety inherent in the risk-based screening framework recommended by that Panel (see also discussion in Section 5.6), the updated MEC/MTL screening results indicates that it is very unlikely that any of the CECs for which current MEC data from California are available have the potential to pose a risk to public health. As discussed in several other places in this report, the Panel notes it is not aware of any evidence suggesting that potable and non-potable reuse following the treatment trains used by California utilities has been linked with adverse human health effects.

The Panel would like to note that the update of CEC monitoring requirements for recycled water summarized in this report also provides a conceptual foundation for consideration of monitoring requirements of DPR projects but that additional factors as discussed in the DPR Expert Panel report (Olivieri et al., 2016) need to be addressed (e.g., the lack of an environmental buffer).

DRAFT

5. Increasing Efficiency and Relevance of CEC Monitoring in Recycled Water

5.1 Introduction

Although the data collected per Attachment A of the Policy was invaluable in assessing the need to continue monitoring of CECs recommended by the Panel in 2010, the current Panel's efforts to update all recommendations delineated in the 2010 report were hampered, as previously discussed, by the State Water Board's lack of progress with implementation of previous Panel recommendations that would have allowed the effort to be more focused and efficient. A number of procedural recommendations regarding the permitting of potable water reuse projects, the management of potable water facility monitoring data (i.e., CEC, bioanalytical, and high-frequency operation data), the need to update CEC monitoring data, the external review of CEC data, and the reporting of potable water operations to the public are outlined in Chapter 2 (section 2.4). Moving forward, the independent Expert Panel for CECs in Recycled Water Applications should be used as a regular (i.e., on a triennial basis) independent peer review panel with the two main tasks of reviewing and, if appropriate, endorsing the State Water Board staff efforts in applying the risk-based framework as well as make recommendations to the State Water Board for Recycled Water Policy updates. Therefore, this chapter focuses on additional key elements the State Water Board should conduct as part of implementation of the risk-based framework in preparation for the next Panel review.

5.2 Timely Periodic Update of the Panel's Risk-Based Framework

Given that thousands of chemicals are potentially present in recycled water and that information about those chemicals is rapidly evolving, the Panel recommended that the State Water Board continue to rely on a transparent, science-based framework to guide prioritization of which CECs should be included in recycled water monitoring programs both now and in the future as additional data become available. The original framework proposed by the 2010 Panel required the following four steps that focused on CECs for which there are occurrence and toxicological information that are relevant to recycled water applications under consideration:

1. Compile MECs for CECs in the source water for reuse projects;
2. Develop a MTL for each CEC (or groups thereof) for which MECs are compiled based on toxicological relevance;
3. Compare the MEC to the MTL. CECs with a MEC/MTL ratio greater than "1" should be prioritized for monitoring. Compounds with a ratio equal to or less than "1" should only be considered if they represent viable treatment process performance indicators, and;
4. Screen the priority list of CECs to ensure that a commercially-available robust analytical method is available for each compound on the list.

As mentioned earlier, the CEC data gathered since the 2010 report following the requirements of Attachment A of the Recycled Water Policy were not readily accessible nor available in an electronic format that allowed for a timely and efficient update following the risk-based framework. However, the Panel did receive and review utility CEC data (see Chapter 4) that indicated MECs in recycled water were below updated MTLs for virtually all CECs with exception of NDMA, NMOR and 1,4-dioxane. Based on the current update, the 2018

Panel recommends that the risk-based approach be modified for future updates to continue to focus on health-based exposure screening but allow more flexibility in the use of non-California CEC data sources and consideration of environmental degradation and treatment process performance to populate the CEC monitoring list.

1. Develop a State Water Board staff protocol to collect CEC data that will allow a more efficient and effective update of the risk-based framework that should be completed and implemented prior to the Panel's next (triennial) review to be conducted in 2021. This protocol should include the following key tasks:
    - Collect MEC data for health- and performance-based indicator CECs and surrogate data in a machine-readable format (e.g., Microsoft Excel) and upload into a database so the data can be easily accessed for review and analysis. (The State Water Board, working with various IPR-GWR utilities, should consider if this effort could be efficiently conducted through modification of the current monitoring programs contained in existing NPDES/Waste Discharge Requirements).
    - Develop and implement an internal staff protocol for the collection and review of non-California MEC data for CECs not monitored in California at the present time.
2. Develop a State Water Board staff protocol to refine and update MTLs and review MEC/MTL results. MTLs for a given CEC derived using methods other than those recommended by the Panel in 2010 can vary for a variety of reasons, including differences in assumptions of exposure or toxicity, or both. The Panel recommends further refinement of both exposure and toxicity assumptions to improve consistency and basis of MTLs for all CECs.
    - The MTLs used by the Panel to update the list of CECs are based on exposure and toxicity assumptions developed by each original source (e.g., USEPA, Australia, Germany). The exposure pathways included in the development of the PNECs, and the assumptions specific to each pathway (e.g., water ingestion rate, RSC), vary between sources. The Panel recommends State Water Board staff update all PNECs such that they are based on exposure assumptions recommended in the 2010 Expert Report. If necessary, those assumptions can be reviewed and updated during the triennial review by the next Panel in 2021.
    - The acceptable daily intakes (ADIs) used to derive MTLs for all CECs should be updated to be consistent with the methods recommended in the 2010 Expert Report. The Panel recognizes this update represents a substantial effort. Such an update could be prioritized to focus on the CECs with the greatest potential to pose a risk to human health and on CECs with the greatest uncertainty regarding toxicity. To identify CECs with the greatest potential to pose a risk to human health State Water Board staff could rank CECs according to ADI and give higher priority to those CECs with the lowest ADIs. The difference between ADIs developed by different sources represents a measure of uncertainty surrounding toxicity. CECs with the largest range in ADIs would be given higher priority than CECs with a small range. Additionally, CECs with a single ADI value would be given higher priority than CECs with a small range (see Textbox 5.1).
    - State Water Board staff should combine updated ADIs with the exposure assumptions recommended in the 2010 Expert Report to develop MTLs and provide those to the next Panel for review and approval.

- Collect updates to MTLs through the collection of PNEC and health advisory data for constituents currently not listed in Table 5.1 and utilize the priority weighting protocol described in Section 4.3.2 to identify MTLs.
- State Water Board staff should review new CEC data collected from non-CA sources and develop MEC/MTL ratios to determine whether those CECs should be considered for inclusion on the monitoring list.

  State Water Board staff should review whether indicator CECs should remain on the list or be removed from the list based on the data collected and the protocol utilized by the Panel. The Panel is concerned that if CECs are added or removed from the list only every 5-7 years, several years of data may be collected unnecessarily if several quarterly samples document that the MEC/MTL ratio is consistently equal to or less than 1. Conversely, a concern might arise where data collected either in California or elsewhere suggest that MEC/MTL or PEC/MTL ratios could be greater than 1 but that such CECs are added to the list only upon Panel review. The CEC listing and de-listing process needs to be responsive to new data and developments as they occur and, ideally, not depend on a Panel triennial review.

  At the same time the Panel appreciates the importance of its role as a peer review and approval body in providing the public and stakeholders confidence that the CECs on the list, and changes proposed by State Water Board staff, are defensible and appropriate. As a compromise, the Panel recommends that State Water Board staff prepare a list of new CECs to be added to or removed from the monitoring list but that these on-ramps and off-ramps are subject to the next triennial Panel review in 2021 before the CEC list is ratified and changes in monitoring implemented on a statewide-basis.

3. <u>Develop a State Water Board staff protocol for collection and review of treatment process and special study data</u>.
   - Develop a staff protocol that provides a consistent framework for the consideration of factors related to the fate and transport of CECs in environmental buffers and the removal/reduction of CECs and bioactivity as measured by bioanalytical tools through potable reuse treatment trains and identify potential constituents that may pass the required treatment barriers. The Panel suggests working through utility trade organizations (e.g., WE&RF) and independent research groups (e.g., NWRI, SCWRRP, SFEI) to develop the protocol format and summarize available data.
   - Review of any special study investigations resulting from screening of MEC/MTL $\geq$ 100.
   - Collect and review available CEC production data from sources like high-production volume (HPV) chemical database (e.g., USEPA TSCA) to identify potential new CECs relevant to potable water reuse applications.
   - Continue to compile recommended analytical methods and MDLs for potential CECs and bioanalytical endpoints that will be added to the monitoring list.

Textbox 5.1 Toxicological Relevance of Caffeine

Caffeine provides an example of a CEC that would have been removed from the health-based indicator list even absent updated (lower) 2018 MEC data. The 2010 Expert Report used an MTL of 350 ng/l for caffeine based on a single PNEC developed by Australia (Australia (2008)). Australia's allowable intake of caffeine (1.5 ug/kg/day) is not derived from studies specific to caffeine but rather application of a Threshold of Toxicological Concern (TTC) based on caffeine's structure and anticipated mode of action. More recently, the European Food Safety Authority (EFSA, 2015) completed a review of the safety of caffeine and concluded that daily exposure to caffeine as high as 5.7 mg/kg/d from all sources in adults including lactating women, and of about 2.8 mg/kg/d in pregnant women, does not raise safety concerns. EFSA indicates that data from adults suggest that acute intakes of 3 mg/kg/d or less can serve as a basis to derive intakes of no concern for children and adolescents. EFSA also points out that exposures as low as 1.5 mg/kg/d may increase sleep latency in children and adolescents. The more recent EFSA review suggests that a more appropriate MTL for caffeine may be as much as 1000 times higher than the interim MTL used by the Panel in 2010. Even a 10-fold increase in the 2010 MTL would eliminate caffeine as a CEC requiring monitoring, even absent the more recent (lower) occurrence data.

5.3 New Strategies for Broader Screening of Relevant CECs in Recycled Water

To assist the State Water Board in responding in a timely fashion to rapid changes regarding CEC production, fate, transport, treatment and toxicological relevance as well as not yet identified CECs that might occur in recycled water, a formal process should be established that can help to identify and incorporate new information on occurrence and toxicity of potentially relevant CECs in recycled water.

5.3.1 Consideration of screening studies of CECs reported outside California

During the 2018 Panel's review process, limited information of MEC of CECs in secondary/tertiary treated effluents reported in studies outside California or the U.S. was available to the Panel. If these MECs are considered and extrapolated to California treatment plant effluents (i.e., accounting for different per-capita water consumption and therefore dilution of municipal wastewater), the MEC/MTL ratio may exceed 1 for five other CECs (such as benzotriazole, gabapentin, oxipurinol, valsartan acid, metformin). No information was available about measurement of these compounds in recycled water in California. However, this exercise underscores the fact that broader screening studies are a helpful approach to guide a proactive identification of new CECs potentially relevant to potable water reuse practices in California.

5.3.2 Bioanalytical monitoring methods

Bioanalytical methods are *in vitro* (cell or protein-based) and *in vivo* (whole animal) test systems that are capable of targeting a wide spectrum of CECs, and when calibrated, may also provide some indication of adverse effect. For unknown CECs, bioanalytical methods should be used in the future to quantify bioactivity/toxicity in recycled water projects while leading to the identification of previously unidentified chemicals of concern (see discussion below in Section 5.3.3). Chapter 7 provides additional detail regarding appropriate and commercially available bioassays as well as their utility for screening of recycled water quality. This bioanalytical approach is proposed as a screening level monitoring tool, targeting unknown CECs that complements proposed targeted analysis for select (health- or performance-based) indicator CECs as already implemented in Attachment A of the Recycled Water Policy. Thus, this screening level monitoring approach, which is amenable to the same interpretative framework that is applied for targeted CEC monitoring, may offer an additional early warning

safeguard for human health in particular for potable reuse applications, and a valuable tool in assessing levels of bioactivity across treatment trains.

5.3.3 Non-target analysis

To further enhance the screening for potential compounds for monitoring in recycled water, an additional option recommended by the Panel involves non-target analytical (NTA) evaluations. As discussed in detail in Chapter 6, NTA methods hold great promise for the identification of previously unknown substances in recycled water. However, it is important to understand the limitations, complexities, and costs of performing NTA (see Chapter 6). Thus, the Panel recommends that NTA is not suitable as a separate regular monitoring approach for monitoring of recycled water, but might assist in identifying compounds that are biologically active in water (e.g., measurable responses above or near screening trigger levels during bioanalytical investigations) or, similar to bioanalytical tools, to assess overall treatment efficiency of recycled water during special studies.

5.4 Revised Risk-Based Framework for CEC Monitoring

5.4.1 Screening of unmonitored CECs

While the Panel already emphasized the usefulness of the risk-based framework to consider on- and off-ramps in the selection of appropriate CECs for recycled water monitoring, the additional efforts to enhance screening for yet unmonitored CECs as discussed in this chapter result in an update of the risk-based framework (Figure 5.1) and the overall monitoring requirements for potable reuse practices as summarized in Table 5.1.

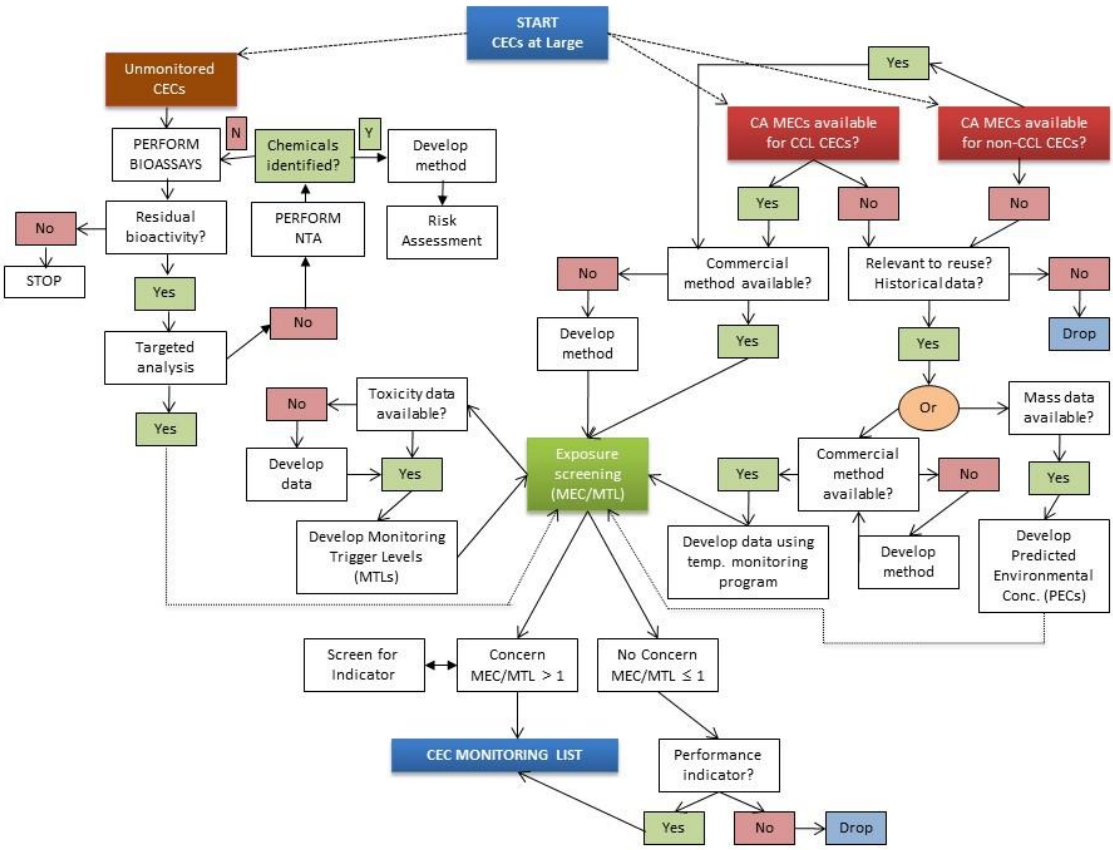

Figure 5.1. Revised risk-based CEC selection framework.

5.4.2 Revised monitoring of performance-based indicator CECs and surrogate parameters

The outcome of the updated human health-based MEC/MTL screening exercise demonstrated the usefulness of engaging in regular, targeted monitoring efforts for CECs in recycled water. This review process also informed the selection of appropriate performance-based indicator CECs, which should ideally occur at concentrations substantially above the method reporting level to demonstrate removal efficiency and exhibit consistent occurrence with low variability in secondary/tertiary effluents that serve as sources for recycled water.

Based on the update of MECs provided by California potable reuse facilities, caffeine was removed as a performance-based indicator due to its low occurrence level. Iopromide was replaced by iohexol, another X-ray contrast agent, which exhibits more consistent MECs and thus is better suited to serve as a performance indicator. DEET, an insect repellent and suggested as a performance-based indicator in the 2010 Panel report, exhibited declining and overall low MECs and was replaced by the antibiotic sulfamethoxazole (Table 5.1).

Surrogate parameters continue to serve as the core means to demonstrate process reliability, and thus continue to be recommended by the 2018 Panel for all Title 22 water reuse practices. For potable reuse applications, specific surrogate parameters are recommended that correlate with the removal of CECs during advanced water treatment processes. These surrogate parameters are augmented by differential UV absorbance and total fluorescence measurements for surface spreading operations. For non-potable reuse practices, the operational UV dose was added to account for reclamation facilities employing UV irradiation for disinfection.

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Table 5.1. Updated monitoring requirements for performance based indicator CECs and performance surrogates for potable and non-potable reuse practices.

Reuse Practice	Health-based indicator	MRL (ng/L)	Bioanalytical methods	MRL (ng/L)	Performance-based Indicator	Expected Removal [6]	MRL (ng/L)	Surrogate	Method	Expected Removal [6]
Surface Spreading Application (SAT)	NDMA[2]	2	ER	0.5	ΔGemfibrozil[3]	>90%	10	ΔAmmonia	SM	>90%
	NMOR[1]	2	AhR	0.5	ΔSulfamethoxazole[4]	>90%	10	ΔNitrate	SM	>30%
	1,4-Dioxane[1]	100			ΔIohexol[3]	>90%	50	ΔDOC	SM	>30%
					ΔSucralose[5]	>25%	100	ΔUVA	SM	>30%
								ΔTotal fluorescence		>30%
Subsurface Application (Direct Injection) and Surface Water Augmentation	NDMA[2]	2	ER	0.5	ΔSulfamethoxazole[4]	>90%	10	ΔConductivity	SM	>90%
	NMOR[1]	2	AhR	0.5	ΔSucralose[5]	>90%	100	ΔDOC	SM	>90%
	1,4-Dioxane[1]	100			ΔNDMA[2]	25-50%	2			
Irrigation (20 practices)					None			Turbidity	SM	
								Cl_2 residual or operational UV dose	SM	
								Total coliform	SM	
Impoundments (1 practice, not including 2 fish consumption exposure practices)					None			Turbidity	SM	
								Cl_2 residual or operational UV dose	SM	
								Total coliform	SM	
Cooling and air conditioning (2 practices)					None			Turbidity	SM	
								Cl_2 residual or operational UV dose	SM	
								Total coliform	SM	
Other uses (18 practices)					None			Turbidity	SM	
								Cl_2 residual or operational UV dose	SM	
								Total coliform	SM	

[1]Industrial chemical; [2]Disinfection byproduct; [3]Pharmaceutical residue; [4]Antibiotic; [5]Food additive; [6]travel time in subsurface two weeks and no dilution, see details in Drewes *et al.*, 2008; SM – Standard Methods; MRL – Method Reporting Limit.

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5.5 Relevance of Safety Factors

The Panel cannot stress strongly enough that the outcome of our review of the existing process, including the above findings, clearly points to the safety of potable and non-potable reuse in California. It is essential that all stakeholders and the public realize that the Panel's finding includes a very large margin of safety. That large margin of safety arises from conservative assumptions that are built into each step of the overall human health CEC screening process.

Derivation of MTLs. The MTLs depend upon assumptions about toxicity (e.g., the ADI) and about exposure assumptions (e.g., RSC, contact rates, exposure frequency and duration). ADIs incorporate several uncertainty factors to extrapolate effects from animals to acceptable intakes in humans. Those uncertainty factors typically range from 100 to 1,000. Most of the MTLs incorporate an RSC of 0.2, which may overestimate exposure from other sources and can lead to a margin of safety as high as 5 for some CECs. The drinking water ingestion rate and exposure duration assumptions used to derive potable use MTLs are upper percentiles and will overestimate potential exposure for most people in the population and provide a 2-10-fold margin of safety for the typical person. The comparison of non-potable use to potable use scenarios suggests that non-potable use MTLs incorporate a margin of safety of 100-fold (or more) for most people.

Point of monitoring (POM) and point of exposure (POE). The process the Panel used to screen CECs considered concentrations measured in secondary or tertiary treated wastewater effluent, not the point of exposure. Attenuation of CECs during advanced water treatment was not given any credit but these processes (including SAT, integrated membrane systems or advanced oxidation processes) represent very effective barriers against a wide range of CECs. As a result of dilution and dispersion, CEC concentrations will be further reduced in an environmental buffer. Post-treatment after abstraction either at the well-head (for GWR) or at a regular surface water treatment plant (for SWA) provide additional barriers to some CECs. Finally, blending with other drinking water sources might occur either prior to or in the drinking water distribution system before this water reaches the point of exposure.

Summary of Margin of Safety. The combination of these explicit and implicit conservative assumptions results in an overall margin of safety of at least 1,000-fold and perhaps exceeding 1,000,000-fold for the average person. The Panel appreciates that there may exist people with one or more exposure characteristics that could lead to higher exposures than assumed by the CEC screening process for that characteristic (e.g., water consumption rate, body weight). However, given the numerous conservative assumptions embodied throughout the screening process, and not just in the exposure assessment, the overall margin of safety is likely to be at least 10- to 100-fold, even for relatively highly exposed individuals.

5.6 Summary

Millions of chemicals are potentially present in recycled water and information about those chemicals is rapidly evolving. The Panel believes the 2018 update of the CEC monitoring list has demonstrated that the 2010 risk-based framework is an effective and dynamic tool for identifying CECs to monitor in recycled water to assure that public health is protected. However, the Panel is concerned that implementation of the framework may not be sufficiently dynamic and responsive to new information if CECs are added or removed from the list only every third year of a triennial review cycle. The Panel believes the CEC listing and

de-listing process needs to be responsive to new data and developments as they occur and, ideally, not depend on a triennial review. At the same time, the Panel appreciates the importance of its role as a peer review and approval body in providing the public and stakeholders confidence that the CECs on the list, and changes proposed by State Water Board staff, are defensible and appropriate. Thus, this Panel recommends that State Water Board staff develop a more responsive and dynamic CEC listing and delisting protocol (using the risk-based framework developed by the 2010 Panel and reinforced by this Panel) for consideration by the Panel during the next triennial review in 2021.

In the interim and until the next triennial review Panel is convened in 2021, this Panel recommends that State Water Board staff employ newly available data in the risk-based framework. If those data result in a MEC/MTL ratio that is greater than 1, State Water Board staff can suggest such CECs be added to the monitoring list for the next Panel review meeting. That Panel can review the data and process followed by State Water Board staff. Similarly, if new data indicate that the MEC/MTL ratio is equal to or less than 1 for a CEC on the current monitoring list, State Water Board staff can recommend removal of such a CEC on a triennial basis to the next Panel. Removal and additions can only occur upon the recommendation of the Panel. Requiring Panel review of CEC removal provides the public assurance that removal and additions can only occur based on expert peer review.

In summary, the key messages are:

- The Panel's risk-based screening framework was effective in evaluating the veracity of CEC monitoring.
- The process of accessing and evaluating existing monitoring data by the Panel was cumbersome and time-consuming, because of inconsistent data formatting and reporting.
- The Panel recommends that the State Water Board take a more active role in procuring and assessing routine CEC monitoring data.
- The Panel recommends that the State Water Board take a more active role in reviewing toxicological thresholds that can change based on availability of new data and interpretation of such data.
- The Panel recommends a more flexible, responsive program to assess and respond to CEC monitoring data.

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6. Monitoring of CECs using Analytical Chemistry

6.1 Introduction

The 2010 Panel provided detailed information regarding the various primary methods applied for the analysis of chemicals in complex environmental matrixes (Anderson et al., 2010). Since that time, a multitude of new reports, manuscripts, and books have been published to further demonstrate the breadth of chemicals that are detectable in the aqueous environment. Today, more than 135,000,000 chemicals are registered with Chemical Abstract Services (https://www.cas.org/) and more than 15,000 are added each day (Snyder, 2014). It is also safe to assume that product sales and usages also likely changed over this time. As a key example, the glucocorticoid drugs triamcinolone acetonide (e.g., Nasacort™) and fluticasone propionate (e.g., Flonase™) were available by prescription only until 2014 when the US Food & Drug Administration (FDA) approved them as over the counter medicines. Thus, it is difficult to say with any degree of certainty which new substances may now be detectable in the wastewaters of California and even more difficult to predict what transformation products may result from new chemistries entering the treatment systems.

Moreover, the diversity in the chemical universe ranges from single atoms (e.g., chloride) to highly complex biomolecules (e.g., lipopolysaccharides) with corresponding molecular weights ranging from single digits to hundreds of thousands of Daltons. The arrangement of atoms also results in polarities from very water-soluble to essentially insoluble and volatility states ranging from gaseous to solids at ambient temperatures. Without question, the chemical world is vast and highly complex and is evolving at a rate that cannot be evaluated by traditional risk assessment paradigms. Nor is a single analytical instrument alone capable of even scratching the surface of the chemical universe. Comprehensive chemical monitoring to identify each and every substance in the aqueous environment is vastly infeasible.

For these reasons, a portfolio of performance indicators and surrogate species are critical for process monitoring along with targeted analyses of known chemicals that may pose risk to public health at concentrations believed to occur in relevant water matrices. Unfortunately, no sample preparation step will capture the vast world of chemical constituents nor will a single analytical instrument be capable of a comprehensive analysis of chemicals in the environment. Thus, numerous extraction techniques under a variety of pH conditions along with a suite of often costly and complex instruments will be necessary to even begin to explore a small fragment of the vast world of chemical constituents present in the aqueous environment.

6.2 Extraction Issues

Nearly all analytical screening of water, including the chemical methods described in Chapter 6 and the bioanalytical tools described in Chapter 7, involves extraction and concentration of organic constituents from water samples prior to analysis. For instance, inorganic substances are rarely considered, including important oxyhalides such as perchlorate and bromate, as well as metals such as arsenic, chromium, cadmium, and others. Another important limitation is that no single extraction technique can capture all potential organic compounds. Most commonly, solid-phase extraction (SPE) is utilized to trap organic constituents within the cartridge, which are subsequently dried and eluted with organic solvents. The SPE technique will not capture all organic substances and volatile chemicals are not feasible to isolate using

SPE techniques. While some studies have advocated for the use of multiple classes of extraction cartridges, there are still limitations in the classes of chemicals that can be captured. In addition, some substances are strongly bound to SPE materials and are very difficult to elute; that is, some chemicals will bind so strongly to extraction cartridges that solvent elution will not completely release them from the media. Lastly, many polymeric SPE materials will leach organic constituents that may interfere with instrumental analyses and may react with *in vitro* bioassay systems to produce false positives or negatives. Liquid-liquid extraction (LLE) is yet another means to capture organic constituents present in water samples, but various solvents would be needed along with water adjusted to basic, acid, and neutral pH in order to extract the widest range of organic chemicals present. Moreover, LLE requires subsequent evaporation of organic solvents leading to losses of volatile chemicals.

As an example, one can consider four very commonly explored trace constituents: perchlorate, 17β-estradiol (E2), nitrosodimethylamine (NDMA), and 1,4-dioxane. Each of these substances requires a different type of extraction (if any) and each a very different methodology for detection. Quite likely, none of these substances would have been detectable by typical instrumental NTA approaches, though E2 was detected quite early in municipal wastewater effluents using sensitive *in vitro* bioanalytical methods (Desbrow et al., 1998; Snyder et al., 2001).

Extraction procedures for *in vitro* bioassays face similar challenges to those of instrumental analyses; however, the final extract generally will be an organic solvent of preferably low toxicity and reasonable water solubility. Most commonly, DMSO or methanol/ethanol is used. However, differences in cellular response may result from different solvents and/or concentration dosed into the cell assay. For instance, DMSO results in high cell membrane permeability and often results in higher bioactivity than the same extract dosed in an alternative solvent.

More recently, increased sensitivity of modern instruments coupled to more automated sample preparation techniques are certainly leading to increased throughput and often more reliable data. Of particular interest are “miniaturized” extraction and direct water injection techniques directly coupled to analytical instruments that tremendously reduce sample volumes required, thus reducing sample collection and handling of larger volumes of water along with savings in time, labor, solvents, and other laboratory supplies.

For instance, online SPE (OSPE) is now nearly the standard for semi-volatile/non-volatile trace organic chemical analysis coupled with LC-MS (Koal et al., 2003; Lopez-Roldan et al., 2004; Kot-Wasik et al., 2006; López-Serna et al., 2010; Anumol and Snyder, 2015). Online SPE allows for sample volumes generally at 2 mL or less to be automatically extracted and introduced directly into the LC-MS instrument, achieving similar method reporting limits (MRLs) to those methods that use conventional SPE, evaporative concentration, and subsequent injection into the LC-MS instrument.

6.3 Targeted Analyses

6.3.1 Advances in environmental chemical analysis

Analytical technologies have continued to evolve and become even more sensitive over the past seven years since the first Panel report was published in 2010. Essentially all vendors have released more sensitive versions of liquid chromatography (LC) and gas chromatography (GC) mass spectrometers, including new source designs which allow more ions to pass into

the mass spectrometer (MS). While the passage of more ions greatly increases sensitivity, it also greatly increases contamination within the MS, which leads to more required maintenance routines and more rapid loss of sensitivity as the source and internal features of the instrument become contaminated.

Beyond SPE or LLE, numerous methods are considering the direct analysis of water without any sample preparation. While direct analysis of water by GC is possible, it is far less amenable than LC since water has a very high expansion volume in the gas phase and salts and non-volatile residues would quickly contaminate GC inlets and columns. However, direct injection (DI) of water into an LC-MS system offers great promise for rapid and sensitive analysis using the latest generation of MS equipment (Anumol et al., 2015). When DI is applied to LC-MS, generally a void time is employed whereby the chromatography effluent is diverted to waste to allow salts and other non-retained highly-polar materials to bypass the MS. After a prescribed amount of time, the chromatography column effluent is sent to the MS for analysis. This intrinsically leads to loss of potentially important substances such as oxyhalides, metals, and highly water-soluble organics. Regardless, many semi-volatile or non-volatile organics and metalloids can be analyzed by DI methods. It is important for DI methods to employ a filtration step before injection in order to remove particles that may readily clog chromatography columns, particularly ultra-high performance liquid chromatography (UPLC) columns with quite small particle sizes. Losses have been observed through filtration methods, both from substances bound to particles and from sorption of target compounds on filter materials, thus spike/recovery studies are critical when developing or implementing OSPE and/or DI methodologies.

6.3.2. Quality assurance and quality control

The quality assurance and quality control (QA/QC) aspects of environmental analyses were explained in detail previously (Anderson et al., 2010). However, two critical aspects regarding the extraction/concentration of chemical constituents and calculation of MRLs in complex aqueous matrices deserve additional discussion. While a few USEPA-approved methods are available for a limited number of trace organic chemicals, some of these methods are specific to drinking water. The Panel assumes that methods applicable to finished drinking water would be equally applicable to water produced for drinking during potable reuse applications (i.e., water produced by advanced water treatment processes).

Potable water reuse intrinsically involves trace analytical measurements of water qualities ranging from raw sewage to highly-purified water. As described previously, analytical reliability becomes increasingly challenging with more complex aqueous matrices. Raw sewage often contains total organic carbon (TOC) concentrations of 35 mg/L or higher, while RO-UV/AOP product water from potable reuse generally contains less than 0.5 mg/L TOC. Thus, measurement of trace organic chemicals from “source to finish” in potable reuse will be far more complicated than methods developed and optimized for only finished drinking water. Recovery of CECs from raw sewage suffers from numerous analytical challenges, but perhaps the most important is the ion suppression resulting from application of LC-MS methodologies. While isotope-dilution with surrogate standards is often used to correct for losses throughout the analytical process (Vanderford and Snyder, 2006), the absolute recoveries are often far from 100%. If the method reporting limit was determined in purified water, the achievable MRL in more complex matrices often will be far higher due to suppression from the sample matrix. Thus, “non-detectable” levels will be far greater than the MRL determined from purified water. This is especially problematic with electrospray

ionization, where charge competition occurs and co-eluting substances will thus compete for the limited charge available for ionization (King et al., 2000). A potential solution is that the MRLs be established in the most complex water matrices to be analyzed or MRLs are determined for each matrix to be evaluated. Perhaps the most appropriate technique is to adjust the MRL based upon the recovery of the isotopically-labeled surrogate standard, preferably for each target analyte (Anumol et al., 2013). Only through reflection of the true MRL can the performance indicator approach be accurately applied (Dickenson et al., 2009). Otherwise, erroneous results and false negatives may lead to unjustified conclusions. Likewise, trace analysis can similarly lead to false positives that also can obfuscate reality. As a general rule, QA/QC criteria included in USEPA Standard Methods for matrix spikes, duplicates, blanks, and internal/surrogate standards should be applied to CEC analyses in recycled water.

There are many lists of CECs that are monitored by various water agencies, regulatory bodies, and water research teams. However, it is important to consider that modifying a list to add "new" substances is not an easy task. This is commonly asked when a new "emerging" contaminant is reported. Targeted analyses are performed on methods that are optimized for the substances monitored (depending on the type of instrument employed). While it may seem trivial to add some new compounds to an existing list of analytes, the truth is far from the myth. Adding a new compound will require that: a) the substance is retained on the SPE/OSPE, b) the chromatography is sufficient to retain/separate the substance, c) the ionization of the mass spectrometer is plausible for the compound of interest, and/or d) the substance is stable throughout the sample collecting, holding times, and preservation techniques employed (see Anderson et al., 2010). Moreover, not all instruments are created equally. This is especially true for ionization source designs of LC-MS systems. The source on LC-MS, be it electrospray ionization (ESI) or atmosphere pressure chemical ionization (APCI) is among the most unique feature that differentiates the instruments from various vendors. These sources can vary dramatically in relationship to the types of chemicals ionized and thus will impact sensitivity and to a lesser extent selectivity. While ionization source designs also vary for GC-MS, electronic ionization (EI) is by far the dominant technology utilized and molecular fragmentation patterns are generally common among instruments. Therefore, many databases (such as the National Institute of Standards and Technology (NIST) MS database) are commonly employed among GC-MS instruments allowing for high-degrees of structural matching for both targeted and non-targeted analyses. One article estimated that approximately 200,000 spectra were available for GC-EI-MS databases (Schymanski et al., 2015). Comparatively, very few databases are available for soft-ionization techniques (such as those employed for LC-MS), however, numerous private firms and researchers are developing such databases. Even so, databases for soft-ionization techniques will remain challenging since instrument parameters, mobile phase modifiers, and adducts can greatly change the resulting molecular and fragmentation ions using this technique.

There are relatively few standardized methods available for unregulated chemicals in environmental waters. However, the Panel evaluated five methods that may have particular applicability for CECs in recycled water, namely: Standard Method 6810, and USEPA methods 539, 542, 1694, and 1698. Table 6.1 provides the target substances for each these methods.

Table 6.1. Target analytes of different standardized methods.

EPA542	EPA1694	SM6810	EPA539	EPA1698
Carbamazepine	Acetaminophen	Acetaminophen	Estriol	Androstenedione
Diazepam	Albuterol	Bisphenol A	17β-Estradiol	Androsterone
Diclofenac	Ampicillin	Caffeine	17α-Estradiol	Campesterol
Enalapril	Anhydrochlor-tetracycline	Carbamazepine	17α-Ethinylestradiol	Cholestanol
Eythromycin	Anhydrotetra-cycline	Diclofenac	Testosterone	Cholesterol
Fluoxetine	Azithromycin	Fluoxetine	Estrone	Coprostanol
Gemfibrozil	Caffeine	Gemfibrozil	Androstenedione	Desmosterol
Naproxen	Carbadox	Ibuprofen	Equilin	Desogestrel
Phenytoin	Carbamazepine	Naproxen		17α-Dihydroequilin
Sulfamethoxazole	Cefotaxime	Primidone		Epi-Coprostanol
Triclosan	Chlortetracycline	Sulfamethoxazole		Equilenin
Trimethoprim	Cimetidine	Triclosan		Equilin
	Ciprofloxacin	Trimethoprim		Ergosterol
	Clarithromycin			17α-Estradiol
	Clinafloxacin			17α-Ethinylestradiol
	Cloxacillin			17β-Estradiol
	Codeine			β-Estradiol-3-benzoate
	Cotinine			Estriol
	Dehydronife-dipine			Estrone
	Demeclocycline			Mestranol
	Digoxigenin			Norethindrone
	Digoxin			Norgestrel
	Diltiazem			Progesterone
	1,7-Dimethylxanthine			beta-Sitosterol
	Diphen-hydramine			beta-Stigmastanol
	Doxycycline			Stigmasterol
	Enrofloxacin			Testosterone
	4-Epianhydrochlortetracycline			
	4-Epianhydrotetra-cycline			
	4-Epichlortetra-cycline			
	4-Epioxytetra-cycline			
	4-Epitetracycline			
	Erythromycin			
	Erythromycin anhydrate			

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EP542	EP1694	SM6810	EPA539	EPA1698
	Flumequine			
	Fluoxetine			
	Gemfibrozil			
	Ibuprofen			
	Isochlortetra-cycline			
	Lincomycin			
	Lomefloxacin			
	Metformin			
	Miconazole			
	Minocycline			
	Naproxen			
	Norfloxacin			
	Norgestimate			
	Ofloxacin			
	Ormetoprim			
	Oxacillin			
	Oxolinic acid			
	Oxytetracycline			
	Penicillin V			
	Penicillin G			
	Ranitidine			
	Roxithromycin			
	Sarafloxacin			
	Sulfachloro-pyridazine			
	Sulfadiazine			
	Sulfadimethoxine			
	Sulfamerazine			
	Sulfamethazine			
	Sulfamethizole			
	Sulfamethoxazole			
	Sulfanilamide			
	Sulfathiazole			
	Tetracycline			
	Thiabendazole			
	Triclocarban			
	Triclosan			
	Trimethoprim			
	Tylosin			
	Virginiamycin			
	Warfarin			

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Standard Method 6810 (SM6810), entitled "Pharmaceuticals and Personal Care Products", includes 13 compounds and is based on previously published methods (Ternes et al., 2001; Gros et al., 2006; Vanderford and Snyder, 2006) and a funded project from the Water Research Foundation (Vanderford et al., 2012). SM6810 uses SPE followed by two LC-MS/MS methods and is designed for wastewater, recycled water, and drinking water. The method is claimed to achieve detection limits ranging from 1 to 2,000 ng/L. However, the method specifically notes that *"reporting limits may vary according to matrix"*.

EPA method 542 (EPA542) is entitled *"Determination of Pharmaceuticals and Personal Care Products in Drinking Water by Solid Phase Extraction and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS)"* and was promulgated in September 2016. This method is specifically purposed for finished drinking water, and thus likely is not applicable to recycled water prior to drinking water treatment processes. This method includes 12 analytes, eight of which are common to SM6810 (Table 6.1). From a single laboratory, the lowest MRLs achievable ranged from 0.27 to 5.0 ng/L in finished drinking water. EPA542 requires 1-liter of water to be extracted and subsequently concentrated into 10 mL of reagent grade water. Analyses are performed using LC-MS/MS. EPA542 is not an isotope-dilution method for all analytes as it relies on only two surrogate standards and three internal standards.

EPA method 1694 (EPA1694) entitled *"Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS"* was promulgated in December of 2007. This method targets 74 analytes, the majority of which are antibiotics/antimicrobials and their corresponding metabolites. EPA1694 includes triclosan, triclocarban, gemfibrozil, naproxen, fluoxetine, carbamazepine, and sulfamethoxazole, which are also included in EPA542 and SM6810. Thus, these seven PPCPs can be analyzed by any of the standardized methods for pharmaceuticals. However, EPA1694 can be applied to aqueous samples, biosolids, and solid matrices. While designed for applications under the Clean Water Act, EPA1694 states *"other applications are possible".* EPA1694 can achieve MRLs ranging from approximately 1-500 ng/L in aqueous samples, with the majority ranging from 1-50 ng/L. However, EPA1694 specifically states that the MRLs reported are *"the levels at which the analytes can be determined in the absence of interferences"* and that the achieved levels will depend on the analytical instrumentation applied. For water samples, EPA1694 requires two extractions using SPE, one at pH 2 and another at pH 10. The method also requires analysis by two LC-MS/MS analyses, one with electrospray positive ionization and one with electrospray negative ionization.

EPA method 539 (EPA539) was promulgated in November of 2010 and is entitled, *"Determination of Hormones in Drinking Water by Solid Phase Extraction (SPE) and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS)".* As the name implies, EPA539 is a method developed with the intent for application to finished drinking water. The method is designed for seven steroid hormones, five estrogens and two androgens. The MRLs for the method, based on a single laboratory, range from 0.06 to 4.0 ng/L. EPA539 requires four internal standards and one surrogate, though the laboratory may choose between deuterium labeled ethinylestradiol and bisphenol A for the single surrogate standard required. Sample volumes between 500 – 1,000 mL are extracted using SPE disks of C18 stationary phase. The volume extracted depends on the sensitivity of the mass spectrometer applied.

EPA method 1698 (EPA1698) was promulgated in December of 2007 and is entitled, *"Steroids and Hormones in Water, Soil, Sediment, and Biosolids by HRGC/HRMS"*. The method addresses 27 target analytes, which include androgens, estrogens, progestins, and others. This method was developed for Clean Water Act applications and is performance based, meaning several modifications are permissible. For water samples, LLE with dichloromethane is applied to 1-L water samples through separatory funnel extraction or through continuous LLE. For samples from more complex matrices (e.g., wastewater effluents), the extract should undergo a clean-up step where a layered alumina/Florisil column is recommended. The resulting extract is concentrated to approximately 0.1 mL then derivatized to trimethylsilyl-ethers using N,O-Bis(trimethylsilyl) trifluoroacetamide with trimethylchlorosilane (BSTFA:TMCS). The derivatized extract is injected into a GC-High Resolution Mass Spectrometer (HRMS; minimum resolving power of 5,000 at 10% valley). The EPA shows MRLs from 0.1 to approximately 20 ng/L for the target steroids and hormones.

It is interesting to note that several of the aforementioned methods are listed as isotope-dilution methods, however, generally only a few of the target compounds are spiked as isotopically-labeled standards, and all other substances are corrected according to those few labeled compounds added. Therefore, the Panel does not consider these as true isotope-dilution methods whereby each analyte would include an isotopically-labeled version spiked into the raw sample (as quickly from time of collection as possible) and followed through the entire procedure.

In addition to the standardized methods discussed previously, additional methods for non-regulated substances do exist from EPA and others, including those for perfluorinated alkyl acids (EPA method 537), perchlorate (EPA methods 314, 332, and 6850), NDMA (EPA method 521), and other substances. However, to the best of the Panel's knowledge, these substances already have notification limits and/or action limits in California and/or health advisory levels from the USEPA. In addition, as discussed in the previous panel report, essentially all drinking water agencies are required to comply with the USEPA's Unregulated Contaminant Monitoring Rule (UCMR), the latest version is UCMR4 and the target analytes are provided in Table 6.2. Therefore, many CECs are, or will be, monitored through various other state and federal programs.

Table 6.2. Target analytes of the Unregulated Contaminant Monitoring Rule (UCMR4).

Ten Cyanotoxin Chemical Contaminants			
Contaminant	**CAS Registry Number**	**Minimum Reporting Level**	**Analytical Methods**
total microcystin	N/A	0.3 µg/L	EPA 546
microcystin-LA	96180-79-9	0.008 µg/L	EPA 544
microcystin-LF	154037-70-4	0.006 µg/L	EPA 544
microcystin-LR	101043-37-2	0.02 µg/L	EPA 544
microcystin-LY	123304-10-9	0.009 µg/L	EPA 544
microcystin-RR	111755-37-4	0.006 µg/L	EPA 544
microcystin-YR	101064-48-6	0.02 µg/L	EPA 544
Nodularin	118399-22-7	0.005 µg/L	EPA 544
anatoxin-a	64285-06-9	0.03 µg/L	EPA 545
Cylindrospermopsin	143545-90-8	0.09 µg/L	EPA 545
Two Metals			

Germanium	7440-56-4	0.3 µg/L	EPA 200.8, ASTM D5673-10, SM 3125
Manganese	7439-96-5	0.4 µg/L	EPA 200.8, ASTM D5673-10, SM 3125
Eight Pesticides and One Pesticide Manufacturing Byproduct			
alpha-hexachlorocyclohexane	319-84-6	0.01 µg/L	EPA 525.3
chlorpyrifos	2921-88-2	0.03 µg/L	EPA 525.3
dimethipin	55290-64-7	0.2 µg/L	EPA 525.3
ethoprop	13194-48-4	0.03 µg/L	EPA 525.3
oxyfluorfen	42874-03-3	0.05 µg/L	EPA 525.3
profenofos	41198-08-7	0.3 µg/L	EPA 525.3
tebuconazole	107534-96-3	0.2 µg/L	EPA 525.3
total permethrin (cis- & trans-)	52645-53-1	0.04 µg/L	EPA 525.3
tribufos	78-48-8	0.07 µg/L	EPA 525.3
Three Brominated Haloacetic Acid (HAA) Groups 3,4			
HAA5	N/A	N/A	EPA 552.3 or EPA 557
HAA6Br	N/A	N/A	EPA 552.3 or EPA 557
HAA9	N/A	N/A	EPA 552.3 or EPA 557
Three Alcohols			
1-butanol	71-36-3	2.0 µg/L	EPA 541
2-methoxyethanol	109-86-4	0.4 µg/L	EPA 541
2-propen-1-ol	107-18-6	0.5 µg/L	EPA 541
Three Other Semi-volatile Chemicals			
butylated hydroxyanisole	25013-16-5	0.03 µg/L	EPA 530
o-toluidine	95-53-4	0.007 µg/L	EPA 530
quinoline	91-22-5	0.02 µg/L	EPA 530

6.4 Non-Targeted Analyses (NTA)

6.4.1 Introduction

One technique to identify previously unknown chemicals in water is the use of non-targeted analysis (NTA). While bioanalytical techniques are another type of NTA (see Chapter 7), this section pertains specifically to analytical chemistry techniques with an emphasis on mass spectrometric (MS) detection. Several articles provide review information on the types of instruments available for NTA; however, the Panel especially recommends two articles for general information on the subject (Schymanski et al., 2015; Gosetti et al., 2016). It is important to differentiate "suspect screening" from true NTA. In many examples that will be discussed, mass spectrometers are operated in scan mode and then databases are used to match peaks identified based on mass spectral libraries and/or retention time indices. In other cases, single chemicals are subjected to various types of water treatment and subsequent transformation products identified using MS. Both of these examples are better termed "suspect screening" (Schymanski et al., 2015) since *a priori* information is used to guide the analyses and/or basic structural information is already available. However, suspect

screening generally infers that analytical standards are not readily available and/or have not been previously procured. According to one manuscript reviewed, *"an unequivocal identification of trace-level compounds in environmental systems is in most cases not possible by HRMS alone without the application of additional knowledge, complementary techniques, or an authentic reference standard"* (Gosetti et al., 2016). For the purposes of this report, we will focus on NTA whereby databases are not available, retention times are unknown, and generally authenticated standards are not readily available.

NTA has been applied to water for decades. Some of the earliest discoveries related to water pollution came from non-targeted analytical techniques. These discoveries include trihalomethanes (THMs) and legacy contaminants such as the pesticide DDT. Perhaps ironically, the earliest reports on pharmaceuticals and steroid hormones in the environment came from NTA applied in the 1970's (Garrison et al., 1975; Keith et al., 1975). The same is true for nitrosoamines such as NDMA, which also have been studied in water since the 1970's (Fine et al., 1975). Historically, NTA was primarily performed using gas chromatography (GC) with a variety of detectors; however, mass spectrometry has been the favored detection technique because of the specificity in determination of molecular weights and predictable fragmentation patterns that allowed for mass spectrographic matching through various databases. Because of limitations encountered with LLE and SPE of aqueous samples along with often rapid variability in constituent concentrations over time, some reports also have demonstrated the value of passive sampling for NTA (Allan et al., 2013), which could be especially valuable for more lipophilic substances. It is important to consider that all of the challenges associated with NTA will also be equivalent challenges for sample preparation for bioanalytical tool applications (see Chapter 7).

NTA also has been specifically employed within the regulatory structure of California water reuse and in non-regulatory applications of the USEPA. The specific application of NTA was referred to as tentatively identified compounds (TICs). The USEPA has defined a TIC as chemical observed during a standardized method analysis, yet is not part of a targeted analyte list (https://www.epa.gov/sites/production/files/2015-06/documents/tics.pdf). In other word, a specific EPA method is utilized for targeted analysis and additional chromatographic peaks observed are compared to mass spectrometric databases for tentative identification through software matching. This EPA document specifically addresses the identification of 1,4-dioxane and methyl-*tert*-butyl ether as TICs from contaminated site investigations. Those compounds identified must be listed as an estimated concentration (if at all) since accurate quantification is not plausible with TICs. The State of California has similarly defined TICs and methodology for analysis of volatile (https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/documents/drinkingwaterlabs/nt-vocs.pdf) and semi-volatile (https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/documents/drinkingwaterlabs/nt-svocs.pdf) compounds. The application of TIC methodology in California is described in textbox 6.1.

6.4.2 Recent developments and applications of NTA

More recently, advances in LC-MS have led to a steady rise in publications focusing on NTA for more polar and water-soluble molecules (Sultan and Gabryelski, 2006). Of these publications, the majority seems to focus on transformation products from oxidation, where a known chemical is exposed to an oxidant under controlled conditions and determinations of transformation structures are elucidated using (generally) high-resolution tandem MS,

predominantly Quadrupole-Time-of Flight (QTOF) and Orbitrap MS systems. For instance, Vanderford et al. (2008) utilized a direct infusion of triclosan with a mixing tee containing a chlorine solution to evaluate the near real-time formation of transformation products of triclosan and atorvastatin (Vanderford et al., 2008). This work showed not only formation of terminal transformation products, but also the formation and subsequent reaction of transformation product intermediates and terminal products. In a similar work, Mawhinney et al. (2012) investigated the near real-time formation of benzotriazole transformation products through the injection of ozone residual in a contact vessel directly connected to an LC-QTOF instrument. This work was able to show that various intermediate oxidation transformation products would form from benzotriazole, but ultimately a dominant dicarbaldehyde, which was stable throughout the contact times applied, was formed.

Algal toxins have also been evaluated for the formation of largely unknown transformation products during water treatment (Yan et al., 2016). Numerous other examples can be observed in the literature where a single compound is exposed to a laboratory based treatment process in a synthetic water or natural water of a single quality and the identification of numerous transformation products was accomplished. While these types of works are considered NTA, it should be noted that these examples generally begin with single synthetic water quality at high concentrations of the target molecule. Thus, the NTA to determine the structures formed during oxidation are relatively easy and predictable as compared to wide screening of complex aqueous mixtures with little/no *a priori* information as to the breadth of depth or potential organic substances present. Thus, while many NTA research projects do seek to identify previously unknown metabolites, the actual process often involves a single compound at relatively high concentration in an artificial matrix.

It is interesting that many of the transformation products identified have no toxicological information and those pose a tremendous challenge to regulators and water agencies to explain to the public the relevance, or lack thereof, related to these newly identified structures. To truly deconvolute the thousands of structural features detectable in a single extraction/injection into a modern LC-MS system involves highly complex/expensive software and extremely experienced analysts (Merel et al., 2015). The injection of an SPE extract from a WWTP effluent can easily contain thousands of molecular features (Merel et al., 2015), the vast majority of which will likely remain unidentified, which in turn can create potential public communication challenges as those data are disseminated.

Textbox 6.1

The State Water Board established a draft regulation around 2002 for groundwater replenishment reuse projects (GRRPs) that required annual NTA for tentatively identified compounds (TICs) at systems where the recycled water contribution was greater than 0.5; however, the draft requirement was removed by approximately 2008. US EPA methods for volatile and semi-volatile compounds were used for the analyses (i.e., EPA Methods 8270C and EPA method 524.2, respectively) have been used for this TIC requirement. Agencies conducted TIC annual analysis noted that results were mostly non-detect, there was significant uncertainty in the results, and compounds in analytical blanks confounded results. According to TIC monitoring information provided to the panel, in 5 years of required TIC analyses: 2013 had 7 unknown detects, 2016 had one unknown, and 2015 had 17 unknowns and one identified 2-Phenyl-3-(4-fluorophenyl) TIC. While the TIC requirement remains in some GRRP Regional Board permits, it has been removed by others. It is uncertain if TIC analyses have revealed any novel and/or relevant new contaminants deserving further investigation.

Numerous additional articles, beyond transformation products, have been published over the past 20 years. In 2009, an article was published that investigated 11 samples collected from

various European river systems and subsequently liquid-liquid extracted then analyzed by GC-MS (Schwarzbauer and Ricking, 2010). Many compounds were detected, with the majority having been previously reported (such as pharmaceuticals and personal care products), but some new structures were also found. These include 1,1-dichloro-2,2diethoxyethane, mono- and dibrominated (methoxyphenyl) propionic acids, 4-chloro-2-(trifluoromethyl)aniline, di-isopropylurea, and N,N-diethyldithiocarbamic acid.

Another publication also describes a GC-MS methodology that provides for targeted and non-targeted analyses (Gómez et al., 2009). According to this publication, *"most of the published methods for pharmaceutical care product ingredients and related compound analyses in wastewater, surface waters and groundwater are based on GC–MS"*. In this study, the authors' used LLE for water samples acidified to pH 3 using n-hexane and noted that *"LLE has the advantage that particles and surfactants do not usually influence the extraction very much and sometimes a decrease in recovery rates is experienced when using SPE for very lipophilic compounds compared to LLE"*. The study was performed using a GC-MS (single quadrupole) instrument with peak locking for known analytes using selective ion monitoring (SIM) while operating in scan mode for other areas of the chromatogram for NTA. Resulting full-scale spectra were compared to the NIST database. Through this work, 14 target analytes were identified and quantified in environmental samples while an additional 12 compounds were identified from the NIST database. Interestingly, most of the 12 "new" compounds identified by NTA are well known to occur in the aqueous environment (e.g., DEET, BHA, diazinon, benzophenone, and others). This publication also provides an interesting discussion regarding signal enhancement with GC-MS (up to 49% in wastewater matrixes) due to impacts to irreversible binding of organic contaminants to active sites on the GC inlet liner, whereas, samples from actual matrices such as wastewater seemingly deactivate the liner and result in signal enhancement. However, the authors point out that this same phenomenon may result in false positives from leaching of various analytes. The authors then suggest that matrix matched samples be used for QA/QC and calibration.

Perhaps the most interesting article regarding NTA identified by the Panel was from an international study of HRMS techniques applied to a single sample extract from the Danube River (Schymanski et al., 2015). In fact, the Panel strongly recommends agencies considering NTA to review this article in order to better appreciate the complexities and limitations of NTA. Eighteen different institutions from 12 European countries analyzed this single extract using both GC and LC coupled to HRMS. Interestingly, the authors concluded even though the general workflow for the analyses was similar among groups, data processing remained time-consuming and often disparate. The authors state that *"the objective of a fully automated identification workflow remains elusive in the short term"* and that *"non-target screening of environmental samples is becoming increasingly complex"*. The authors also conclude that *"it is clear that not all of the up to several thousand unknown peaks can be identified"*. To facilitate more comprehensive screening, the authors suggest open exchanges of suspect screening approaches and exchanges of target and suspect lists across labs. In total, 354 of the 622 target compounds were reported at least by one laboratory using LC-HRMS, while the maximum reported by any single laboratory was 167. Several complicating issues were identified, including the use of the exact mass for a salt that dissociates in water, isobaric substances, adducts, and others. It also should be noted that in this study, one extract was created and split among the participating laboratories, without question, the results would have even more variability if each laboratory performed its own extraction procedure.

In a study at the University of Arizona, identical municipal wastewater effluent samples were extracted identically to produce extracts for both targeted and NTA (suspect screening) in order to compare the results from LC-triple quadrupole (QQQ) targeted analysis to NTA using LC-QTOF. Listed in Table 6.3, the 34 target analytes were selected based on an online SPE-LC-MS/MS method published by (Anumol and Snyder, 2015). In this case, the LC-QTOF was operated in scan mode for all ions and the dominant ions extracted from the total ion chromatogram. Of the 34 target analytes, 20 were detected in targeted LC-MS/MS analysis. However, the effluent sample handled identically resulted in only 13 detected compounds from the same list of 34 and using the same concentration factor as OSPE. In this experiment, the HPLC system and columns were identical, thus, providing a finite example that many compounds detected using targeted analysis are actually not detectable using NTA (suspect screening). On the other hand, the LC-QTOF data (operated in both ESI- and ESI+ resulted in more than 600 molecular features (essentially molecules), of which only 13 were suspect screening targets.

Table 6.3. Comparison of the detectability of CECs using targeted LC-QQQ vs. non-targeted LC-QTOF on identical wastewater samples.

Compound	Targeted Analysis (Online-SPE-LC-MS/MS)	Non Targeted Analysis (NTA - LC-QTOF)	Compound	Targeted Analysis (Online-SPE-LC-MS/MS)	Non Targeted Analysis (NTA - LC-QTOF)
Atenolol	✓	✓	DEET	✓	✗
Atrazine	✗	✗	Naproxen	✗	✗
Benzophenone	✓	✓	Norgestrel	✗	✗
Benzotriazole	✓	✓	PFHxA	✗	✗
Bisphenol A	✓	✓	PFOS	✓	✓
Caffeine	✗	✗	PFOA	✗	✗
Carbamazepine	✓	✗	Primidone	✓	✗
Clofibric acid	✗	✗	Propranolol	✗	✗
Diclofenac	✓	✗	Propylparaben	✗	✗
Diltiazem	✓	✗	Simazine	✗	✗
Diphenhydramine	✓	✗	Sulfamethoxazole	✓	✓
Fluoxetine	✓	✓	Testosterone	✗	✗
Gemfibrozil	✓	✓	Triclocarban	✗	✗
Hydrochlorothiazide	✓	✓	Triclosan	✓	✓
Hydrocortisone	✗	✗	Trimethoprim	✓	✓
Ibuprofen	✓	✓	TCEP	✓	✓
Meprobamate	✗	✗	TCPP	✓	✗

6.4.3 Conclusions regarding the use of NTA for CEC monitoring

Without question, NTA methods hold great promise for the identification of previously unknown substances in recycled water. However, it is important to understand the limitations, complexities, and costs of performing NTA. The use of GC-EI-MS for relatively volatile/semi-volatile species has been employed for many decades. This is largely due to availability of robust databases that reflect the consistency of fragmentation of gas phase molecules by EI. However, database matching is not perhaps true NTA but rather “suspect screening”. For completely unknown organic constituent identification, likely additional structural information will be required, such as nuclear magnetic resonance (NMR). In addition, the extraction technique employed, instrument sensitivity, source design, sample handling, and software employed for deconvolution will all impact the detection and identification of unknown compounds during NTA. Using modern software platforms coupled with significant true replicates, it is possible to use statistical software tools such as principle component analysis (PCA) to detect changes within or among samples. For instance, samples before and after ozonation were analyzed by LC-QTOF and using Principal Component

Analysis, mass features were clearly visible for substances attenuated by ozone and substances created (transformed) by ozone (Merel et al., 2015). However, the next step to actually provide definite structural identification would be an arduous and labor-intensive process that likely would require additional instrumentation.

Lastly, it is important to remember that the same issues of ion-suppression and contamination of inlets/sources may very well lead to lack of detection of many substances present in the aqueous environment, especially since NTA methods are intrinsically less sensitive than optimized targeted analytical techniques. Moreover, to even approach comprehensive instrumental NTA, GC (with both volatile and semi-volatile interfaces), LC, and ion chromatography interfaces would be required for both low resolution, high resolution, and inductively-coupled plasma mass spectrometers. Additionally, mass spectrometers would need to be operated in both positive and negative ionization modes, likely with chemical, electrospray, and electron ionization modes (at a minimum). In summary, NTA methods hold promise for identifying new structures, but it should not be viewed as a comprehensive "silver bullet". Moreover, stakeholders will also be challenged in communicating the detection of compounds with little/no toxicological information and/or dubious chemical structures. Thus, as described in the following chapter, the use of bioanalytical methods cannot only inform NTA and suspect screening, but also provide some information as to the biological relevance associated with newly discovered organic contaminants.

DRAFT

7. Role of Bioanalytical Methods to Assess the Relevance of Unknown CECs

7.1 What Are Bioanalytical Tools and How Can They Help?

As opposed to targeted chemical methods that quantify individual chemicals (see Chapter 6), bioanalytical tools are non- or semi-targeted methods that utilize *in vitro* (cell or protein-based) and *in vivo* (whole animal) test systems (broadly referred to herein as "bioassays") that are capable of detecting a wide spectrum of CECs, and when calibrated, may also provide some indication of adverse effect. While targeted methods focus on known compounds, bioanalytical methods include the ability to integrate unknown compounds and mixture interactions within complex environmental matrices. Similarly, toxicity evaluations of single chemicals will generally miss the synergistic, additive, or antagonistic potential found in mixtures, thus providing a false sense of security or false indication of a potential risk. The idea is that one can measure, by concentration addition, chemicals in a mixture that act by the same mechanism, i.e., that behave as if they were dilutions of each other, depending on their potencies. In addition, with recent movement by regulatory agencies worldwide toward a mode of action approach in risk assessment paradigms, several bioassays have been developed for the screening of compounds for specific biological target activities such as dioxin-like activity (van den Berg et al., 1998), endocrine responses (i.e., estrogen, androgen, thyroid activities), and genotoxicity. For example, USEPA has invested significant resources for high throughput cell bioassays, particularly for the Endocrine Disrupter Screening Program, and a number have been through rigorous QA/QC evaluations (Table 7.1).

Table 7.1. Commercially available EDSP Tier I Bioassays with adequate quality assurance guidelines ***https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-tier-1-battery-assays***

Test Environment	Endpoint	Assay
In vitro	Estrogen receptor (ER) binding	Rat uterine cytosol
	Estrogen receptor (hERα) transcriptional activation	Human cell line (HeLA-9903)
	Androgen receptor (AR) binding	Rat prostate cytosol
	Steriodogenesis	Human cell line (H295R)
	Aromatase	Human recombinant microsomes
In vivo		Uterotrophic (rat)
		Pubertal female (rat)
		Pubertal male (rat)
		Amphibian metamorphosis (frog)
		Fish short-term reproduction

For chemicals that behave as hormone mimics, bioanalytical tools could play a role as an initial screen for CECs, which if measured levels of bioactivity prove problematic, could then direct follow-up investigation such as toxicant identification using targeted or non-targeted chemical analysis, or increasingly relevant biological/toxicological measurements. For example, if a recycled water sample failed to demonstrate detectable estrogenic activity in one of the assays described below, the measurement of difficult analytes by targeted analytical methods (see Chapter 6) may not be necessary. On the other hand, additional management action could be advised if a recycled water sample exceeds a preset level of concern (see Chapter 5 and below). For unknown CECs, bioanalytical methods offer an

additional safeguard for the protection of human health by quantifying bioactivity/toxicity while leading to the identification of previously unidentified chemicals of concern. This dual screening approach using bioanalytical tools is illustrated in Figure 7.1.

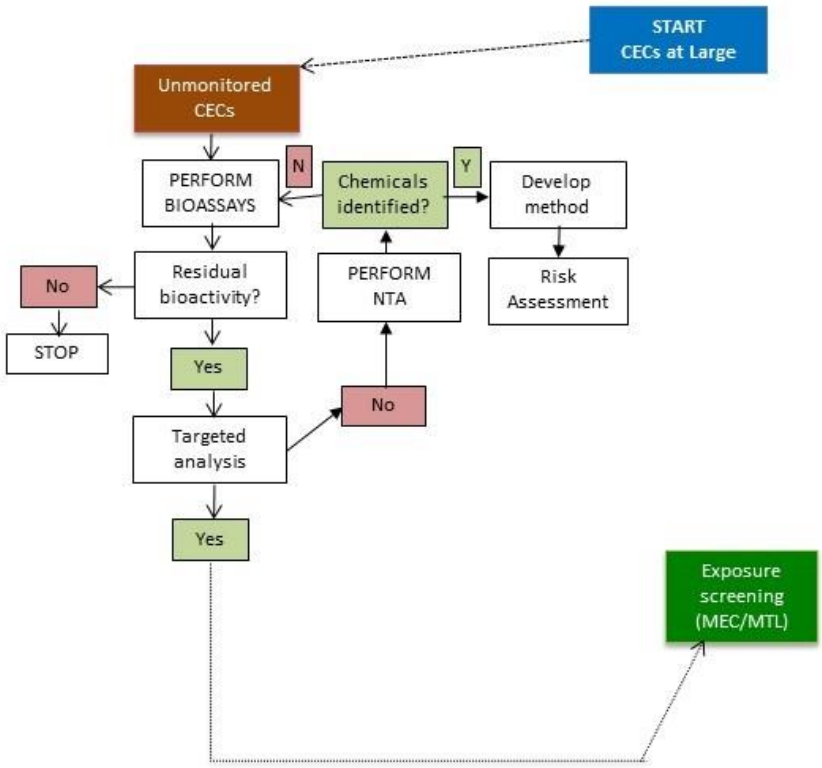


Figure 7.1. Screening approach for unmonitored CECs using bioanalytical tools and non-targeted chemical analysis (NTA) in recycled water.

The application of bioanalytical tools to recycled water monitoring and investigation has been the topic of numerous workshops and meetings since 2012 (Leura, Australia, State Water Board, NWRI, WE&RF, etc.). These meetings have been largely positive in their support for the development of bioanalytical tools for screening water quality for unknowns. *In vitro* bioassays have been used, for example, to assess total estrogenicity in source water for 25 U.S. drinking water treatment plants (Conley et al., 2017). In addition to reporting good correspondence of *in vitro* bioscreening response to actual 17β-estradiol (E2) measurements made by liquid chromatography-Fourier Transform mass spectrometry (LC-FT-MS), Conley et al. (2017) also discovered that nine of the 25 plants showed no estrogen equivalence activity and the ones that did were all less than 0.5 ng/L estrogen equivalency. A similar battery of *in vitro* bioassays was recently implemented in assessing water quality in a recycled water demonstration pilot project (Di Paolo et al., 2016; Carollo Engineers, 2017).

The recent Direct Potable Reuse (DPR) Expert Panel; however, was more critical of bioanalytical tools, raising concerns regarding lack of standardization, interpretation of results, and regulatory applications, which ultimately may limit their use (Olivieri et al., 2016). For bioanalytical tools to be effective, these issues need to be adequately addressed prior to acceptance by the recycled water community for monitoring of recycled water applications. This chapter addresses these concerns, one by one, followed by recommendations for applications during the next data collection period and future research and development.

7.2 Bioanalytical Methods are Standardized for Applications Worldwide

Standardization can have multiple meanings under varied contexts. In terms of this report, we define standardization as the ability of a bioassay to conform to measurement standards for a recycled water sample, such that they provide utilities and regulators confidence in the comparability of results for recycled water. Validation of a standardized method is the next step in the process of method development and application, which typically entails additional exercises that are meant to provide the highest level of confidence in terms of data accuracy and comparability. The tools themselves have been "standardized" for use in the evaluation of numerous other matrices. For example, USEPA has approved a method to screen for dioxin-like chemicals in sediment (USEPA, 2014b). OECD has also published validated protocols to screen chemicals (such as pesticides) as estrogen and androgen agonists and antagonists using commercial and non-commercialized cell assays:

- Test No. 457: BG1Luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists (https://archive.org/details/bub_gb_y91joFQCzXUC).
- Test No. 455 Performance-Based Test Guideline for Stably Transfected Transactivation *In vitro* Assays to Detect Estrogen Receptor Agonists and Antagonists (OECD, 2016a). (http://www.oecd.org/publications/test-no-455-performance-based-test-guideline-for-stably-transfected-transactivation-in-vitro-assays-to-detect-estrogen-receptor-9789264265295-en.htm)
- Test No. 458 Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals (OECD, 2016b). (http://www.oecd.org/env/test-no-458-stably-transfected-human-androgen-receptor-transcriptional-activation-assay-for-detection-of-androgenic-agonist-9789264264366-en.htm).

For chemicals that may function through the aryl hydrocarbon receptor (AhR), the European Union has also established guidelines for sample preparation and testing. In addition to traditional analytical methods, the use of bioanalytical methods is also accepted as indicated by the Commission Regulation (EU) No 589/2014 and Commission Regulation (EU) No 709/2014. The assay parameters are clearly established in a document by the European Union Reference Lab in Freiburg, Germany (http://www.crl-freiburg.eu/dioxin/screening.html). In addition, other vendors have approved AhR assays including BioDetection Systems (BDS), which offers its PAH-CALUX assay for quantification of chemicals that work through the AhR (Pieterse et al., 2013) and INDIGO Biosciences, which offers a similar assay (Indigo, 2016).

In fact, the use of *in vitro* bioassays for water quality monitoring has been advocated since the mid-seventies (WHO, 1975). In recent years, more comprehensive reviews have provided numerous examples of the application of bioanalytical tools for recycled water over the last 50 years (Leusch and Snyder, 2015). Moreover, several recent publications have demonstrated the robustness of standardized protocols using commercially available cell lines (including ER) for analyses of water samples through inter-laboratory exercises (van der Burg et al., 2010; Besselink, 2015; Mehinto et al., 2015; Kunz et al., 2017). The commercial assays tested included ThermoFisher/LifeTech (Mehinto et al., 2015 and Escher et al., 2014; Kunz et al., 2017) and BDS (van der Burg et al., 2010; Besselink, 2015; Kunz et al., 2017).

Thus, while standardization of bioassays is possible and has been achieved for multiple endpoints, the commercial availability of test products (e.g. cell lines and/or kits) is limited,

and the number of commercial service labs that offer bioanalytical testing for matrices of interest to the recycled water community remains small. Commercial sources for *in vitro* bioassay kits that can be used by laboratories with basic microbiological capabilities and expertise include ThermoFisher/LifeTech (service available upon request) and INDIGO Biosciences. Examples of laboratories that currently provide services, including bioanalytical screening of organic extracts of water samples, include INDIGO Biosciences (indigobiosciences.com), Attagene Inc. (attagene.com), and BDS (http://www.biodetectionsystems.com). The Panel has confirmed that commercial laboratories are willing to prepare recycled water sample extracts using procedures recommended by the Panel for bioanalytical screening, which are essentially the same as those employed for targeted CEC monitoring (see sections 6.2 and 7.3.2).

As a follow-up to the 2010 Panel recommendations to develop bioanalytical tools for screening of unknown CECs in water, the State Water Board commissioned an investigation to adapt and standardize commercially available *in vitro* bioassay (IVB) technologies for recycled and ambient water monitoring applications (State of California Agreement No. 10-096-250). The project investigators optimized the response of commercially available "freeze and thaw" cell assay kits, selected reference toxicants by which quantitative, comparable results could be generated (i.e. bioanalytical equivalent concentrations (BEQs)) and demonstrated that multiple labs could converge on comparable results for split extracts of aqueous samples from fully operational and/or pilot scale recycled water facilities. The results of this Phase 1 effort resulted in the standardization of several IVBs, including proposed QA/QC that target endocrine active chemicals, including the Estrogen Receptor (ER) and glucocorticoid receptor (GR) assays (see Table 7.2).

At the present time, the State Water Board is in a position to build on the Phase 1 accomplishments and parallel efforts (e.g. WRRF 10-07) by funding Phase 2 of the bioanalytical toolbox development and application effort. By teaming with WE&RF and the Phase 1 investigators, the Phase 2 project titled "*Standardizing In Vitro Bioanalytical Tools for Ambient and Recycled Water Applications*" will identify, develop, optimize and standardize an expanded suite of bioanalytical tools for monitoring and assessment of ambient and recycled waters. In addition to the optimization and standardization steps of Phase 1, Phase 2 will go a step further and feature an inter-comparison exercise for commercial and utility labs for bioanalytical endpoints that graduate through the multi-stages of development (see Table 7.2), providing validation of already standardized methods such as ER-α and AhR. Participating labs will be required to demonstrate adherence to QA/QC guidelines that mirror those that are standard practice for analytical chemistry measurements (see Table 7.3). The Phase 2 project will also optimize sample collection, handling and extraction protocols to ensure that sample extracts subject to bioscreening analyses are uniform and representative of the CECs targeted.

With an expected start date of mid-2018 and duration of 3 years, the schedule for the Phase 2 bioanalytical toolbox development and application project will be synchronized with the data collection period resulting from the 2018 Panel's recommendations. The anticipated completion date for the Phase 2 project will coincide with the next Panel review, which will occur in 2021 if a triennial schedule is followed. Since the next amendment for the Policy is also scheduled for mid-2018, guidance for acquisition of high quality bioanalytical data by recycled water facilities in CA can be coordinated with the team of investigators and advisors slated for the Phase 2 study. Moreover, the validation of bioanalytical data collected by CA

utilities per the Panel's recommendations will be enhanced by participation in the Phase 2 interlaboratory comparison exercise, which is currently planned to be conducted in 2020.

With the future development and expansion of bioscreening assays as a useful water quality monitoring tool, in part supported by the State Water Board, the Panel expects the private sector will respond offering measurement services as well as materials for *"do it yourself"* bioassay measurement.

7.3 Interpreting Bioanalytical Results

The Panel recognizes that all analytical methods, whether chemical or biological, are subject to erroneous results (e.g., false positives and false negatives). Similarly, targeted non-regulated CECs and non-targeted organic chemical analytical screening also suffer from the same question as to what a "hit" means and how it can be interpreted in the context of human health. Thus, it is absolutely necessary to clearly define the thresholds used to evaluate the results as has been presented in the 2010 and 2018 CEC reports. In addition, the Panel reiterates that a tiered strategy be used to evaluate "hits" (Section 7.5.3) as recommended in several reviews (e.g., Leusch and Snyder 2015).

7.3.1 What do in vitro assays measure exactly?

Many cell-based (or *in vitro*) assays are based on binding and activation of a chemical (natural or xenobiotic) to a specific receptor on or in a cell. Genetic manipulations in fast growing cell-lines can link the binding and activation of the receptor to a color change or physicochemical event that can be quantified in high throughput. A standard curve is normally constructed using a strong agonist for the receptor, for example, 17β-estradiol (E2) for the ER, typically over a range of nine concentrations.

The output from the bioassays are subsequently referenced to a strong agonist for the receptor to generate BEQs. BEQs are generated from a standard curve. For example, E2 is a strong agonist for the ER. Similar to results for targeted chemical analysis, BEQs are expressed in mass (ng/L) or molar concentration units. This value is typically derived by comparing the 50 percentile (EC50) or 10 percentile (EC10) responses of the test sample with the same EC value of the standard curve (Escher et al., 2008; Mehinto et al., 2015; Conley et al., 2017). Once a BEQ has been obtained it is then compared to a threshold guideline in water for the agonist, which is already present as an Acceptable Daily Intake (ADI). For example, because there is already a guideline value for E2 in drinking water, the preferred equivalency agonist for the ER is 17β-estradiol. Hence any BEQ that exceeds this guideline (i.e. a bioassay "finding") would be considered for appropriate action in the proposed tiered scheme for bioanalytical measurements (Section 7.5.3). If a "finding" occurs, because it is above the ADI guideline, then additional testing would be initiated either to confirm the response or to potentially identify the causative agent. A detectable response (or "finding") above the guideline in this regard does not suggest that the bioanalytical response should be used in the same fashion that MCLs are applied to targeted chemical monitoring. If the goal of water quality professionals is to assess the potential hazards of recycled water, then use of these tools under "worse-case" scenario exposures (assuming 100% exposure) represents the most conservative exposure assessment approach. If bioactivity is not detected under the most conservative approach, then no further testing is needed. An illustration of a decision tree for the use of bioanalytical tools is provided in Appendix F.

7.3.2 How are samples extracted for bioscreening analyses?

Nearly all cellular bioassay screening of water involves extraction and concentration of organic constituents from water samples followed by dosing into the cell media at a very low solvent concentration (less than 1%). As discussed in Chapter 6, this extraction technique poses intrinsic and obvious limitations. The SPE technique will not capture all organic substances, particularly volatile and highly-water soluble chemicals. While some studies have advocated for the use of multiple classes of extraction cartridges, there are still limitations in the classes of chemicals that can be captured. Polymeric SPE materials will leach organic constituents that may interact with the cellular bioassay system, thus true field blanks are equally important to bioassays as they are to instrumental analyses.

Perhaps one of the greatest challenges is the determination of appropriate limits of quantification (LOQ) for bioanalytical techniques. Many *in vitro* bioassays are extremely sensitive; however, only a limited amount of solvent is tolerated (generally less than 0.5% solvent/media v/v). One technique to describe the amount of organic material added to the cellular assays is the relative enrichment factor, or REF (Escher et al., 2014; Jia et al., 2016ö; Mehinto et al., 2015) or the sample enrichment fold, or SEF (Jia et al., 2016). Often, cells will be dosed at concentrations that encompass REF/SEFs of 1 (equivalent to environmental concentration) and higher. Aqueous sample volumes of approximately 1 liter are generally required to allow for ample concentration factors, replicate analyses, and assuming approximately 4 different bioassays. Thus, the extraction process can be quite labor intensive as compared to targeted analysis and NTA, which are quickly moving towards automated on-line and direct injection analyses (see Chapter 6). In addition, during the extraction and concentration step, some compounds will become immiscible (precipitate), bind to glassware, and/or volatilize. Therefore, QA/QC procedures are critical to understand the true recovery of potential agonists and antagonists within a particular extraction/concentration procedure and bioassay.

However, there are numerous studies that indicate that bioassays can be employed successfully and reproducibly in recycled water monitoring programs (Leusch and Snyder, 2015; Mehinto et al., 2015; Carollo Engineers, 2017). While some limitations for comprehensive sample preparation remain (see Chapter 6), it is critical to consider that robust analytical methods do exist for the key agonist chemicals for several bioassays. Specifically, EPA Methods 539 and 1698 (see Chapter 6) for estrogenic hormones and EPA Methods 613 and 1613B for dioxins and PCBs, provide extensive standardized procedures for sample preparation steps for the key agonists for the ER and AhR bioassays, respectively. The Panel has verified that commercial laboratories within California are capable and willing to conduct the extraction procedures for these methods and provide the extract to the recycled water utility for bioassay analysis at other commercial laboratories. The use of standardized sample handling and extraction methods offers another important advantage in that a sample extract with screening results of interest using the ER or AhR bioassays could be further analyzed using the same EPA methods used for sample preparation. Thus, a list of key target analytes could be investigated and compared for bioassay activity-chemical balance.

Beyond the QA/QC specified within the EPA methods suggested, the Panel strongly recommends matrix spike samples be performed regularly wherein the strong agonist used to estimate BEQs for each particular bioassay is spiked into a water sample (see Table 7.3). These should be done in true duplicate samples, to determine the recovery in each matrix evaluated. In addition, blank water spiked with the same strong agonist should also be

analyzed to determine recovery and reproducibility. The Panel also suggests that sample names be encoded with no information immediately identifiable to the analyst as to blank and spike samples to reduce bias. In summary, there are adequate sample preparation and analysis procedures established and standardized to allow recycled water utilities to begin using the ER and AhR bioassays through a combination of EPA methods for sample preparation and existing commericial laboratories for bioassay analyses.

The Phase 2 bioanalytical tool development and application project described in section 7.2 will further standardize sample collection, storage and extraction protocols for bioscreening analysis of water samples, focusing on matrices of interest for recycled water utilities. By testing a range of source and product water qualities in concert with target and/or surrogate agonists (chemicals) at the bench scale, this effort will deliver standard operating procedures that provide the broadest range and most robust measurement of response for a number of bioassays, including ER and AhR. There are opportunities for additional future research whereby optimized sample collection and preparation methodologies could be developed to reduce sample volumes and preparation steps, and ultimately, to analyze a water sample without pre-concentration (aka direct water analysis). Realization of direct water analysis, whereby the medium is prepared with the water sample itself, would effectively eliminate target chemical losses and/or discrimination of chemicals present in a given sample. However, direct water analysis is not a simple task and many challenges must be overcome. For example, salt concentration and pH buffering would need to be optimized to avoid cell lysing through differences in osmotic pressure. The high cost of labor associated with direct water analysis would also need to be addressed.

7.4 Regulatory Concerns

While concerns have been expressed that a regulatory requirement will be made upon utilities similar to that of Whole Effluent Toxicity (WET) testing of wastewater discharge, the Panel instead suggests a tiered, “adaptive management” strategy that minimizes regulatory restrictions by utilizing bioanalytical methods as a screening tool in conjunction with chemical analysis to identify if chemicals missed by targeted monitoring are potentially problematic. The Panel also notes that while identification of cause of death from a WET test can lead to significant costs, bioscreening tools that target specific biological responses (e.g. receptor activation or binding) are much less labor intensive, have significantly higher “through-put”, and are considered to be lower cost alternatives than standard Toxicity Identification Evaluation (TIE) analyses conducted under WET evaluations. With appropriate coupling to NTA (see Figure 7.1 and discussions in Chapter 6), causative agents can be identified. Success stories include identification of estrogens in wastewater responsible for ER activation in studies from the 1990s (Snyder et al., 2001; Jobling et al., 1998), and more recent successes such as identification of highly potent glucocorticoid steroids in recycled water (Jia et al., 2016).

Studies that have coupled bioassays with NTA and other analytical chemistry methods have shown that with the notable exception of estrogen active compounds where correlations between chemistry and *in vitro* biological activities can be as high as 90%, less than 5% of the biological activity of other receptors (e.g. glucocorticoid, androgen) can be measured by chemistry, demonstrating the lower selectivity but greater sensitivity of bioassays in evaluating water extracts (Leusch et al., 2014; Leusch et al., 2017; Conley et al., 2017).

With regard to unknown CECs, the Panel would like to also note the use of bioassays to assess novel disinfection byproducts produced from water treatment as some treatments (e.g. chlorination) can create byproducts with greater toxicity (Neale et al., 2012; Bulloch et al., 2014; Denslow et al., 2016).

7.5 Panel Recommendations for Implementation of Bioanalytical Tools

There is simply no way that chemical-by-chemical monitoring can keep pace with the discovery of new chemicals, either manufactured intentionally or produced unintentionally as transformation products of water treatment practices. While Panel members are supporters of the new mechanistic paradigms for toxicology testing as described by the Adverse Outcome Pathway concept (Ankley et al., 2010) and by Toxicology in the 21st Century recommendations for chemical safety testing as described by the National Research Council (NRC, 2007), the Panel does not believe this process could be used to set *in vivo* water safety guidelines at this point in time. Rather, the Panel adheres to the paradigm of using the Adverse Outcome Pathway framework to identify specific molecular responses that can be developed, standardized and applied to screen for the bioactivity of compounds and mixtures in water that are relevant for protecting human health. Adverse endpoints of cancer or reproductive dysfunction can be inferred by measuring activation of one or more molecular initiating events, and it is this linkage of events that supports the use of bioanalytical tools. Thus, the Panel proposes use of *in vitro* assays to evaluate more comprehensively the gamut of potential exposures to contaminants, and to establish screening (i.e. early warning) trigger levels, rather than to establish regulatory numeric standards for compliance. The benefits of this strategy for water quality monitoring and assessment is the prediction of *in vivo* adverse outcomes from high throughput receptor-driven molecular initiating events. Measuring activation of the molecular initiating events in a mass-balance approach can be used in conjunction with preset guidelines for screening water for contaminants. The Panel acknowledges that a significant amount of work remains before a useful collection of assays is ready for regulatory compliance application. For the near-term, the best use of cell assays in their current state of development is to complement analytical chemistry, particularly in a screening approach to help identify known and unknown contaminants in reclaimed water at concentrations that may have the potential to pose a risk to human health or the environment (Figure 7.1). The Panel's vision is that as knowledge of Adverse Outcome Pathways broaden, and more cell assays are developed, standardized and validated to screen for chemicals based on mode of action, a bioanalytical toolbox will become an essential monitoring and assessment component in protecting human health from exposure to chemicals in water.

DRAFT

7.5.1 Which in vitro assays are ready for screening of recycled water quality?

At present, the Panel believes there are several cell assays that are relevant for water quality monitoring, but that are at various stages of development and readiness for routine monitoring (Table 7.2).

Table 7.2. I*n vitro* assays that screen for CECs by mode of biological action.

Endpoint Activity	Relevant CECs	Adverse effect	Development Stage[a]
Endocrine disrupting chemicals (EDCs)			
Estrogen receptor (ER)	Estradiol, bisphenol A, nonylphenol	Feminization, impaired reproduction, cancer	4
Anti-estrogen receptor (ER-)	Pyrethroids	Disrupted reproductive development, impaired reproduction	2
Anti-androgen receptor (AR-)	Musks, phthalates, pesticides	Androgen insensitivity, impaired reproduction, cancer	2
Glucocorticoid receptor (GR)	Anti-inflammatory steroids	Development, immune diseases, diabetes	3
Progesterone receptor (PR)	Progestins	Cancer, hormone resistance syndrome, impaired reproduction	2
Carcinogenic chemicals			
Aryl hydrocarbon receptor (AhR)	Dioxin-like chemicals, polycyclic aromatic hydrocarbons, pesticides	Cancer, impaired reproduction	5
Tumor suppressor protein Response Element (p53RE)	Dioxin-like chemicals, PAH metabolites	Oxidative stress, tissue and DNA damage, cancer	1
Immunosuppressants, neurotoxins and other chemicals of concern			
Thyroid receptor (TR)	Pesticides, bisphenol A	Impaired metabolism, auto-immune diseases	1
Peroxisome proliferator activated receptor (PPAR)	Pharmaceuticals, phthalates	Metabolic disorders, impaired immune function, cancer	1
Acetylcholine receptor	Neonicotinoid and other pesticides	Neurotoxicity, behavior	1

Stage 1 - exploratory: is endpoint amenable to WQ screening?
Stage 2 - optimization: is performance consistent with monitoring goals?
Stage 3 - standardization: can SOPs and thresholds be developed?
Stage 4 - pilot evaluation: does it provide value in practice?
Stage 5 - implementation: is it available commercially and certified by the Environmental Laboratory Accreditation Program?

In particular, the Panel believes that those that have been fully standardized and subjected to pilot evaluation (Stage 4) are ready to be applied for initial monitoring of recycled water applications. This would presently include the ER and aryl hydrocarbon receptor (AhR) *in vitro* assays. In addition to testing of water at the point of application and/or compliance for monitoring, the Panel also recognizes the utility of bioanalytical screening tools, as pointed out by the recycled water research community, in assessing treatment efficacy of individual unit operations or the entire treatment train (Figure 7.2). Several studies have demonstrated the utility of bioassays in the evaluation of treatment and have consistently shown that biological activity can effectively be used to evaluate treatment. For example, reverse osmosis treatment has been shown to essentially remove all detected biological activity in water (Escher et al., 2011; Leusch et al., 2014; Mehinto et al., 2015; Leusch et al., 2017; Carollo Engineers, 2017).

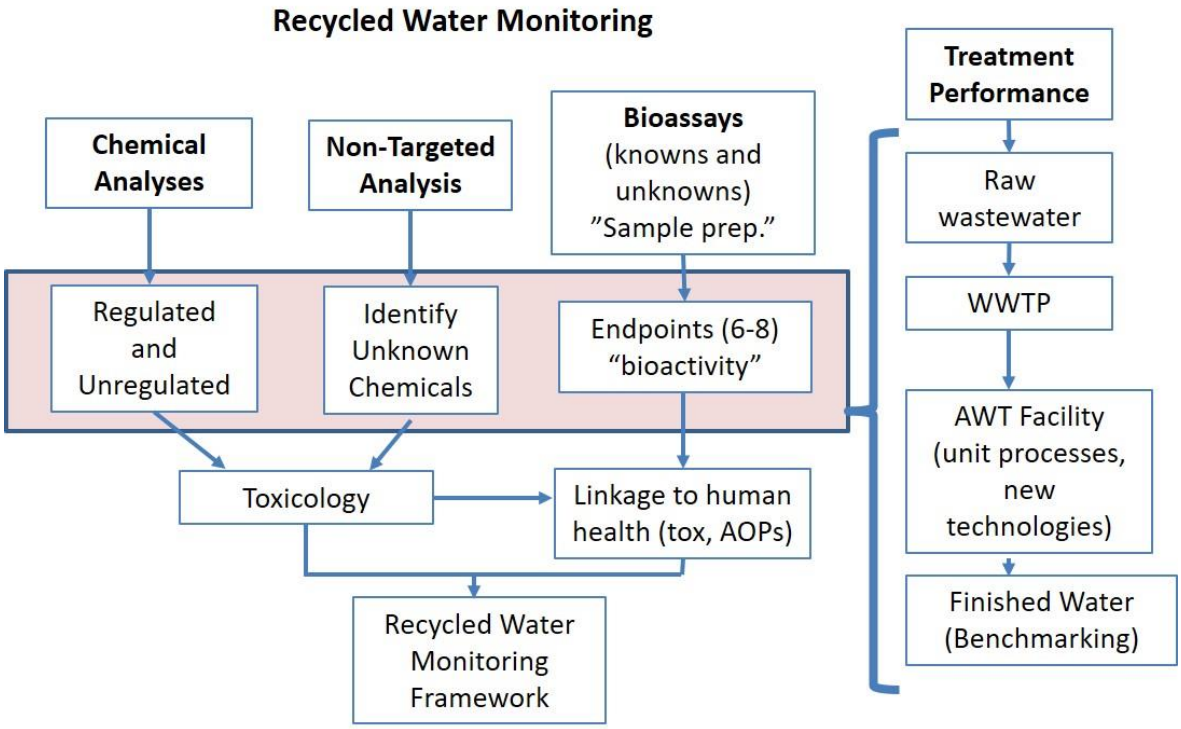


Figure 7.2. Application of bioanalytical tools complements chemical analyses in monitoring and assessment of recycled water quality and treatment performance.

This is significant because biological activity was also shown to be more sensitive than chemical analyses in many of these studies. In addition to evaluating RO treatment efficiency, bioassays have been used to assess granular activated carbon (GAC) filtration, ozonation (Beresford et al., 2016), advanced oxidation processes (Escher et al., 2011; Carollo Engineers, 2017), and wetland treatment of effluents (Nivala et al., 2018). In a potable reuse demonstration pilot project in Altamonte Springs, FL, six *in vitro* assays were used to demonstrate effective treatment with biological aerated filter, GAC and UV AOP effluents (i.e. finished water) confirming that the pilot treatment train eliminated most, if not all bioactivity represented by the assays, and by association eliminated chemical pollutants associated with these bioactivities (Carollo Engineers, 2017).

The Panel recognizes that cell assays currently available cannot be used to evaluate all possible mechanisms of adverse biological impacts. However, for focused mechanisms linked to adverse outcomes, cell bioassays are proposed as a more comprehensive monitoring tool

to expand and complement already existing water quality evaluation techniques. Robust, reproducible and high throughput assays have been developed for these applications for human medicine and these should be used to screen for known and unknown chemicals of interest. This is one of the primary ways to evaluate the occurrence of unknown/unknown CECs. It is imperative to specify the endpoint of concern in this process. Whereas the USEPA has focused on compounds that interfere with estrogen, androgen, thyroid hormone and steroidogenic responses to date, other potential candidate endpoints of concern for human health include genotoxicity (cancer), immunotoxicity, and neurotoxicity (Table 7.2). Expansion of the bioanalytical toolbox to include these and other endpoints deemed relevant to the protection of human health is a recommendation for future work.

7.5.2 Recommendations for standardized protocols for in vitro assays

Based upon the extraction methods discussed in 7.3.2 and Chapter 6, the Panel concludes that extracts prepared in this fashion can, at a minimum, be sent to commercial laboratories for analyses (see section 7.3). Alternatively, the analyses can be performed by utilities using "kits" available from commercial laboratories that have undergone "round-robin" inter-laboratory optimization and validation (Mehinto et al., 2015). Regardless whether the bioanalytical analysis is performed by a commercial or utility laboratory, some general QA/QC guidelines should be followed that address key analytical parameters and their control levels. Table 7.3 specifies these guidelines for the GeneBLAzer® assay as an example. All parameters proposed by the Panel have analogs for a comprehensive, performance-based validation of protocols for targeted chemical analyses, e.g. blank analysis (background and solvent responses), calibration response (Hill slope and log EC50 value), matrix spike recovery and interlaboratory agreement. In addition, a guideline to test for cell viability is necessary to ensure that the living component of the *in vitro* assays (i.e. the cells themselves) are functional.

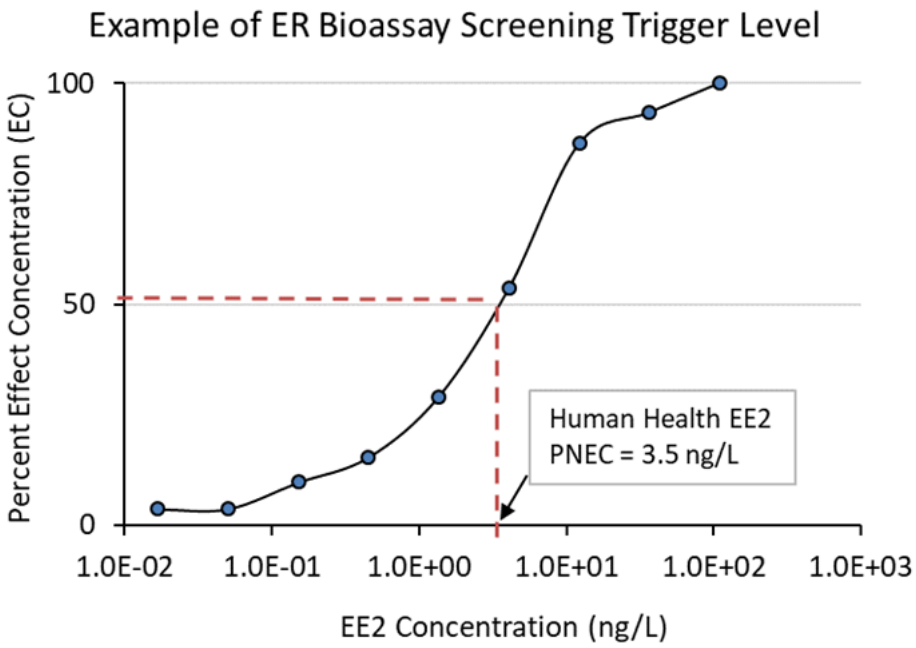
Table 7.3. Quality assurance/quality control (QA/QC) guidelines for the GeneBLAzer® *in vitro* transactivation bioassays adapted and standardized for water quality screening (adapted from Mehinto et al., 2015).

Parameter	Acceptance criteria
Extract cytotoxicity	Cell mortality shall be less than 20%
Background response	Mean response for media only controls[1] shall be at least 25% lower than the mean response for cell and media controls
Solvent effect	Mean response for solvent vehicle-exposed cells shall be less than or equal to the mean response for cell and media controls
Calibration	Hill slope and $\log EC_{50}$ values shall be within the expected range (see Table SI-2); $r^2 > 0.95$
Matrix Spike Recovery	Percent recovery of strong agonist spiked into sample prior to extraction shall be 70% < Percent Recovery < 130%
Intra-laboratory precision	Relative standard deviation (RSD) of triplicate bioassay responses (for a given sample) shall be less than 20%

[1] cell free

Textbox 7.1

A Framework to Use Bioassays to Identify Sources Requiring Further Evaluation. If bioassay response can be linked with PNECs developed for the protection of human health, such linkage could allow for the use of bioassays as a screening tool to determine whether recycled water has the potential to pose a concern to human health and may warrant further, more refined evaluation. The estrogen receptor (ER) bioassay provides an example of such a screening framework given that ER bioassay methods are well developed, accepted and repeatable (Leusch et al., 2017). In addition, PNECs based on mammalian toxicity data have been developed for several of the estrogens that trigger a response in the ER bioassay (Caldwell et al., 2010). If the bioassay response that corresponds to the PNEC can be identified, then that bioassay response can be used as a trigger to distinguish between recycled water that is not expected to pose a risk to human health and therefore does not require further evaluation of potential effects associated with the ER mode of action. The Figure below shows an example of the derivation of such a trigger level based on the PNEC of 3.5 ng/L for 17β-ethinylestradiol (EE2). On that figure the concentration corresponding to the PNEC is superimposed on the ER bioassay dose-response curve (Mehinto et al., 2018; Denslow et al., unpublished data). The PNEC corresponds to approximately the EC50 of the bioassay response, indicating that concentrations of EE2 (or mixtures of other compounds that act by the same mode of action) that lead to bioassay responses of less than about 50% are not expected to pose a risk to human health. A bioassay response of greater than about 50% indicates that either EE2 or a combination of other compounds that act by the same mode of action may be present at levels that may pose a risk to human health. In such cases, further targeted evaluation of recycled water extract is necessary to identify the compound or compounds that are causing the higher than acceptable ER bioassay response (see Figure below). It is important to emphasize that this example illustrates the framework for a screening evaluation of only a single mode of action, estrogen receptor response. As discussed elsewhere in this section, bioassays evaluating numerous modes of action are in various stages of development and refinement. For a recycled water extract to be identified as not requiring further evaluation based on bioanalytical screening tools, similar linkages between bioassay response and human health PNECs need to be established for the other relevant modes of action. The Panel recommends that focused pilot studies evaluating the applicability and reliability of the ER bioassay screening framework described above be conducted and that other modes of action be added as bioassay methods as those modes of action reach maturity.



7.5.3 Decision making logic for bioanalytical screening results

Step 1. Compare bioassay response (as BEQs) to established guideline, such as a PNEC or Action Level (AL) (see Appendix D and Text Box 7.1).

a. If BEQ > AL, GO TO STEP 2.

b. If BEQ < AL, continue with screening level monitoring; GO TO STEP 3

Step 2. Define actions commensurate with degree and persistence of excursions (see Appendix F, Figure F.1).

a. Confirm exceedance within a specified period of time (e.g. 72 h).

b. If confirmed, initiate analysis of samples targeting chemicals known or suspected of eliciting responses consistent with the endpoint in question. Increase frequency *of in vitro* bioassay and/or chemical analysis of samples to determine the temporal variability and persistence of the exceedance. Notify the regulatory agency(s) responsible for oversight of monitoring.

c. Consider the degree of exceedance (i.e. magnitude of BEQ/AL) in determining the appropriate course of action.

Step 3. Review and evaluate monitoring data on a periodic (e.g. annual) schedule, using a tiered decision framework whose management actions are commensurate with the magnitude and consistency of bioassay results (see Appendix F, Figure F.1)

a. For endpoints that consistently exhibit a BEQ/AL <0.1, consider decreasing monitoring frequency or eliminating requirement for inclusion of endpoint.

b. For endpoints that consistently exhibit responses at a minimal level of concern (0.1 < BEQ/AL < 10), continue monitoring to ensure that responses are not increasing over time.

c. For endpoints that consistently exhibit responses at an elevated level of concern (10 < BEQ/AL < 1,000), consider increasing frequency of monitoring, identifying sources and/or instituting alternative treatment options to reduce the bioactivity response into the minimal or no concern level.

d. For endpoints that consistently exhibit responses at a high level of concern (BEQ/AL > 1,000), consider removing the RW supply while increasing frequency of monitoring, identifying sources and/or treatment alternatives.

e. Consult with independent experts to review monitoring data, update toxicological data (PNECs, NOECs) that may support revision of ALs, and recommend improvements in the selection and performance of *in vitro* bioassays.

7.5.4 Recommendations for monitoring of potable reuse projects

The Panel recommends two major uses for bioanalytical assays as a screening tool: 1) to complement targeted CEC monitoring of product water from potable reuse projects, and as voluntary approach 2) to follow the efficacy of the removal of CECs through AWT trains, on a voluntary basis if the product water shows high BEQ values (Figure 7.1).

In the first use, the goal would be to show levels of bioactivity for the ER and AhR assays that are below their respective AL. If expectations are met, then these projects would be deemed good to go and would only continue to be monitored via both bioanalytical assays and analytical chemistry at a relatively low frequency (e.g. biannually). For projects that show BEQ's above the AL through the finished product, they would go to Step 2 in the Panel's workflow (section 7.5.3) to determine the repeatability of the occurrence. Step 3 addresses

the interpretation of the results. The utility could also check on operating/maintenance issues and retest as needed. For each type of potable reuse facility, the frequency of repeating the assays would be biannually to check that source control and treatment remain effective. The assays would also be performed at start-up of a new operation or after a major process change or upgrade is performed. New water reuse facilities should monitor more frequently, at a minimum quarterly, for the first year of the project.

For the second use, the Panel suggests voluntary application of standardized bioanalytical tools to demonstrate efficacy of CEC removal (as represented by ER and AhR) for all IPR groundwater recharge and SWA utilities. The sampling locations, type of reuse project (including treatment processes), and frequency of sampling would all depend on the sampling objective and the type of potable reuse. For example, a utility interested in benchmarking the removal of bioactivity along their treatment train (including the POC) could measure ER and AhR activity at each or after selected points in their treatment train. They could also implement standardized bioanalytical measurements to evaluate the effects of operational and maintenance procedures on water quality.

7.5.5 Guidance for implementation of bioanalytical monitoring

As the application of bioanalytical tools to recycled water quality monitoring and assessment is a relatively new endeavor, the Panel recommends that the State Water Board convene an advisory group to guide utilities and the State Water Board through the initial round of *in vitro* bioassay monitoring, as described in 7.5.4. This "bioanalytical advisory group" could consist of select Panel and SAG members, bioanalytical application experts, State Water Board staff and representatives from the commercial services industry who would ultimately be tasked to perform such measurements. The group would define goals for bioanalytical monitoring, specify protocols for sampling, extraction, measurement and data reporting (discussed in 7.5.2), and provide guidance for interpretation of bioanalytical monitoring results, including QA/QC data (discussed in 7.5.3). To maximize commonality and consistency of the guidance provided, the group would also interact with on-going and future efforts to develop, evaluate and apply bioanalytical tools for water quality screening, particularly those supported by the State Water Board and/or recycled water research organizations working with the State Water Board.

7.6 Challenges for Applying Bioanalytical Tools and Next Steps

7.6.1 Toolbox development and standardization

Although dozens if not hundreds of *in vitro* assays exist for chemical screening applications, few are standardized and routinely applied to water quality monitoring. One of the greatest challenges for the successful application of bioanalytical tools is the development of a robust set of assays that show value and utility in screening for classes of chemicals that are relevant for recycled water applications, and that protect against the specter of deleterious human health effects. The Panel strongly advocates the development of a comprehensive, performance based QA/QC program for Stage 3 *in vitro* assays (Table 7.3), including round robin interlaboratory testing to ensure consistency in performance among assay developers and end users. This has already been done for AhR and ER bioassays (Escher et al., 2014; Mehinto et al., 2015; Leusch et al., 2017), hence their inclusion as Panel-recommended assays for future recycled water monitoring. The Panel further recommends that focused pilot studies evaluating the applicability and reliability of the ER bioassay screening framework

described in Textbox 7.1 be conducted and that other modes of action be added as those assays reach maturity.

7.6.2 Linkage of in vitro responses to in vivo effects

The Panel also recommends that assay endpoints under consideration (see Table 7.2) be mechanistically linked to apical endpoints of toxicity (e.g., cancer, development, immune dysfunction, reproduction) rather than to non-specific biological responses. One option for making *in vitro* to *in vivo* linkages in a high throughput capacity is to couple *in vitro* assays to zebrafish embryo development assays, which provide an *in vivo* assessment of developmental toxicity. Zebrafish have been widely accepted as an alternative model of vertebrate (i.e. human) development. Additionally, inclusion of metabolism or bioactivation is necessary to provide the *in vitro* to *in vivo* linkage. Pretreatment of samples with liver homogenates (i.e. S9) prior to cell exposure has been proposed to estimate the metabolic disposition of known and unknown contaminants in water extracts.

7.6.3 Effect of mixtures on assay performance

CECs that respond weakly to *in vitro* assays ("weak agonists") may not display expected parallelism and may have lower maximal achievable responses. In these cases, it is difficult to get an accurate measurement of the activity of the individual test chemical. One can still standardize by comparing EC50s of the individual chemicals. The problem becomes trickier for environmental mixtures that may be composed of several different weak agonists, or mixtures of strong and weak agonists, and maybe even antagonists at concentrations that preclude performing a complete dose response experiment. In these cases where antagonists are present, the *in vitro* assay may underestimate the potency of the individual agonists in the mixture, but can still aid analytical chemists in identifying mixtures that have additive effects that exceed an action level. The Panel recommends that simple mixtures with agonists and antagonists of various potencies be evaluated. The compounds should be those observed previously in source waters.

7.7 Conclusions

- Bioanalytical tools can enhance monitoring by screening for a broader universe of chemicals than can be measured by targeted monitoring, including unknown CECs.
- Standardized *in vitro* (cell-based) assays are a rapid, cost-effective way to quantify classes of CECs, e.g. endocrine active chemicals.
- Monitoring trigger levels (MTLs) can be established for well-established cell assays (e.g. ER and AhR).
- The Panel recommends implementation of the estrogen and aryl hydrocarbon receptor (ER and AhR) assays for screening of unmonitored CECs in potable reuse projects.
- Additional investment in research and training is needed to provide a robust and comprehensive "bioscreening toolbox" for other biological target endpoints, which are currently at different stages of development for recycled water applications, and to increase capacity for commercial bioanalytical service.

8. Importance of Antibiotic Resistance in Water Recycling

8.1 Introduction

In the 2010 report, the Panel conducted a "…cursory review of antibiotic resistance in relation to water reuse practices..." and concluded that the issue was more complex and required more resources, expertise and time for a thorough review. Based upon their cursory review, the 2010 Panel reached a preliminary conclusion that antibiotic resistance is potentially an issue for any wastewater discharge into the environment and did not appear to be solely an issue with the water reuse practices considered. The Panel recommended that a more thorough review of antibiotic resistance related to reuse practices be conducted.

In 2012, the Science Advisory Panel for CECs in aquatic ecosystems also addressed monitoring for antimicrobial and antibiotic chemicals as well as antibiotic resistance in oceanic, brackish and fresh waters that receive discharges of treated municipal wastewater and storm water effluent [*Monitoring Strategies for Chemicals of Emerging Concern (CECs) in California's Aquatic Ecosystems*, SCCWRP, 2012]. This Aquatic Ecosystems Panel applied a risk-based screening framework to three receiving water scenarios in order to identify CECs for initial monitoring. The framework included no observed effect concentrations (NOEC) and lowest observed effect concentrations (LOECs) for antibiotic chemicals. One antimicrobial chemical (triclosan) and no antibiotic chemicals, were identified for initial screening for the WWTP effluent-dominated inland freshwater waterway using this process. However, this Panel noted that there was no standardized assessment method for antibiotic resistance in receiving water matrices.

In 2016, the DPR Expert Panel addressed issues related to DPR in California including antibiotic resistance (*Evaluation of the Feasibility of Developing Uniform Water Recycling Criteria for Direct Potable Reuse*, Chapter 7: Antibiotic Resistant Bacteria and Antibiotic Resistance Genes. Olivieri et al., 2016). An extensive literature review was undertaken in order to address factors such as sources and exposure routes, methods for assessing antibiotic resistance in water matrices, and occurrence and removal of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) through water and wastewater treatment. The DPR Panel concluded that a combination of secondary wastewater treatment and advanced water treatment processes is likely to reduce ARB and ARG concentrations in recycled water to levels well below those found in conventional treated drinking water. Based on these and other findings, the DPR Panel felt that recycled water (i.e., secondary and advanced treated water) was not a significant disseminator of ARB and ARGs relative to other sources. However due to some uncertainty with that opinion, research was recommended on ARB/ARG risk assessment, methods development and standardization, and characterization of removal using advanced water treatment processes.

California Title 22 treatment requirements, as described in Chapter 3, are some of the most stringent criteria in the nation, if not the world. For example, the tertiary treated water requires a filtered and disinfected wastewater that meets a CT value (product of total chlorine residual and modal contact time measured at the same point) of not less than 450 mg-min/L at all times, with a modal contact time of 90 minutes (based on peak dry weather design flow) or provides a 5-log removal/reduction of MS2 F-specific phage, poliovirus or similar virus. Thus, while the following review information is presented and discussed to consider the general state of knowledge regarding ARB and ARGs, no attempt has been made to compare

the treatment types and performance against the California requirements. Also, no attempt has been made to unify authors various approaches, methods or units of measurement.

It is well known that bacteria exposed to antibiotics will develop resistance, as a way to survive. But, it may not be the only driver for antibiotic resistance. As demonstrated by Knöppel et.al. (2017), selection in the absence of antibiotics can also co-select for decreased susceptibility to several antibiotics, thus indicating that genetic adaptation of bacteria to natural environments may drive resistance evolution by generating a pool of resistance mutations.

8.2 Occurrence and Treatment Efficiencies for Removal of ARB/ARGs in Wastewater

Previous studies have reported concentrations of ARGs found in raw wastewater, activated sludge, secondary effluent and tertiary effluent as well as their log reductions by these treatment processes (see Appendix E-1). Appendix E-2 lists the same information for ARB. Concentrations of clinically relevant ARGs were reported ranging from 10^7 to 10^{11} copies/100 mL in raw wastewater, while culture-based ARB ranged from 10^5 to 10^8 colony forming units (CFU) or most probable number (MPN)/100 mL.

Less than one-$\log_{10}$ unit of individual ARGs is removed by primary treatment, while secondary treatment reduces ARGs by one to three $\log_{10}$ units and ARB between zero and five $\log_{10}$ units. Tertiary treatment can provide up to an additional four-$\log_{10}$ removal of ARGs and ARB. Membrane bioreactor technology incorporating UV disinfection can reduce ARGs and ARB to a greater extent than conventional WWTPs using tertiary sand filtration and disinfection. For unknown reasons, removal of antibiotic resistance determinants may not be consistent since some ARGs and ARB might be found in higher numbers after particular treatments. Available disinfection data for removal of ARB and ARGs were summarized by the DPR Panel (see Appendix E-3). Generally, ARB were reduced by 2-4 $\log_{10}$ during disinfection processes and ARGs by 1-3$\log_{10}$ while ARGs were reduced by 2-7 $\log_{10}$ units for primary through tertiary treatment and ARB were reduced by 1-9 $\log_{10}$ units for the same treatment train. With the addition of disinfection (i.e., chlorine, ozone, UV) the $\log_{10}$ reduction of ARGs ranges from 3-10 and ARB from 3-13.

8.3 Occurrence, Fate and Transport of ARB/ARGs in the Three Major Categories of Recycled Municipal Wastewater

The Title 22 minimum level of treatment required for these uses is shown in Tables 3.1, 3.3 and 3.4. A summary of literature on ARB/ARGs in each of these three categories is given below. The California Department of Water Resources recently completed a new survey of recycled water usage based on 2015 data. The preliminary report is available (http://water.ca.gov/recycling). The percentages of municipal recycled wastewater in this latest survey used for agriculture, landscape irrigation and groundwater recharge were about 30%, 18% and 16%, respectively (see Chapter 3, Figure 3.1).

8.3.1 Agricultural irrigation

Agricultural irrigation represents the largest category of recycled municipal wastewater in California and can be divided into 11 subcategories (Table 8.1). The minimum level of treatment for 5 of these categories where there is little likelihood of human contact with the irrigated products is undisinfected secondary treatment (see Chapter 3.1 for definitions and detailed information on the level of treatment required for each category). Two categories, ornamental nursery stock or sod farms with unrestricted public access and pasture for milk

animals, require treatment at least at the "disinfected secondary-23" level (oxidized and disinfected so that the median concentration of total coliform bacteria does not exceed a most probable number of 23 MPN per 100 mL, and the MPN does not exceed 240/100 mL in more than one sample in any 30-day period.) Three categories of food crops for human consumption where there is no contact of the edible portion with recycled water minimally require "disinfected secondary-2.2" treatment (oxidized and disinfected so that the median concentration of total coliform bacteria does not exceed a most probable number of 2.2/100 mL, and the MPN does not exceed 2/100 mL in more than one sample in any 30-day period). Only one category (i.e. food crops where the edible portion is contacted by recycled water) must minimally use disinfected tertiary treated wastewater.

No published information on ARB and ARGs related to agricultural irrigation in California has been found and the literature for these applications is limited in general. Interpretation of available data is complicated by the lack of uniform targets and standard methods. Much literature pertaining to agricultural use or agroecosystems has been directed toward antibiotic use and occurrence of resistance in animals and the effect of using manure on farmland (Lau et al., 2017; Rothrock et al., 2016; Williams-Nguyen et al., 2016a and 2016b).

A study in Israel (Negreanu et al., 2012) examined ARB and ARGs at four geographically diverse sites where cropland was drip irrigated either with treated wastewater or freshwater. The soil types were 52% clay, 60% clay, dune quartz sand and loam having 20% clay. Three of the sites were irrigated with secondary treated wastewater and one used secondary effluent recharged through soil and the vadose zone. The crops at the sites were avocado, citrus trees, cotton and wheat, and olive trees. The duration of irrigation ranged from 6 to 15 years.

No correlation was found between wastewater effluents and antibiotic resistance. The relative abundance of ARB was never significantly higher in soils exposed to treated wastewater effluents compared to corresponding freshwater plots. The reverse was true on two occasions. Six different ARGs conferring resistance to four different clinically relevant antibiotics were targeted and were detected in the soil exposed to wastewater effluents at levels ranging from 10^4 to 10^6 copies per g/mL. The levels of ARB and ARGs were generally identical or even lower than in freshwater irrigated soils. The authors concluded that the high levels of ARB that enter soils from the wastewater were not able to compete and survive in the soil environment and did not significantly contribute ARGs to soil bacteria. Although data were not shown, soil samples taken directly under the drippers had significantly higher levels of antibiotic resistance than soil samples 50 cm from the drippers. The authors indicated that their results suggest that wastewater effluent-associated bacteria had a negligible impact on the soil microbiome and that the levels of ARB and ARGs in both soils irrigated with wastewater effluents and freshwater were indicative of native antibiotic resistance associated with the natural soil microbiome. These findings could have relevance to the fate and transport of antibiotic resistance and to the role of the environmental buffer in potable reuse as well as non-potable groundwater recharge scenarios.

Table 8.1. Title 22 Treatment requirements for agricultural irrigation (uses 37% of municipal recycled water[a]).

Agricultural Irrigation	Treatment Level			
	Disinfected tertiary	Disinfected secondary-2.2	Disinfected secondary-23	Undisinfected secondary
Food crops where RW contacts edible portion including root crops	X			
Food crops, surface irrigated, above-ground edible portion and not contacted by RW		X		
Ornamental nursery stock and sod farms with unrestricted public access			X	
Pasture for milk animals for human consumption			X	
Orchards with no contact between edible portion and RW		X		
Vineyards with no contact between edible portion and RW		X		
Non-food bearing trees, including Christmas trees not irrigated less than 14 days before harvest				X
Fodder and fiber crops and pasture for animals not producing milk for human consumption				X
Seed crops not eaten by humans				X
Food crops undergoing commercial pathogen-destroying processing before human consumption				X
Ornamental nursery stock, sod farms not irrigated less than 14 days before harvest				X

[a]http://www.waterboards.ca.gov/water_issues/programs/grants_loans/water_recycling/munirec.shtml

Gatica and Cytryn (2013) reviewed a number of studies designed to determine the effects of anthropogenic practices on environmental bacterial communities with an emphasis on the potential effects of reclaimed water irrigation on antibiotic resistance in the soil microbiome. Given the state of the art at the time of the studies, they concluded that while wastewater effluent discharged to freshwater environments tends to expand ARB and ARG levels, reclaimed water irrigation did not seem to impact antibiotic resistance levels in the soil microbiome. While these authors were cautiously optimistic about the future implementation of reclaimed water irrigation, they also indicated that further studies were

needed to determine any possible contribution of reclaimed water irrigation to antibiotic resistant reservoirs in irrigated soils.

Fahrenfeld et al. (2013) quantified ARGs in three western U.S. reclaimed water distribution systems. They reported a broader range of ARGs after the reclaimed water passed through the distribution systems and indicated that it is important to consider bacterial regrowth and the overall water quality at the point of use and not just at the treatment plant. They also did some laboratory microcosm studies with secondary effluent irrigated soil. The prevalence of sulfonamide ARGs was increased in historically manured soil compared to soil irrigated with chlorinated or dechlorinated secondary effluent or deionized water. However, tetracycline ARGs were not affected by irrigation highlighting that there could be different environmental fates for different ARGs.

The effect of irrigation on increasing some ARGs could be due to direct inputs of extracellular ARGs, intracellular ARGs or horizontal gene transfer to native soil bacteria. Fahrenfeld et al. (2013) also indicated that their results were consistent with those of Negreanu et al. (2012) where higher levels of antibiotic resistance were found below the drippers. Soil type and irrigation rate may affect the transport of ARB and ARGs.

Ferro et al. (2015) evaluated a sunlight/H_2O_2 process as an option to other solar driven AOPs for municipal wastewater from small communities (size not defined) to be used for crop irrigation. In a pilot-scale system, they exposed antibiotic resistant bacteria (*E. coli* and *E. faecalis*) to 20 mg/L H_2O_2 in a solar compound parabolic system. *E. coli* were reduced to below the detection limit (2 CFU/mL) after 120 minutes of exposure whereas *E. faecalis* required 240 minutes to reach that limit. When used to irrigate lettuce plots, no bacteria contamination was observed on the lettuce or soil when the bacterial density was below the detection limit in the wastewater. They concluded that a treatment time with this system should be >90 minutes to avoid transfer of pathogens and ARB from the disinfected wastewater to the crops and soil.

Graham et al. (2016) studied archived soil samples for broad-spectrum ß-lactam ARGs from land that received only animal manure or inorganic fertilizer. While this study did not examine the effect of reclaimed water, it does provide information on the fate and variability of ARGs in soil. Manure use for 100 years was found to approximately double ARG abundances in manured soils, thus increasing the probability of broader ARG exposure in drainage water and fodder crops. Dominant ARGs varied over time and roughly paralleled the first reporting of these genes in clinical isolates suggesting an historical interconnection of ARGs in animal manure and humans. When non-therapeutic antibiotic use was banned, *bla*(CTX-M) gene levels declined in manured soils. However, *int*1 gene levels continued to increase despite the ban. The authors speculated that manure use increased the intrinsic potential of the soils for horizontal gene transfer.

Williams-Nguyen et al. (2016a and 2016b) reviewed the state of the science with respect to ARB and ARGs in agroecosystems and addressed literature pertaining to the use of biosolids, manure and wastewater irrigation in agriculture. They stated that there is no evidence that irrigation with wastewater effluent increases the presence of ARB or ARGs in environmental media and cited studies (Negreanu et al., 2012; McLain and Williams, 2014) with long-term exposure to treated effluent that have found no effect. While recognizing the potential for dissemination of ARGs through irrigation, they concluded that the available evidence suggests that the impact on the prevalence of ARB and ARGs in the soil is minimal. They also stated

that less is understood about the fate and transport of ARGs than ARB in soil and water and indicated that extracellular DNA persistence has been reported to range from months to years (Pietramellara et al., 2009; Carini et al., 2016).

Christou et al. (2017) reviewed recent studies on antibiotics, ARB and ARGs in the agricultural environment as a result of reclaimed water irrigation. Their paper primarily addressed antibiotics but also covered antibiotic resistance. These authors indicated that WWTPs have been regarded as "hotspots" and "genetic reactors" for antibiotic resistance. The use of reclaimed water, biosolids and manure can enrich the soil with ARB and ARGs which can persist in the environment or be transferred to human commensals or pathogens with clinical relevance. While the potential is there, how significant this is in disseminating antibiotic resistance is not known. Reclaimed water irrigation may result in releasing ARB and ARGs to natural and agricultural environments, which can potentially cause risk to human health.

Christou et al. (2017) stated that the studies they reviewed show that the dynamics of the reclaimed water-soil-crop continuum with respect to ARB and ARGs are highly complex and that ARB persistence and horizontal transfer of ARG across environmental barriers depends on many biotic and abiotic factors. For example, studies of reclaimed water irrigation of agricultural soil (Fahrenfeld et al., 2013; Negreanu et al., 2012; Gatica and Cytryn, 2013) did not seem to impact the level of antibiotic resistance in the soil microbiome. However, studies of reclaimed water usage in urban parks in China (Wang et al., 2014) and Victoria, Australia (Han et al., 2016) found a higher diversity and an increased abundance of some ARGs compared to those in pristine soil or receiving freshwater irrigation. Christou et al. (2017) suggest that the small amount of sample, the heterogeneity of samples and the fact that environmental bacteria live mainly as aggregates may lead to these contradictory findings.

They also point out that soil contains an abundance of bacteria—one gram can contain 10^8 bacterial cells and more than 10^4 species, while one mL of reclaimed water may contain less than 10^6 bacterial cells of which less than 10^3 contain an acquired resistance gene. Assuming a soil water content of 10%(w/w) the prevalence of acquired ARGs in soil would be 0.0001%. In an unlikely scenario where aggregation or bacterial growth increased the prevalence of ARGs 100-fold, it would then be 0.01%. Pepper et al. (2018) estimated that the application of effluent to soil would only increase ARB by 0.0043% and ARGs by 0.14% over what is naturally found in soil.

Christou et al. (2017) concluded that current knowledge cannot exclude the possibility that environmental ARB can be transmitted to humans. However, assessing the risks is difficult due to technical shortcomings related to detection and quantification of ARB and ARGs in environmental matrices; lack of data on the number of ARB required to colonize humans, and scant information on the paths of dissemination and transmission from the environment to humans.

8.3.2 Landscape irrigation

This Title 22 category is divided into 8 subcategories, four of which require "disinfected tertiary" treated wastewater and four require "disinfected secondary-23" at a minimum (Table 8.2).

Published literature on ARB and ARGs related to reclaimed water use for landscape irrigation is scarce. Wang et al. (2014) examined soil samples from 6 public parks in Beijing, China and one pristine control area for antibiotic levels and ARGs including the *int*1 Class I integron as

an indicator of horizontal gene transfer potential. No data were supplied on ARGs in the reclaimed water or on the volumes and frequency of irrigation. ARGs were detected in all reclaimed water irrigated soil samples and they ranged from 2.6 x 10^5 to 1.69 x 10^8 copies per g of dry soil. The distribution of ARGs was different among park soils using the same reclaimed water. The differences might have been due to multiple factors including irrigation volume, irrigation frequency, abundance of ARGs in the reclaimed water and soil conditions.

Table 8.2. Title 22 treatment requirements for landscape irrigation (uses 24% of municipal recycled water[a]).

Landscape Irrigation	Treatment Level			
	Disinfected tertiary	Disinfected secondary-2.2	Disinfected secondary-23	Undisinfected secondary
Parks and playgrounds	X			
School yards	X			
Residential landscaping	X			
Unrestricted-access golf course	X			
Cemeteries			X	
Freeway landscaping			X	
Restricted-access golf courses			X	
Non-edible vegetation with access control to prevent use as a park, playground or school yard			X	
Groundwater Recharge (uses 12% of municipal recycled water[a])	Allowed under special case-by-case permits by Regional Water Boards			

[a]http://www.waterboards.ca.gov/water_issues/programs/grants_loans/water_recycling/munirec.shtml

The *sul*I ARG was detected at 1.69 x 10^8 copies per g of dry soil in irrigated soil samples but also at 9 x 10^7 copies per g of dry soil from a natural scenic resort in Ling Mountain. The *int*I1 gene was also present at a high abundance in pristine soil (2.61 x 10^7 copies per g of dry soil). The *tet*G, *sul*I and *sul*II genes had a significant positive correlation with the *int*I1 gene, suggesting that this gene could play a role in disseminating ARGs and indicate that horizontal gene transfer could be an important pathway for ARG proliferation. Soil pH was >7 at all sites and was negatively correlated with the abundance of ARGs, indicating that neutral soils were more suitable for microbial growth. These authors concluded that they could not establish a direct link between ARGs and public health concerns and indicated a need for more research on reclaimed water irrigation in urban parks, exploring the transfer of ARGs from soil to humans and establishing a suitable risk assessment model in order to determine the possible hazard to human health.

Han et al. (2016) examined the diversity, abundance and composition of ARGs in 12 urban parks with and without reclaimed water irrigation in Victoria, Australia. Forty unique ARGs were detected across all park soils with genes conferring resistance to ß-lactam being the

most prevalent. The total numbers and fold changes of the ARGs were significantly increased in urban parks by reclaimed water irrigation compared to natural parks not irrigated with reclaimed water. However, the article did not thoroughly address other sources of ARG in an urban environment. There were also shifts in ARG patterns and a significant change in the soil bacterial community structure in reclaimed water irrigated parks compared to those not irrigated by reclaimed water. Significant positive correlations between fold changes of the integrase *int*I1 gene and two ß-lactam resistance genes were noted. The absence of significant impacts of reclaimed water irrigation on the abundances of the *int*I1 and the transposase *tnp*A genes indicated that reclaimed water irrigation did not improve the potential of horizontal gene transfer of soil ARGs. These authors suggested that reclaimed water irrigation of urban parks could influence the abundance, diversity and compositions of clinically relevant soil ARGs.

Echeverria-Palencia et al. (2017) examined soil, air and drinking water from 6 parks in each of four California cities (Los Angeles, San Diego, Bakersfield and Fresno) for four antibiotic resistance genes. The *sul*1 gene was selected because it has been proposed as an urbanization marker, its ability to persist in the environment and the amount of previously collected data; the bla_{SHV} gene has a close relationship to genes suggested for environmental monitoring and had been shown to be increasing in soils, and the *erm*B and *erm*F genes are two of several that have been proposed as indicators for monitoring the antibiotic resistance status of a particular environment (Berendonk et al., 2015).

In soil, there were statistically significant differences in the bla_{SHV} levels with Bakersfield being highest, followed by San Diego, Fresno and Los Angeles. In drinking water, bla_{SHV} was detected in all San Diego samples but fluctuated in the other three cities. In air, bla_{SHV} was highest in Fresno while the other three cities were comparable to each other and about 50% lower than Fresno. The *sul*1 gene was consistently detected in soil in all parks and cities. The *sul*1 gene per liter of water was highly variable for San Diego, Bakersfield and Fresno having been detected in 30%, 21% and 13% of samples, respectively, while it was detected in 100% of Los Angeles samples. There were no statistically significant city-to-city relationships for the *erm*B and *erm*F genes. The results of the current study placed *erm*F above and *erm*B well below those currently reported in the literature.

Echeverria-Palencia et al. (2017) indicated that there are little quantitative data available on ARG occurrence and that studies often focus on fold increases due to anthropogenic activity. They state that studies reporting ARG quantities in the environment are difficult or impossible to compare because results are reported in different units and address different ARGs. In addition, a broad resistance profile is rare with many studies only looking at a single environmental compartment. Echeverria-Palencia et al. (2017) did not determine if any of the 24 parks were irrigated with reclaimed water. If none of the parks received reclaimed water, then this study might serve as baseline data and provide an opportunity for additional research. The main purpose of this study appeared to be to present a monitoring approach that, if standardized, might allow a more thorough global assessment of antibiotic resistance in the environment.

8.3.3 Groundwater recharge

Under Title 22, groundwater recharge is allowed under special case-by-case permits from the Regional Water Quality Control Boards. Reclaimed water used for this purpose must be filtered and receive disinfected tertiary treatment at a minimum as specified in the

Groundwater Replenishment regulation (SWRCB, 2014). California requirements are detailed in Chapter 3. Note (see Figure 3.2) that surface spreading application requires tertiary treatment with the CT 450 and subsurface application requires reverse osmosis treatment in addition to tertiary.

As mentioned previously in this report, the DPR Panel considered ARB and ARGs and assembled data on their occurrence and removal by wastewater treatment processes (see Appendix B). These data can provide some indication as to what would be going into a groundwater recharge system given the level of ARB and ARGs in the WWTP effluent and the configuration of the treatment train.

Published literature on antibiotic resistance in groundwater recharge systems is almost non-existent. Böckelmann et al. (2009) investigated fecal indicator bacteria, bacterial pathogens, and ARGs in three European artificial groundwater recharge systems. They selected 6 ARGs conferring resistance to ampicillin, methicillin, penicillins and cephalosporins, tetracycline, erythromycin and vancomycin. The ARGs (*ampC*, *mecA*, *blaSHV*-5, *tetO*, *ermB*, and *vanA*) were selected due to their abundance. The three recharge sites were in Spain, Italy and Belgium and only the Belgian site used tertiary treated wastewater (ultrafiltration and reverse osmosis). Recharge was by an infiltration pond and the water was extracted from wells at least 35 m from the pond. Abstracted water went through conventional drinking water treatment with aeration, rapid sand filtration and UV disinfection prior to entering the distribution system.

At the Spanish site, secondary treated wastewater was discharged to a river and recharge was through the riverbed. Recharged water was recovered in a mine, treated with UV and chlorine and used for irrigation of an urban park and street cleaning. At the Italian site, secondary effluent from a municipal treatment plant and surface draining water was recharged through a sinkhole into karst. The recharged water was used for agricultural irrigation. The *tetO* and *ermB* were the only two ARGs detected at all three sites. Some correlation was noted between the occurrence of enterococci in the reclaimed water and the *tetO* gene. This result is consistent with those reported by other investigators (Ferreira da Silva et al., 2006). Böckelmann et al. (2009) stated that there was no clear trend in the extent of contamination by ARGs at the three sites but the lowest level of ARGs at all sites was found during the summer sampling campaign. They concluded that the three investigated sites had different capacities for removal of ARGs. Because they found *tetO*, *ermB* and *mecA* in groundwater derived from artificial recharge, they suggested that the recharge groundwater might be a potential source of antibiotic resistance in the food chain.

McLain and Williams (2014) examined resistance to 16 antibiotics in Enterococcus in sediments from an Arizona recharge basin fed with reclaimed water for 20 years. As a control, they looked at resistance to the same antibiotics in sediments from a nearby groundwater-filled pond with no history of exposure to treated wastewater. The recharge site had 7 basins filled on a rotating basis with tertiary treated municipal wastewater. The control site had been filled only with groundwater originating from an on-site well. The soils at both sites were geomorphically similar well-drained soils deposited by alluvial materials long weathered under arid conditions. Soil samples were collected at 0-5, 10-15 and 25-50 cm depths in a dry basin over a two-year period (2009 - 2010). Inlet water samples showed no viable Enterococcus suggesting that isolates found in the sediments were from natural reservoirs and not deposited by reclaimed wastewater. Since there were no between year differences in the data at any depth for both the study and control sites, the 2009 and 2010 datasets were

combined. High levels of resistance to some antibiotics, including lincomycin, ciprofloxacin and erythromycin were found in sediments regardless of the water source, i.e. groundwater or reclaimed water. Higher antibiotic resistance was not found in reclaimed water sediments compared to the control groundwater sediments. Resistance to multiple antibiotics was actually lower in isolates from reclaimed water sediments. The authors stressed the importance of including appropriate control sites and considering naturally occurring resistance when evaluating the effect of reclaimed water use on the environment.

8.3.4 Fate and transport of ARB/ARGs during advanced wastewater treatment processes

Zhang et al. (2015) used metagenomics analyses to study the removal of ARGs through mesophilic and thermophilic anaerobic digestion using bench-scale reactors. The relative abundance of ARGs shifted from influent to effluent sludge but mesophilic or thermophilic treatment did not cause a measurable change in the abundance of total ARGs or their diversity. The feed sludge contained 35 major ARG subtypes and >90% of 8 and 13 ARGs were removed by thermophilic and mesophilic digestion, respectively. In contrast, *aad*A, *mac*B and *sul*1 were enriched during thermophilic anaerobic digestion and erythromycin esterase Type 1, *sul*1 and *tet*M were enriched during mesophilic anaerobic digestion.

Recent work on ozone suggests that this treatment may be altering and selecting antibiotic resistance elements. Alexander et al. (2016) evaluated ozone treatment (0.9 ± 0.1 g DOC) of secondary treated wastewater to determine its impact on clinically relevant ARB and ARGs. Enterococci were reduced by almost 99% but were still present in the bacterial population after ozone treatment. *Pseudomonas aeruginosa*, in contrast, had only minor changes in abundance indicating that microorganisms have different mechanisms for dealing with ozone bactericidal effects. The *erm*B erythromycin resistance gene was reduced by two orders of magnitude but two other clinically relevant ARGs (*van*A and *bla*VIM) increased. While bacterial diversity decreased, GC-rich bacteria survived after ozone treatment.

Pak et al. (2016) studied ozonation for removing ARB and pB10 plasmids under different TSS and humic acid concentrations after testing chlorination as a reference disinfection process. Chlorine at 75 mg/L and 10 min contact time removed about 90% ARB and 78.8% pB10 plasmids. The estimated CT (concentration x time) value for ozone (C zero = 7mg/L) for 4-log pB10 removal was 127.15 mg·min/L and that was 1.04 and 1.25-fold higher than required for ARB (122.73 mg·min/L) and nonantibiotic resistant *E. coli* K-12 (101.4 mg·min/L), respectively. Ozonation prevented pB10 plasmid transfer better at higher concentrations of humic acid and low pH. These authors only looked at individual disinfection processes and not a full plant and from that concluded that the CT concept might not be appropriate for antibiotic resistance control in wastewater treatment. California regulations include a requirement for multiple barriers including CT requirements.

Ferro et al. (2016) performed laboratory-scale experiments to evaluate the effect of an AOP UV/H_2O_2 process on antibiotic resistance transfer potential. They exposed wastewater samples to UV doses ranging from 0 to 2.5 x 10^2 mJ/cm^2 and H_2O_2 at 20 mg/L to determine the inactivation of antibiotic resistant *E. coli* and ARGs. Although the ARB were inactivated and there was a decrease in the ARGs after 60-minute treatment, the UV/H_2O_2 was not effective in removing ARGs from the water suspension. The authors concluded that this AOP may not be effective in minimizing the potential spread of AR in the environment since inactivated bacterial cells may release DNA into the treated water and lead to AR transfer to other bacteria.

Li et al. (2016) assessed the removal of antibiotics, ARGs and bacteria in two WWTPs and found that their abundance and removal rate varied significantly. Biological treatment mainly removed antibiotics and ARGs while physical techniques reduced ARB by about 1 log for each one; UV disinfection did not significantly enhance removal efficiency. Antibiotics in the effluents had diverse influences on a downstream lake. Concentrations of sulfamethazine and sulfa resistant bacteria increased enormously while total ARGs increased about 0.1 log due to the effluent input to the lake.

Moreira et al. (2016) used photocatalytic ozonation in a continuous mode with TiO_2-coated glass Raschig rings and light emitting diodes to treat municipal wastewater. Microorganisms and ARGs (*intI*1, *bla*TEM, *qnr*S, *sul*1) were efficiently removed but after storage total heterotrophic and ARB (to ciprofloxacin, gentamicin, meropenem), fungi and *intI*1 increased to close to the pretreatment levels. The *bla*TEM, *qnr*S, *sul*1 ARGs were reduced to levels below or close to the quantification limit even after 3-days storage of wastewater.

Li et al. (2017) investigated the removal of two sulfonamide and three tetracycline ARGs and the intI1 integron gene from wastewater by $FeCl_3$ and polyferric chloride coagulants. They also examined the removal of dissolved organic carbon, NH_3-N and total phosphorous by coagulation. ARG removal by coagulation ranged from 0.5 to 3.1-logs. Based on observed correlations, the authors indicated that the co-removal of dissolved organic carbon, NH_3-N and different ARGs played an important role in ARG loss by Fe-based coagulation.

Quantitative and qualitative changes in ARGs were examined in two WWTPs that treated livestock or industrial wastewater as well as municipal wastewater (Lee et al., 2017). Only the treatment plant receiving poultry livestock wastewater showed an increase in *sul*, *qnr*D and *bla*TEM ARGs. Biological treatment with secondary clarification and coagulation processes resulted in dynamic shifts in the patterns of ARG occurrence. The relative abundance of *tet* increased by up to almost 358% during biological treatment at both WWTPs, whereas *erm*B decreased by up to 92%. The relative abundance of *tet* decreased during secondary clarification at both WWTPs by up to 86% and by up to almost 76% by coagulation. UV disinfection removed up to 75% of ARB but there was no reduction in ARGs at a dose of 27 mJ/cm^2. The WWTP receiving livestock wastewater discharged an estimated 4.2×10^{18} ARG copies per day while the other WWTP (industrial and municipal wastewater) discharged about 5.4×10^{16} copies per day.

Leddy et al. (2017) applied DNA-based next-generation sequencing (NGS) to characterize the microbial communities in a California advanced water treatment facility (AWTF). This technology has the potential to provide high throughput, culture independent results to give a more complete, accurate and rapid indication of treatment train effectiveness for removal of microorganisms and antibiotic resistance elements. Samples were collected from the secondary treated influent to the AWTF and the biofilms on the feed side of the MF filters and the RO units. For parasites, the influent water was found to predominantly contain paramecia, some diatoms and lesser amounts of amoebae. The MF membranes only contained paramecia. No parasite DNA was found on the RO membranes. *Cryptosporidium* and *Giardia* DNA was not detected and that may have been due to the sample extraction method used. With respect to fungal diversity, 31, 16 and 8 species were detected in the influent water, and on the MF membranes and RO membranes, respectively. The DNA of 6 species was common to all three sampling points. Only one species, *Mycena chlorophos*, was present in both the influent water and the RO biofilm.

Bacterial diversity results showed 855, 554 and 89 different species in the influent water, MF membranes and RO membranes, respectively. About 1,000 of the total species detected require additional study to confirm identification. Opportunistic pathogens (*Mycobacterium*, *Pseudomonas* and *Sediminibacterium*) were identified in all samples. Indicator bacteria were not identified in the RO biofilms but were detected in the influent water and MF membranes. Bacteriophage and virus DNA was detected in the influent water and MF membranes but not in the RO biofilms with bacteriophages being more abundant than human viruses. A sequence associated with Adenovirus was detected in the MF membranes but not the influent water. A total of 141 ARGs were found in the influent water, 85 in the MF membranes and 9 in the RO biofilms. Four ARGs (*ant2-la*, *sul1*, *mexF* and *tetC*) were found in all samples and four were specific to the RO biofilm (*em*40, *aph3*Node, *rbp*A and *tet*K).

The authors concluded that NGS and metagenomics can be used to characterize microbial communities and antibiotic resistance in secondary treated effluent and MF and RO biofilms and that the approach has promise for assuring public health safety in water reuse scenarios. Additional studies are needed to apply the techniques to RNA viruses and to determine temporal and spatial variations in treated wastewater and biofilms.

The Los Angeles County Sanitation Districts (LACSD) recently completed two studies on antibiotic resistance in wastewater. The first one assessed the occurrence of carbapenem resistant enterobacteria (CRE) in treated wastewater effluents (LACSD, 2017). Chlorinated secondary treated effluent samples from the Joint Water Pollution Control Plant (JWPCP) and tertiary treated effluent samples from the Long Beach and San Jose Creek Water Reclamation plants were tested for four carbapenems. CRE were not detected in any of the tertiary treated effluent samples. They were detected in secondary JWPCP effluent at low concentrations (0.08 to 0.16% of the total coliform population). Low concentrations (non-detectable to 0.06% of the total coliform population) of carbapenamase producing (CP)-CRE were detected in two of three JWPCP sampling events. CP-CRE can transfer their resistance genes to other bacteria. Four carbapenems were tested for and resistance to ertapenem was the most prevalent (85% of all CRE detected).

The second study (Quach-Cu et al., 2018) examined the effect of primary, secondary and tertiary wastewater treatment processes on ARG concentrations and total bacterial biomass in both the solid and dissolved wastewater fractions. Two indicator ARGs were selected for this study: $bla_{SHV/TEM}$ and *sul*1 and samples were collected at the San Jose Creek East Water Reclamation Plant (WRP). Full-scale WRP treatment with tertiary media filtration and chlorine disinfection reduced the raw influent ARG concentrations by about 4 logs (Table 8.3). The concentrations of ARGs and total biomass decreased in the dissolved fractions with each successive stage of treatment (raw>activated sludge (AS)>secondary effluent >final effluent). The $bla_{SHV/TEM}$ ARG occurred in lower concentrations than the *sul*1 all wastewater matrices tested (raw, AS, secondary effluent, final effluent). Tertiary filtration with chlorine disinfection was the most effective process for reducing ARGs in the full-scale treatment plant (Table 8.4).

Pilot-scale experiments showed that filtration and disinfection reduced the ARG plasmid concentration by greater than 4 logs in both the solid and dissolved fractions whereas with filtration alone the reduction was about 0.9 log. Filtration increased plasmid removal by chlorine by about two logs compared to chlorinated non-filtered secondary effluent. In the full-scale WRP samples, most of the ARGs were in the dissolved fraction of the final effluent

while the activated and secondary effluent waters had a higher proportion of genes in the solids-associated fractions. The quantities of all three targets (*bla*$_{SHV/TEM}$ and *sul*1 ARGs and 16S rDNA) increased in the activated sludge stage corresponding with an increase in total biomass. The *bla*$_{SHV/TEM}$ ARG did not increase as much as the *sul*1 and 16S genes. Low pressure UV irradiation was not effective in removing ARGs from wastewater.

Table 8.3. The quantity of *blaSHV/TEM, sul1* and bacterial 16S genes in full-scale water reclamation plant (WRP) treatment processes: solids fraction (based on LACSD data).

WRP matrix[a]	*blaSHV/TEM* (copies/L)[b]	positive samples	*sul1* (copies/L)[b]	positive samples	16S rDNA (copies/L)[b]	positive samples
Raw	$1.41x10^7$ $+3.31x10^6$	3/3	$7.61x10^7$ $+4.43x10^7$	3/3	$6.58x10^9$ $+5.14x10^9$	3/3
AS	$1.95x10^7$ $+1.06x10^7$	9/9	$1.82x10^{10}$ $+2.36x10^{10}$	9/9	$1.21x10^{12}$ $+7.22x10^{11}$	9/9
SE (Unconc.)	$<1.18x10^6$ [c]	0/9	$3.96x10^6$ $+4.94x10^6$	7/9	$4.43x10^7$ $+5.13x10^7$	7/9
SE (HFF)	$1.09x10^5$ $+9.24x10^4$	3/3	$3.03x10^7$ $+1.85x10^7$	3/3	$5.77x10^8$ $+1.74x10^8$	3/3
FE (Unconc.)	$<1.18x10^6$ c	0/6	$<1.87x10^5$ [c]	0/6	$<2.27x10^6$ [c]	0/6
FE (HFF)	$<5.30x10^3$	0/6	$7.89x10^3$ $+9.18x10^3$	3/6	$4.57x10^5$ $+6.30x10^5$	6/6
Filter backwash	$1.55x10^6$ $+1.91x10^6$	5/5	ND	-	ND	-

a Samples were collected from SJC-east WRP. Final effluent refers to tertiary-treated water that was chlorinated and de-chlorinated. Backwash was collected from the filtration tanks during the backwash cycle after approximately 24-hours of continuous use. AS: activated sludge; SE: secondary effluent; FE: final effluent; Unconc.= unconcentrated.

b The averages and standard deviations were calculated based on the following sampling events set one: 8/1/16, 8/8/16, 8/29/16; set two 5/7/16, 9/19/16, 9/28/16, 10/11/16, 10/18/16, 10/24/16, and 12-22-10 to 2-1-11 for filter backwash. Results are in qPCR copies per L of original matrix. Final effluents were obtained as grab samples from SJC-east (not from any of the hose spigots). ND= Not done. None of the samples displayed any gross inhibition (greater than three cycles from the control SPC reaction) in qPCR. Averages were calculated using the qPCR concentrations of positive samples and the limit of detection for all samples that were negative.

c "Less than" values denote all samples were negative and the concentration given represents the LOD for each assay. This was determined as the lowest concentration that could be detected greater than 90% of the time.

Table 8.4. Log reductions through treatment (based on LACSD data).

Treatment[a]	log reduction *sul1*-solids	log reduction *sul1*-dissolved	log reduction *bla*$_{SHV/TEM}$ - solids	log reduction *bla*$_{SHV/TEM}$ – dissolved	Treatment[a]
Raw to AS	-2.38	1.46	-0.14	0.83	*Raw to AS*
AS to SE	2.78	1.28	2.25	2.01	*AS to SE*
SE to FE	3.58	2.05	>1.31[b]	>0.24[b]	*SE to FE*

a AS: activated sludge; SE: HFF concentrated secondary effluent; FE: HFF concentrated final effluent after chlorine disinfection.

b All samples tested were below the detectable limit of the method and were assigned the value of the assay's detection limit for log removal calculations.

8.4 Recommendations from Recent Scientific Conferences on Antibiotic Resistance in the Environment

Wuijts et al. (2017) reported the findings of a WHO workshop convened to develop a research agenda on antibiotic resistance. There were three main conclusions of the workshop: 1) guidance is needed on how to reduce the spread of AR to humans from the environment and to introduce effective intervention measures; 2) human exposure to AR via water and its health impact should be investigated and quantified, and 3) a uniform and global surveillance strategy, including analytical methods that can be used by low-income countries as well, is needed in order to monitor the magnitude and dissemination of AR.

In a paper addressing the safety aspects associated with AR and the reuse of treated wastewater, Hong et al. (2018) identified challenges that need to be resolved including improving methodologies to identify and quantify ARB and ARGs; identifying the ARB and ARGs to monitor that best relate to occurrence of disease burden; determining how to assess risk associated with AR and reuse; developing strategies for preventing ARB and ARGs from entering the wastewater.

The 4th International Symposium on the Environmental Dimension of Antibiotic Resistance (EDAR) was held in Lansing, Michigan from August 13-17, 2017. At that symposium, there were three roundtable discussions: one on advances, gaps and path forward in basic science; one on advances, gaps and path forward in agriculture, aquaculture, food safety and manufacturing, and the third on advances, gaps and path forward in water sanitation and the treatment domain. White papers summarizing each of these three roundtables are in preparation and they are expected to be published in a special issue of FEMS Microbial Ecology in the near future. The symposium was international in scope and some of the identified needs may be more applicable to less developed countries.

The roundtable on water and sanitation (Bürgmann et al., 2018) indicated the need for new risk assessment frameworks adapted to water and sanitation sources of antimicrobial resistance (AMR) and that effort was needed to quantify the relative contributions of various sources and routes of dissemination of AMR originating in human waste to human health. The participants in this roundtable also indicated that there are unresolved questions about the microbial ecological processes occurring in wastewater treatment plants and the extent to which they attenuate or amplify ARBs and ARGs. In addition, research on the fate of ARBs and ARGs in wastewater management systems and intended receiving environments or reuse applications is needed.

Similar recommendations on risk assessment and research needs were made by antimicrobial resistance workshop participants at an Association of Environmental Engineering and Science Professors (AEESP) Biennial Conference (Pruden et al., 2018). In addition, workshop participants specified the need for standardized methods and reporting and the identification of priority monitoring targets.

The EDAR roundtable on advances, gaps and path forward in agriculture, aquaculture, food safety and manufacturing (Topp et al., 2018) focused on antibiotic use and identified antibiotic stewardship, and the pre-treatment of manure and sludge to abate antibiotic resistant bacteria as being critical control points for reducing antibiotic emissions from agriculture. Antibiotics are sometimes added to fish and shellfish production sites and this is a direct route of contaminating the aquatic environment. Vaccination of high value (e.g. salmon) production systems could reduce the need for antibiotics. Consumer and regulatory

pressure were indicated for reducing high concentrations of antibiotics from pharmaceutical manufacturing. Development of technologies, practices and incentives to reduce antibiotic use together with evidence-based standards for antibiotic residues in effluents were identified as research priorities.

The above recent roundtables/workshops/forums on antimicrobial resistance have indicated the need for selecting relevant targets, developing methods and assessing risks related to ARBs and ARGs in the environment. Many studies have reported the occurrence of antimicrobial resistance elements in water, sewage and wastewater. However, most data are from locations outside of California and even outside the U.S. There is a lack of data on ARB/ARG in Title 22 reclaimed water and environmental application sites.

8.5 Summary

Numerous reports have documented the occurrence of ARB and ARGs in wastewater and their removal by various treatment processes. Much less is known about the fate and transport of antibiotic resistance elements in the environment and the significance of positive findings in disseminating antibiotic resistance in water, soil and the population. In the absence of standard methods and targets, many investigators have tended to focus on clinically relevant ARB and ARGs, and these vary from one locale to another; other investigators have used metagenomics which can detect a variety of ARGs. Different metrics have been used to report antibiotic resistance results, including plate counts, most probable number, relative abundance and number of gene copies per unit of sample. Some studies have included control sites and considered the contribution from sites not impacted by anthropogenic activities.

The published literature on disinfection removal of antibiotic resistance is minimal. Disinfection processes for ARB have usually been as effective as those for bacteria that are not antibiotic resistant while effectiveness for ARG removal appears to vary depending upon the particular ARG. UV disinfection is effective for bacterial inactivation but it has not been uniformly effective for ARG removal with reductions ranging from <1 to 4 logs. Recent studies on ozone have shown little effect with some bacteria while ARGs could either increase or decrease after ozone treatment. Given the uncertainty in this field of investigation it appears that additional research is needed to understand and apply the results of the work.

8.6 Research Recommendations

The following research recommendations should be carried out through research organizations to advance the risk assessment field for ARB and ARGs in recycled water applications in California:

- Microcosm and field-scale studies using culture-based and molecular analyses to determine the abundance and patterns of ARGs and mobile genetic elements (MGEs) should be conducted. These data can support the assessment of potential MGE risks of propagating antibiotic resistance (AR) through the wastewater-agricultural soil-crops-human path. Some environments have AR genes in the absence of anthropogenic activities and little is known about the antibiotic resistomes of most environmental bacteria. A greater understanding of the environmental reservoirs of AR and their potential impacts on clinically important bacteria is needed.
- A protocol should be developed to determine what ARB, ARG and MGE targets (AR elements) should be measured for a given locale and how the results should be

reported. Standard methods for measuring these targets should be established and a consensus reached on appropriate metrics and reporting of results.

- Risk assessment methods are needed in order to assess the significance of finding AR elements in water and soil environments in order to determine the safety and public health impacts of recycling wastewater. Additional studies are needed on the efficiency of physical and chemical wastewater treatment processes for removing/inactivating AR elements.
- The fate and transport of AR elements in agricultural irrigation, landscape irrigation and potable reuse applications should be determined with attention given to adequate controls for naturally occurring AR elements. Considering the quantities of recycled water used by each of the 19 subcategories in agricultural and landscape irrigation together with the Title 22 treatment requirements for the subcategories, the levels of AR elements in the wastewater, their fate and transport and the frequency of irrigation may help to determine relative risk of disseminating antibiotic resistance in the environment and the population.
- The important aspects in studies on the background and baseline antibiotic resistance levels in environmental media (water and soil) should be defined. Within study normalization should consider the different aspects of these ecosystems in order to address the experimental questions. Between study normalization should consider accurate and effective comparison of results.

The State Water Board can encourage the collection of data in reclaimed water and sites within California while waiting for the above scientific advances. Although the study by Echeveria et al. (2017) at 6 irrigated parks in four California cities lacked desirable detail (e.g., whether or not any of the parks were irrigated with reclaimed water), it may provide a framework for more extensive studies. Any such studies should include information on the amount of irrigation, if it was with reclaimed water, the levels of ARB/ARG in the water and soil and similar data at appropriate control sites. While there is no general agreement on targets at the present time, a rationale can be developed for selecting targets based on results from the Echeveria study, those found at the Orange County GWRS by Leddy et al. (2017) and a listing of bacterial groups and genetic determinants suggested as possible indicators to assess the antibiotic resistance status in environmental settings (Berendonk et al., 2015). Similar recommendations on risk assessment and research needs were made by antimicrobial resistance workshop participants at an Association of Environmental Engineering and Science Professors (AEESP) Biennial Conference (Pruden et al., 2018). In addition, workshop participants specified the need for standardized methods and reporting and the identification of priority monitoring targets.

The EDAR roundtable on advances, gaps and path forward in agriculture, aquaculture, food safety and manufacturing (Topp et al., 2018) focused on antibiotic use and identified antibiotic stewardship, and the pre-treatment of manure and sludge to abate antibiotic resistant bacteria as being critical control points for reducing antibiotic emissions from agriculture. Antibiotics are sometimes added to fish and shellfish production sites and this is a direct route of contaminating the aquatic environment. Vaccination of high value (e.g. salmon) production systems could reduce the need for antibiotics. Consumer and regulatory pressure were indicated for reducing high concentrations of antibiotics from pharmaceutical manufacturing. Development of technologies, practices and incentives to reduce antibiotic

use together with evidence-based standards for antibiotic residues in effluents were identified as research priorities.

Recent roundtables/workshops/forums on antimicrobial resistance have indicated the need for selecting relevant targets, developing methods and assessing risks related to ARBs and ARGs in the environment. Scientific advances in these areas are outside the scope of the California authorities. Many studies have reported the occurrence of antimicrobial resistance elements in water, sewage and wastewater. However, most data are from locations outside of California and even outside the U.S. There is a lack of data on ARB/ARG in Title 22 reclaimed water and environmental application sites. While waiting for the above scientific advances, the State Water Board can encourage the collection of data in reclaimed water and sites within California.

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9. Recommendations for CEC Monitoring Programs for Water Reuse Practices in California

9.1 Panel Charge

The 2018 Panel was charged to identify the need for CEC monitoring while evaluating the potential human health risks associated with exposure to CECs in indirect potable reuse applications including groundwater recharge (IPR-GWR) and surface water augmentation (SWA) as well as all non-potable reuse applications currently allowed under Title 22 in California. In addition, the Panel was asked to comment on the state-of-the-science regarding the likelihood of human health impacts posed by antibiotic resistant bacteria/antibiotic resistance genes (ARB/ARGs) in recycled water.

The Panel emphasizes that evaluating CECs in water reuse requires a dynamic process. This process needs to account for new chemicals coming into commerce, better treatment methods to tailor water quality to various reuse applications, new water reuse practices, and constantly changing and more sensitive analytical tools (both chemical and bioanalytical), with the overall goal of assuring that public health is protected.

To achieve these goals, this chapter provides the Panel's recommended next steps regarding adequate protection of public health through permitting of non-potable and potable water reuse projects, the management of potable water facility water quality (i.e., acquisition of CEC, bioanalytical, and high-frequency operation data), the need to update CEC monitoring data, the external review of CEC data, and the reporting of potable water operations to the public.

9.2 Need for CEC Monitoring for Non-Potable Reuse Practices and Surface Water Augmentation Projects

In response to the expanded charge to evaluate all non-potable use Title 22 scenarios, the 2018 Panel developed an approach that relies on comparing the exposure to CECs in recycled water for non-potable Title 22 reuse scenarios to exposure to CECs in water produced for potable reuse; considered a conservative assumption because treatment levels at the point of application are similar to those for most non-potable uses. In addition to ingestion of potable recycled water, incidental (i.e. non-intentional) exposure via several other pathways (e.g., absorption through skin, inhalation) was considered for all non-potable Title 22 applications. This comparison revealed that potential exposures and potential human health risks associated with CECs in non-potable use scenarios are expected to be 10% or lower than exposure to CECs in water intentionally consumed in the conservative potable reuse scenario, and are likely to be less than 1% for most CECs[21]. Thus, CEC monitoring is not recommended for any non-potable reuse applications currently approved under Title 22.

For SWA projects the same CEC monitoring requirements should be considered that currently apply to indirect potable reuse projects practicing direct injection (subsurface application). The point of monitoring to meet these requirements is the end of the advanced water treatment train prior to discharge to a surface reservoir.

[21] A possible exception are CECs that have the potential to bioaccumulate in fish living in impoundments that are used for fishing and are supplied by recycled water (see Section 3.2.1.3).

9.3 Relevance of Antibiotic Resistance to Recycled Water

While antibiotic resistance is still a major challenge and potentially an issue for any wastewater discharge into the environment, information to date is not complete and seems to indicate that the causes for antibiotic resistance are still not well known and the current studies do not show that antibiotic resistance transmission is a consequence of water reuse practices considered in this report. The lack of standardized methods for investigating the occurrence and removal of, and risks associated with, ARB and ARGs hinders the assessment of the severity of ARB and ARGs as an issue for recycled water applications in California. Focused investigations are needed to better understand the occurrence, fate and risks associated with ARB and ARGs in recycled water applications across California. The Panel recommends that the State Water Board consider the results of more definitive research showing an actual relationship of antibiotic resistance to reused water before changing its current policy.

9.4 Updated 2018 CEC Monitoring Recommendations for Potable Reuse Practices

The Panel encourages the State Water Board to continue using the risk-based CEC selection framework which is based on state-of-the-art data assessment (and includes off-ramps and on-ramps). Continued CEC monitoring is recommended for potable reuse projects including groundwater recharge and surface water augmentation.

For indirect potable water reuse practices (i.e. GWR and SWA), the Panel updated monitoring trigger levels (MTLs) based on toxicological information gathered from several new sources, including state, federal, industry and international organizations, as well as based on the Panel's own professional judgment. Regarding the selection of specific MTLs, the Panel made minor modifications to the process developed by the 2010 Panel. Greatest priority continues to be assigned to drinking water thresholds developed by the State of California followed by USEPA. *The result of this update was a revised set of MTLs, some higher and some lower than MTLs used in 2010, and others included for the first time.*

The Panel also updated measured environmental concentrations (MECs) based on more recent data collected by water reuse facilities in California. The Panel retained its conservative assumption of considering MECs for CECs measured in secondary/tertiary effluent as feed water for recycled water facilities. In addition, the Panel reviewed available monitoring data for individual treatment processes and product water for GWR applications as well as some select CEC monitoring studies outside of California. Because of wide variation in analytes reported, frequency of monitoring, and time period and duration of monitoring, the 2018 Panel compiled and reported 90th percentile concentration values to retain the conservatism established by the 2010 Panel.

The updated MECs and MTLs were employed to screen a total of 489 CECs (increased from 418 in 2010) using the same screening framework used by the 2010 Panel to identify candidate compounds for monitoring (Figure 5.1). This exercise indicated that regular monitoring of three of four 2010 health-based indicator CECs (17β-estradiol, triclosan and caffeine) is no longer necessary, as the monitoring data set collected over the past several years (2008-2017) indicate that concentrations are consistently below MTLs (i.e., the MEC/MTL ratio is equal to or less than 1). In contrast, the collected monitoring data indicated that concentrations of NDMA were eight times higher than the MTL and, therefore, *NDMA should be retained as a human health-based indicator*. Of the remaining CECs screened, the

90th percentile MECs for two compounds, *N-Nitrosomorpholine (NMOR) and 1,4-dioxane*, exceed their respective MTLs by factors of 9 and 7, respectively, thus warranting their addition as human health indicators.

The very small percentage of CECs that are recommended for monitoring (3 of 489 or < 1%) reinforces the inherent low risk of CECs in recycled water to human health currently attributable to most Title 22 uses and potable reuse surface water augmentation under current regulatory practices. Table 9.1 summarizes the updated 2018 health-based and performance-based indicators for CECs and performance surrogates.

While the Panel's risk-based framework is clearly effective in identifying CECs for which pertinent data are available, the framework cannot capture all possible new compounds that may be entering the market, nor does it adequately address their transformation products. To help identify such compounds that may occur in recycled water and their potential, if any, to affect human health, the Panel believes that bioanalytical screening methods are a critically important tool whose value and applicability needs to be explored over the next few years in a series of special studies. *The Panel recommends that the Estrogen Receptor alpha (ER-α) and the Aryl hydrocarbon Receptor (AhR) bioassays be used to respectively assess estrogenic and dioxin-like biological activities in recycled water*. These two *in vitro* bioassays were selected because each has clear adverse outcome pathways that allows specific molecular responses to be adequately standardized for screening recycled water quality at potable reuse projects.

Monitoring requirements for bioassays should mirror current requirements to monitor individual indicator CECs in potable reuse projects in the secondary/tertiary effluent and at the point of monitoring as specified in Attachment A of the Policy. Bioanalytical tools might also offer opportunities to evaluate the treatment efficacy of individual treatment processes.

Non-targeted (chemical) analysis (NTA) holds promise as a powerful tool for identifying previously unidentified chemicals in recycled water samples. However, at this time, unlike some bioanalytical tools, NTA remains highly complex, labor and capital cost intensive. The Panel recommends these be attempted and/or applied on a voluntary basis and with clear goals (e.g. as guided by the responses from bioanalytical tools) as part of investigative type studies.

9.5 Administrative Adjustments to Improve the State Water Board's CEC Monitoring Program

To support future updates of the CEC monitoring program, the Panel recommends that the State Water Board consider taking several procedural steps regarding permitting of potable water reuse projects, the management of potable water facility monitoring data (i.e., CEC, bioanalytical, and high-frequency operation data), and the reporting of potable water operations to the public. These might also include to issue drinking water permits for potable reuse projects that includes enhanced source control measures.

A more flexible and responsive program should be developed to update CEC monitoring recommendations in response to rapidly emerging science, technology advances and monitoring (screening) data collected. This would require that internal protocols are developed for DDW staff review and response to CEC and bioanalytical data, source control data, and high-frequency operational monitoring data. The revised process would also benefit from consistent permittee electronic reporting requirements. For internal staff and external

utility communication, protocols should be developed as well as guidance for providing the public an annual report summarizing performance of potable reuse projects.

The Panel recommends that the State Water Board take a more active role in procuring, managing and assessing CEC monitoring data and associated toxicological thresholds that are subject to rapid/continual evolution as specified in the report.

Finally, the Panel recommends that the State Water Board reconvene an independent Panel to review proposed changes to CEC monitoring recommendations and to make further recommendations for the use of the framework every three years starting such a review cycle in 2021.

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Table 9.1. Revised monitoring requirements for health-based and performance-based indicator CECs and performance surrogates for potable and non-potable reuse practices.

Reuse Practice	Health-based indicator	MRL (ng/L)	Bioanalytical methods	MRL (ng/L)	Performance-based Indicator	Expected Removal[6]	MRL (ng/L)	Surrogate	Method	Expected Removal[6]
Surface Spreading Application (SAT)	NDMA[2]	2	ER	0.5	ΔGemfibrozil[3]	>90%	10	ΔAmmonia	SM	>90%
	NMOR[1]	2	AhR	0.5	ΔSulfamethoxazole[4]	>80%	10	ΔNitrate	SM	>30%
	1,4-Dioxane[1]	100			ΔIohexol[3]	>90%	50	ΔDOC	SM	>30%
					ΔSucralose[5]	>25%	100	ΔUVA	SM	>30%
								ΔTotal fluorescence		>30%
Subsurface Application (Direct Injection) and Surface Water Augmentation	NDMA[2]	2	ER	0.5	ΔSulfamethoxazole	>90%	10	ΔConductivity	SM	>90%
	NMOR[1]	2	AhR	0.5	ΔSucralose	>90%	100	ΔDOC	SM	>90%
	1,4-Dioxane[1]	100			ΔNDMA	25-50%	2	ΔUVA	SM	>50%
Non-potable reuse practices					None			Turbidity	SM	
								Cl_2 residual or operational UV dose	SM	
								Total coliform	SM	

[1]Industrial chemical; [2]Disinfection byproduct; [3]Pharmaceutical residue; [4]Antibiotic; [5]Food additive; [6]travel time in subsurface two weeks and no dilution, see details in Drewes *et al.*, 2008; SM – Standard Methods; MRL – Method Reporting Limit.

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Appendix A – Biographies of Panel and Stakeholder Advisory Group Members

HUMAN HEALTH TOXICOLOGIST

Dr. Paul Anderson
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Education:
Postdoctoral Fellowship, Harvard School of Public Health, Interdisciplinary Programs in Health
Postdoctoral Fellowship, Harvard University, Biology Department
Ph.D., Biology, Harvard University
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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 active pharmaceutical ingredients (APIs) and personal care products in surface water. His research in the area of constituents of emerging concern (CECs) began with the development of a screening level model (the Pharmaceutical Assessment and Transport Evaluation (or P*h*ATE™) model) that predicts the concentration in surface water of human-use pharmaceuticals and other compounds released from wastewater treatment plants (WWTPs) across the United States. The model has since been corroborated and was published in Environmental Science and Technology in 2004. Additionally, Dr. Anderson helped develop 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 all historical information easily accessible to users. Dr. Anderson and his 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 US 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 to establish the range of likely EE2 concentrations; an evaluation of the potential for estrogens (both prescribed and naturally occurring) in drinking water to pose a potential risk to humans in the United States; and characterization of the potential ecological risk associated with EE2 in surface water. More recently, Dr. Anderson has expanded his research to include two comprehensive reviews of existing information and ongoing research efforts. The first was a review of the state-of-the-science of endocrine disrupting compounds (EDCs) and the implications of the presence of such compounds for wastewater treatment, published by the Water Environment Research Foundation in 2005. It described the sources of EDCs in wastewater, their fate in WWTPs, and impacts in the environment as a result of discharges. The second project, published in 2008, expanded the 2005 work on EDCs to include the full range of organic compounds 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.

ENVIRONMENTAL TOXICOLOGIST

Dr. Daniel Schlenk
Professor
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Education:
Postdoctoral Fellow, Duke University
Ph.D., Biochemical Toxicology, Oregon State University
B.S., Toxicology, Northeast Louisiana University

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 ß-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. 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. A Fellow of AAAS, he has served on two Scientific Advisory Panels supported by the California State Water Board in the USA focused on the monitoring of recycled and surface waters for Emerging Contaminants. Since 2016, he has been a permanent member of the USEPA Chemical Safety Advisory Committee, and from 2007-2014, he was a permanent member of the USEPA FIFRA Science Advisory Panel, which he Chaired from 2012-2014. He is currently an Associate Editor for *Environmental Science and Technology*, and *ES&T Letters.* He was co-editor-in chief of *Aquatic Toxicology* from 2005-2011 and currently serves on its editorial board as well as the editorial boards of *Toxicological Sciences*, and *Marine Environmental Research*. He has published more than 250 peer reviewed journal articles and book chapters on the identification of Molecular Initiating and Key Events within Adverse Outcome Pathways for emerging and legacy contaminants in wildlife and humans. His expertise is in the linkage of molecular and bioanalytical responses associated with neuroendocrine development and whole animal effects on reproduction, growth and survival.

EPIDEMIOLOGIST/RISK ASSESSOR

Dr. Adam Olivieri, P.E.
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Education:
Postdoctoral Fellow, School of Public Health, University of California, Berkeley
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B.S., Civil Engineering, University of Connecticut

Dr. Olivieri has over 35 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 Environmental Assessor with the State of California. Dr. Olivieri has extensive experience in the area of microbial risk assessment and the application of such models to make engineering and public policy decisions. Recently he served as Principal Investigator on the development of a user friendly microbial risk assessment tool (MRAIT) for the Water Environment Research Foundation. Dr. Olivieri served as 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. 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), San Diego State University and several laboratories of the California Department of Public Health Services. The project involved research in pathogenic viruses, parasites, and bacteria (including indicator organisms), chemical screening of volatile and semi-volatile organics, metals, PCBs, dioxins, TOC, and TOX, genetic toxicity bioassays including the Ames Assay, Micronucleus tests, and Cellular Transformation Assay, fish biomonitoring, wastewater treatment plant reliability, chemical risk assessment of both carcinogenic and non-carcinogenic substances, epidemiology of reproductive outcomes, vital statistics, and neural tube defects, and developing a long-term health effects monitoring plan. The San Diego Health Effects investigations have been recognized by the Science Advisory Board and a special publication by the Water Environment Federation and the American Water Works Association covering the use of recycled water to augment potable water resources. The San Diego Health Effects investigations have also been recognized and used by the Australian government and the University of New South Wales in the development of water reuse guidelines. Dr. Olivieri has and continues to serve on a number of national technical review panels, including one for Orange County (CA) evaluating alternative disinfection options along with potential public health implications related to recreation exposure, and a second for 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
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Education:
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Ph.D., Biochemistry and Molecular Biology, University of Florida
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. 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 developed monoclonal antibodies against vitellogenin for a number of different fish species and was involved in early studies on feminization of fish in the US. She has published more than 200 peer-reviewed publications and has led research projects supported by NIH/NIEHS, NSF, USEPA, USGS, and the US Army Corps of Engineers. Dr. Denslow has served as Associate Editor for *Comparative Biochemistry and Physiology Part D Toxicogenomics* and *Ecotoxicology and Environmental Safety*. She received the Pfizer Award for Research Excellence in 2007, a UFRF professor designation for 2009-2012 and for 2017-2020, the SETAC Founders Award and was inducted as a Fellow of SETAC in 2016. 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 on the leadership team of two specialty sections, Molecular and Systems Biology and Reproduction and Developmental Toxicology, where she currently serves as the Treasurer/Secretary. 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

Prof. Dr.-Ing. Jörg E. Drewes *(Panel Chair)*
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Education:
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Dr.-Ing., 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 25 years. For the last 20 years, Dr. Drewes has been conducting research on 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 and pathogens in these systems. Before joining TUM in 2013, he serves as Full Professor of Civil and Environmental Engineering at the Colorado School of Mines, USA (2001-2013) and Director of Research for the National Science Foundation Engineering Research Center on *Reinventing the Nation's Urban Water Infrastructure (ReNUWIt)*. Dr. Drewes has published more than 350 journal papers, book contributions, and conference proceedings. He served on multiple science advisory panels and chaired blue ribbon panels on topics related to public health, engineering, and reliability of water reuse projects in the U.S., Australia, and the EU. He was awarded the 2007 AWWA Rocky Mountain Section Outstanding Research Award, the Quentin Mees Research Award in 1999, the Willy-Hager Dissertation Award in 1997, and the 2003 Dr. Nevis Cook Excellent in Teaching Award. In 2008 and 2013, he was appointed to the U.S. National Academies/National Research Council Committees on *Water Reuse as an Approach for Meeting Future Water Supply Needs (2008-2012)* and *Onsite Reuse of Graywater and Stormwater (2013-2015)*, respectively. He also serves on the Research Advisory Council of the Water Environment and Reuse Foundation (Alexandria, VA) and on the State of California's expert panel on direct potable reuse. Professor Drewes currently serves as the chair of the International Water Association (IWA) Water Reuse Specialist Group. Since 2007, Dr. Drewes has held Adjunct Professor appointments at the University of New South Wales, Sydney, Australia, the King Abdullah University of Science and Technology, Saudi-Arabia, and the Colorado School of Mines, USA.

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

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Dr. Shane Snyder is a Professor of Chemical & Environmental Engineering, and holds joint appointments in the College of Agriculture and School of Public Health, at the University of Arizona. He also co-directs the Arizona Laboratory for Emerging Contaminants (ALEC) and the Water & Energy Sustainable Technology (WEST) Center. For over 20 years, Dr. Snyder's research has focused on the identification, fate, and health relevance of emerging water pollutants. Dr. Snyder and his teams have published over 200 manuscripts and book chapters on emerging contaminant analysis, treatment, and toxicology. He currently serves as an editor-in-chief for the international journal *Chemosphere.* Dr. Snyder has been invited to brief the Congress of the United States on three occasions on emerging issues in water quality. He is a Fellow of the International Water Association and a member of the World Health Organization's Drinking Water Advisory Panel. He has served on several USEPA expert panels and is currently a member of the EPA's Science Advisory Board drinking water committee and the USEPA's Board of Scientific Counselors Sustainable Water committee. He was a member of the US National Academy of Science's National Research Council Committee on Water Reuse and currently serves on the WHO's guiding committee on development of potable reuse guidelines. Dr. Snyder also is a Visiting Professor at the National University of Singapore and an Adjunct Professor at the Gwangju Institute of Science and Technology in South Korea.

SCIENTIST/ENGINEER FAMILIAR WITH THE ORIGINS, FATES AND RISKS ASSOCIATED WITH ANTIBIOTIC RESISTANCE

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Education:
Graduate training in Epidemiology, University of Minnesota
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B.S. in Pharmacy, Brooklyn College of Pharmacy, Long Island University

Mr. Jakubowski is a private consultant with more than 50 years of experience working with waterborne pathogens, especially enteric viruses and protozoa such as *Giardia* and *Cryptosporidium*, and whose current interests involve microbiological issues related to indirect and direct potable reuse of wastewater. Recent projects include being a co-editor of the protist section for the UNESCO Global Water Pathogen Project and serving on California's direct potable reuse (DPR) panel. In this latter activity, he was the lead in preparing the DPR panel's antibiotic resistance (ABR) issue paper. Mr. Jakubowski also presented an invited paper on ABR at the 2016 Clarke Prize Conference and has been invited to be a member of the ABR panel at the 2017 IWA Water Reuse Conference. He has served as a consultant to the World Health Organization on pathogenic intestinal protozoa (for development of the International Drinking Water Guidelines), and to the Pan-American Health Organization on environmental virus methods. He was instrumental in conducting the first international symposium on *Legionella* and Legionnaire's Disease at the Centers for Disease Control. He initiated landmark studies on the human infectious dose of *Cryptosporidium* and chaired the Joint Task Group on Pathogenic Intestinal Protozoa for *Standard Methods for the Examination of Water and Waste Water* from 1978 to 2005. He was a charter member of USEPA's Pathogen Equivalency Committee and served on that committee until his retirement from the U.S. Public Health Service/Environmental Protection Agency in 1997. He has research publications on hospital pharmacy; on microorganisms in oysters and clams under the federal Shellfish Sanitation Program, and numerous peer-reviewed publications on determining the health effects and public health significance of pathogens, especially intestinal protozoa and viruses, in drinking water, wastewater and municipal sewage sludge.

STAKEHOLDER ADVISORY GROUP (SAG)

Sean Bothwell is the Policy Director of the California Coastkeeper Alliance (CCKA), and works to implement statewide initiatives to enhance California's water quality and supplies. Sean is the lead environmental advocate on the State Water Board's key statewide policies, including the desalination policy, the trash policy, stormwater permitting, and water recycling. Sean leads CCKA's legislative program by representing the organization in Sacramento at legislative hearings and committee expert panels; and cultivating relationships with the Governor's Office, legislative members, and legislative committee staff. Prior to joining CCKA, Sean provided legal expertise to the San Francisco Bay Conservation and Development Commission.

Ann Heil is head of the Reuse & Compliance Section at the Sanitation Districts of Los Angeles County (LACSD), with oversight of permitting, monitoring, and reporting for the Sanitation Districts' 11 treatment plants with a total design capacity of 650 MGD, as well as oversight of the Sanitation Districts' recycled water and biosolids management programs. She also has extensive experience with source control, with particular focuses on chlorinated solvents, pharmaceuticals, pesticides, salts, and mercury. Ann is a past chair of the legislatively-appointed California Pollution Prevention Advisory Committee and past Board Member for the California Water Environment Association and for the Western Regional Pollution Prevention Network.

Roberta (Bobbi) Larson is the Executive Director of the California Association of Sanitation Agencies (CASA), and is responsible for overall management of the association. Bobbi has worked to raise the level of professionalism and leadership that CASA offers its members through building a high-functioning, cohesive team of staff and consultants; strengthening conference programs, and delivering balanced budgets each fiscal year. Her goal is to position CASA as the most trusted, credible and effective advocate for California wastewater agencies. Prior to assuming her current role, Bobbi served as CASA's contract director of legal and regulatory affairs as a shareholder with Somach Simmons & Dunn, a Sacramento law firm specializing in water quality law.

Mark Millan is is a Councilmember and former Mayor of the Town of Windsor. Mark is the founder and principal of Data Instincts, Public Outreach Consultants, a professional consultancy serving public agencies and engineering firms with outreach efforts for water and recycled water. His assignments as a councilmember include serving as vice-chair for the Water Advisory Committee and the Russian River Watershed Association. Mark has been involved with numerous community-based organizations, including the League of California Cities, WateReuse Association and Research Foundation, International Water Association, Tomorrow's Leaders Today (TLT), and the Town of Windsor Planning Commission.

Jeff Mosher is the Chief Research Officer at Water Environment and Reuse Foundation (WE&RF), a 501c3 organization whose theme is to provide exceptional water research to advance science and technology. Before joining WE&RF, he served as the Executive Director for the National Water Research Institute (NWRI) and was responsible for advancing NWRI's mission of creating new sources of water through research and technology and to protecting the freshwater and marine environments. Mr. Mosher has worked in the water industry for over 18 years, providing scientific and technical support on a variety of water-related issues, such as human risk assessments; chemical and microbial occurrence in water; the fate and transport of contaminants in the environment; water treatment technology performance evaluations; and cost-benefit analyses.

Megan Plumlee, Ph.D., P.E. is the Director of Research at the Orange County Water District (OCWD) where she manages the Research and Development (R&D) Department. The R&D Department conducts applied research to support the District's mission to manage and replenish the Orange County Groundwater Basin, including studies of the advanced recycled water facility operated by OCWD, technology evaluations, and many collaborations with universities and topic experts. She previously served as the manager of applied research at Kennedy/Jenks Consultants and has worked on projects and research spanning non-potable and potable water reuse, contaminants of emerging concern and other water quality topics, costs for advanced treatment, groundwater resources, constructed wetlands, and pilot testing for treatment technologies.

Toby Roy is Water Resources Manager for the San Diego County Water Authority, a wholesale water supplier that supports a $150 billion economy and over 3 million residents. She is responsible for developing and reviewing policies and legislation on water conservation, recycling and integrated planning, coordinates with member and state agencies on regulatory issues, and takes a lead role in advocating regulatory and legislative changes, and remedies to encourage local water supply development, and to improve water quality and public health protection. Previously, Toby worked for the California Department of Health Services, serving in a regulatory role for public drinking systems and recycled water use in San Diego, Riverside and Imperial Counties.

Jennifer West is the Managing Director for WateReuse California, a nonprofit organization with over 140 member agencies whose mission is to advance the beneficial and efficient use of water resources through education, sound science, and technology using reclamation, recycling and reuse. Previously she worked for almost 20 years advancing water and recycled water policy in the California Legislature and before California's regulatory agencies. During this time, she served as the Director for Water for the California Municipal Utilities Association. In the 1990s and early 2000s, she was a legislative and regulatory advocate representing a variety of water clients.

Debbie Webster is the Executive Officer of the Central Valley Clean Water Association (CVCWA), whose mission is to effectively represent the interests of wastewater organizations that are regulated by the Central Valley Regional Water Quality Control Board. The goal of the organization is to assure that regulations are protective of environmental quality, are based on sound science, and reflect a fair and reasonable economic basis.

Appendix B – Monitoring Program and Suggested Response(s) for Indirect Potable Reuse Projects (adopted from Anderson et al., 2010)

Due to time and resource constraints, the guidance provided regarding a start-up and baseline monitoring program does not address all situations that the regulator and regulated entity will need to address. Under these circumstances, the Panel recommends that the affected stakeholders consult experts to recommend a plant or regional-specific solution.

To carry out the monitoring program for the indicator CECs identified above, the Panel recommends a multi-tiered approach for implementing and interpreting results from CEC monitoring programs for recycled water. While the Panel provides recommended thresholds for each of these tiers, conservative values were selected because of the limited toxicological information available and the interim nature of the initial MTLs. When drinking water benchmarks (MCLs) or ADIs derived by the State of California are available, those should be used to update and establish MTLs. The Panel also understands that differences in recycled water quality and facility operations will occur by region and that investigation of chronic exceedances will need to be tailored on a region-by-region or case-by-case basis.

The following discussion provides the Panel's recommended guidance on the monitoring, response and the subsequent review/updating of those plans for potable reuse projects used for drinking water augmentation. This guidance has been adopted from the 2010 Expert Panel Report.

B.1 Guidance on Start-up and Baseline CEC Monitoring Programs for Potable Reuse Projects

The sampling location, type of IPR project (including treatment processes), CEC constituent(s), and frequency of sampling all depend on the sampling objective. Two types of monitoring are suggested, start-up and baseline monitoring. Also, the suggested constituents contained in Table 9.1 have been identified as either an indicator of health relevance, overall plant efficacy or a surrogate to represent treatment process performance. Based on the above, the Panel provides the following guidance:

- Overall Treatment Plant Efficacy - In general, sampling for CEC indicators should occur at the point of monitoring (POM). To meet the groundwater recharge reuse regulations additional sampling is typically necessary from downgradient wells, from monitoring wells representing the underlying groundwater and/or from shallow lysimeter wells. The location and monitoring criteria for selection and use of these sampling locations are site-specific and need to be defined on a case-by-case basis. The guidance provided within this report should be used to supplement the monitoring conducted as part of compliance with the regulations;
    - Plant Start-up Monitoring Frequency - Initial start-up monitoring should include, at a minimum, quarterly analyses of the compounds identified as Indicator CECs (see Table 9.1) for the first year of project operation. The surrogates identified in Table 9.1 should be monitored using online devices, where feasible.
    - Baseline Monitoring Frequency - Baseline monitoring should occur twice per year for all indicator CECs at the POM for a minimum of three years. Consistent water recycle plant operation should produce final effluent IPR project source

water containing Table 9.1 CEC concentrations that are consistently less than 5 times the ratio of MEC/MTL. The surrogates identified in Table 9.1 should be monitored using online devices, where feasible.

- Treatment Unit Process Performance - The following guidance is provided for monitoring the surrogates and indicators during start-up and baseline operations.
  - Plant Start-up Monitoring Frequency - Initial start-up monitoring should include, at a minimum, quarterly analyses of the compounds identified as indicator CECs (see Table 9.1) for the first year of project operation. The surrogates identified in Table 9.1 should be monitored using online devices, where feasible. To provide certainty that the individual treatment processes are performing according to their technical specifications, monitoring (depending on the type of IPR project) should occur at the following representative locations. The following example is for a direct injection based IPR (i.e., using RO/AOP). Duplication of effort at the POM is not the intent, but just shown for completeness.
    - Between secondary and membrane treatment processes;
    - Between membrane and advanced oxidation treatment; and
    - Final effluent after advanced oxidation and prior to groundwater injection (POM).

    The following sampling locations are suggested for an IPR using surface spreading. As noted above the selection of monitoring and lysimeter wells are site-specific and need to be selected consistent with regulations.
    - Final effluent after tertiary treatment and prior to release to the groundwater spreading basin (e.g., POM).
    - At monitoring wells representing the underlying groundwater and/or from shallow lysimeter wells.
    - At down-gradient well(s) representing the potable source water prior to the potable water treatment plant.
  - Baseline Monitoring Frequency - Baseline monitoring should occur twice per year for all indicator CECs at the POM for a minimum of three years. Consistent water recycle plant operation should produce final effluent IPR project source water containing Table 9.1 CEC concentrations that are consistently less than 5 times the ratio of MEC/MTL. The surrogates identified in Table 9.1 should be monitored at the various treatment unit locations noted above using online devices, where feasible.
- Increasing Monitoring: If indicator CECs exceed the suggested thresholds during start-up or baseline monitoring, the Panel recommends that the recharge agency work with State and Regional Water Boards to identify the need for and extent of increased monitoring to confirm the presence of problematic CEC(s), source identification studies, and/or toxicological studies. If appropriate, increased monitoring might involve engineering removal studies and/or modification of plant operation if found to be warranted.

- Commercial Laboratory Conditions: Methods used to quantify indicator CECs need to meet stringent QA/QC measures, including blanks, replication, and matrix spikes. The Panel recommends the use of isotope-dilution and tandem mass spectrometry whenever possible. A detailed description of analytical considerations is provided in Chapter 6.

B.2 Response to Monitoring Results

Should there be positive baseline monitoring results, the recharge agency, Regional Water Boards and State Water Board needs to consider whether the result is of concern. Consideration should entail topics such as: review of the basis of the (initial) MTL; what is known and what is not known about the particular chemical, the chemical's potential health effects at the given concentration, the source of the chemical, as well as possible means of better control to limit its presence, treatment strategies if necessary, and other appropriate actions.

The Panel provides the following guidance relative to defining positive monitoring results and the potential associated follow-up action(s). While the Panel provides guidance on thresholds for each of these tiers, conservative values were selected because of the limited toxicological information available. The guidance is provided based on the assumption that the Panel's conceptual framework, utilized within this report, includes a minimum safety factor of approximately 10,000-fold. The Panel recommends that the recharge agency confer with the State Water Board and the appropriate Regional Water Board to develop a response plan with specific actions to be implemented by the recharge agency as part of interpreting appropriate responses to the monitoring results.

- If no more than 25 percent of the samples during phase-2 monitoring exceed a MEC/MTL ratio of 0.1, the Panel recommends that State Water Board consider deleting the compound from further monitoring, if requested by the permitted agency. In cases where a reduction of monitoring is requested, the MTL(s) should be updated, if feasible, as part of reviewing the request.
- If 1<MEC/MLT< 10: data check, continue to monitor, until 1 year and the MEC/MLT < 1 and preferably is consistently less than 5 times the ratio of MEC/MTL.
- If 10<MEC/MLT< 100: data check, immediate re-sampling and analysis to confirm MEC, continue to monitor, until 1 year and the MEC/MLT< 1 and preferably is consistently less than 5 times the ratio of MEC/MTL.
- If 100<MEC/MLT< 1000: all of the above plus enhance source identification program. Also monitoring at a point in the distribution system closer to the POE to confirm attenuation of the CEC is occurring and to confirm the magnitude of assumed safety factors associated with removal efficiency. The POE should be selected consistent with the groundwater replenishment regulations[22].
- MEC/MTL>1000: all of the above plus immediately confer with the State Water Board and the Regional Water Boards to determine the required response action. Confirm plant corrective actions through additional monitoring that indicates the CEC levels are below at least an MEC/MTL of 100.)

[22] Refer to Title 22 Code of Regulations, articles 5.1 and 5.2 (DDW, 2014).

Please note that the baseline monitoring recommended by the Panel and additional follow-up monitoring to investigate and address positive findings should not be considered for compliance and/or regulatory purposes, but for investigation and potential use for additional follow-up actions only as part of conferring with the State Water Board and the Regional Water Boards.

B.3 Review/Update of Monitoring and Response Plans

In addition to the above suggested monitoring and results-based responses, the Panel suggests the following actions relative to updating and confirming the plant data as well as the list of indicator CECs for monitoring purposes.

- Once every five years, one additional round of CEC monitoring should be conducted to confirm monitoring results. The monitoring list should reflect suggestions of an independent panel, preferably a single non-project based panel, following a selection process outlined in this report. The monitoring results should be submitted, along with all of the previous monitoring data, as part of the five year State Water Board report (see groundwater replenishment regulations, Code of Regulations).
- The independent panel should review and update the list of indicator CECs at least triennially. The review and update should include the following:
    - Collect and review readily available toxicity data and update MTLs;
    - Collect and review California advanced treatment plant effluent data including IPR monitoring data collected as part of State Water Board permitted projects and update MECs;
    - Update list of indicator CECs to include newly identified CECs where the MEC/MTL>1 and remove CECs where updated data indicate that the current MEC/MTL<1;
    - 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;
    - Review and update guidance on sampling frequency and location;
    - Review and update conclusions regarding laboratory analytical methods;
    - Review and update biological and chemical screening methods, as discussed in Section 6, and provide guidance on potential new monitoring methods/tools that would significantly enhance chemical conventional chemical monitoring methods;
    - Develop guidance for the State Water Board for updating the monitoring requirements in groundwater recharge project permits; and
    - Review and update Panel guidance on selecting viable surrogate parameters and performance indicator CECs.

Appendix C – Water Reuse Practices and Public Health Considerations

Table C.1. Recycled water uses allowed in California [1]

Irrigation	Disinfected Tertiary	Disinfected Secondary-2.2	Disinfected Secondary-23	Undisinfected Secondary
Food crops where recycled water contacts edible portion of crop, including all root crops	**Allowed**	Not allowed	Not allowed	Not allowed
Parks and playgrounds	**Allowed**	Not allowed	Not allowed	Not allowed
School yards	**Allowed**	Not allowed	Not allowed	Not allowed
Residential landscaping	**Allowed**	Not allowed	Not allowed	Not allowed
Unrestricted access golf	**Allowed**	Not allowed	Not allowed	Not allowed
Any other irrigation uses not prohibited by other provisions of Calif. Code of Regulations	**Allowed**	Not allowed	Not allowed	Not allowed
Food crops where edible portion is produced above ground and not contacted by recycled water	**Allowed**	**Allowed**	Not allowed	Not allowed
Cemeteries	**Allowed**	**Allowed**	**Allowed**	Not allowed
Freeway landscaping	**Allowed**	**Allowed**	**Allowed**	Not allowed
Restricted access golf courses	**Allowed**	**Allowed**	**Allowed**	Not allowed
Ornamental nursery stock and sod farms	**Allowed**	**Allowed**	**Allowed**	Not allowed
Pasture for milk animals	**Allowed**	**Allowed**	**Allowed**	Not allowed
Non-edible vegetation w/ access control to prevent use as a park, playground or school yard	**Allowed**	**Allowed**	**Allowed**	Not allowed
Orchards w/ no contact between edible portion & recycled water	**Allowed**	**Allowed**	**Allowed**	**Allowed**
Vineyards w/ no contact between edible portion and recycled water	**Allowed**	**Allowed**	**Allowed**	**Allowed**
Nonfood-bearing trees incl. Christmas trees not irrigated <14 days before harvest	**Allowed**	**Allowed**	**Allowed**	**Allowed**
Fodder crops (e.g. alfalfa) and fiber crops (e.g. cotton)	**Allowed**	**Allowed**	**Allowed**	**Allowed**

(Continued-2)

Seed crops not eaten by humans	**Allowed**	**Allowed**	**Allowed**	**Allowed**
Food crops that undergo commercial pathogen-destroying processing before consumption by	**Allowed**	**Allowed**	**Allowed**	**Allowed**
Ornamental nursery stock, sod farms not irrigated <14 days before harvest	**Allowed**	**Allowed**	**Allowed**	**Allowed**
Impoundments	**Disinfected Tertiary**	**Disinfected Secondary – 2.2**	**Disinfected Secondary – 23**	**Undisinfected Secondary**
Non-restricted recreational impoundments, with supplemental monitoring	**Allowed** [2]	Not allowed	Not allowed	Not allowed
Restricted recreational impoundments and publicly accessible fish hatcheries	**Allowed**	**Allowed**	Not allowed	Not allowed
Landscape impoundments without decorative fountains	**Allowed**	**Allowed**	**Allowed**	Not allowed
Cooling or Air Conditioning	**Disinfected Tertiary**	**Disinfected Secondary – 2.2**	**Disinfected Secondary – 23**	**Undisinfected Secondary**
Industrial or commercial cooling or air conditioning involving cooling tower, evaporative condenser, or spraying that creates a mist	**Allowed** [3]	Not allowed	Not allowed	Not allowed
Industrial or commercial cooling or air conditioning not involving a cooling tower, evaporative condenser, or spraying that creates a mist	**Allowed**	**Allowed**	**Allowed**	Not allowed

(Continued-3)

Other Uses	Disinfected Tertiary	Disinfected Secondary – 2.2	Disinfected Secondary – 23	Undisinfected Secondary
Groundwater recharge	**Allowed under special case-by-case permits by Regional Water Board[4]**			
Flushing toilets and urinals	**Allowed**	Not allowed	Not allowed	Not allowed
Priming drain traps	**Allowed**	Not allowed	Not allowed	Not allowed
Industrial process water that may contact workers	**Allowed**	Not allowed	Not allowed	Not allowed
Structural fire fighting	**Allowed**	Not allowed	Not allowed	Not allowed
Decorative fountains	**Allowed**	Not allowed	Not allowed	Not allowed
Commercial laundries	**Allowed**	Not allowed	Not allowed	Not allowed
Consolidation of backfill material	**Allowed**	Not allowed	Not allowed	Not allowed
Artificial snow making for commercial outdoor uses	**Allowed**	Not allowed	Not allowed	Not allowed

[1] Table prepared by WateReuse Assoication as a guide. Refer to the full text of the latest version of Title-22.

[2] With "conventional tertiary treatment." Additional monitoring for two years or more is necessary with direct filtration.

[3] Drift Eliminators and/or biocides are required if public or employees can be exposed to mist.

[4] Refer to Groundwater Recharge Guidelines, California Department of Health Services.

Appendix D – Updates to the Master List of CECs Considered by the 2018 Expert Panel

Table D.1. CECs added to the CEC master list during the 2018 Panel's review process.

CEC	MTL (ng/L)	Reference
10,11-Dihydroxy-carbamazepine	3.0E+02	German Environment Agency[a]
2-Chloroethanol	4.0E+05	German Environment Agency[m]
2,4-Di-tert-butylphenol	3.0E+03	German Environment Agency
Acesulfame	2.0E+08	Science Panel[b, c]
Acyclovir	3.0E+02	German Environment Agency
Alendronate	6.0E+03	MDH[d, e]
Allopurinol	7.0E+05	MDH
Amitriptyline	2.0E+03	MDH
Amlodipine	1.0E+04	MDH
Amphetamine	4.0E+02	MDH
Ampicillin	1.0E+05	MDH
Androstenedione	na[f]	Science Panel
Aspartame	3.0E+08	Science Panel[l]
Benzatropine	1.0E+02	MDH
Candesartan	3.0E+02	German Environment Agency
Carisoprodol	3.0E+05	MDH
Carvedilol	3.0E+04	MDH
Celecoxib	7.0E+04	MDH
Clavulante	9.0E+04	MDH
Clonazepam	1.0E+02	MDH
Clonidine	2.0E+02	MDH
Clopidogrel	9.0E+04	MDH
Cyclobenzaprine	5.0E+02	MDH
Diethylstilbestrol	5.1E-02	Science Panel[g]
Doxepin	9.0E+03	MDH
Drospirenone	1.0E+02	MDH
Duloxetine	1.0E+04	MDH
Escitalopram	1.0E+03	MDH
Ethyl N,N-diphynylcarbamate	3.0E+02	German Environment Agency
Ezetimibe	1.0E+04	MDH
Fenofibrate	6.0E+03	MDH
Fluconazole	4.0E+03	MDH
Furosemide	2.0E+03	MDH
Gabapentin	3.0E+06	MDH
Gabapentin lactam	1.0E+03	German Environment Agency
Glipizide	5.0E+02	MDH
Glyburide	4.0E+01	MDH

Hydrochlorothiazide	4.0E+02	MDH
Hydrocodone	7.0E+02	MDH
Hydrocortisone	2.0E+02	MDH
Imipramine	1.0E+03	MDH
Lamotrigine	3.0E+02	German Environment Agency
Levothyroxine	1.0E+02	MDH
Lisdexamfetamine	4.0E+03	MDH
Lisinopril	6.0E+02	MDH
Lomefloxacin	2.0E+05	MDH
Lorazepam	2.0E+02	MDH
Losartan	6.0E+04	MDH
Lovastatin	4.0E+02	MDH
Mefenamic acid	1.0E+05	MDH
Meloxicam	3.0E+03	MDH
Memantine	7.0E+03	MDH
Methylisothiocyanate	1.2E+05	Science Panel[h]
Methylphenidate	7.0E+03	MDH
Methylprednisolone	5.0E+01	MDH
Minocycline	2.0E+03	MDH
Montelukast	2.0E+04	MDH
Nebivolol	9.0E+02	MDH
Neotame	1.8E+06	Science Panel[k]
Nifedipine	1.0E+04	MDH
Ofloxacin	5.0E+04	MDH
Olanzapine	1.0E+02	MDH
Olmesartan	3.0E+02	German Environment Agency
Olmesartan medoxomil	2.0E+04	MPH
Oxycodone	2.0E+02	MDH
Oxypurinol	3.0E+02	German Environment Agency
p-Chlorobenzene sulfonic acid	2.0E+06	Science Panel[g]
Pentoxyifylline	5.0E+05	MDH
Phenobarbital	3.0E+02	German Environment Agency
Pioglitazone	5.0E+02	MDH
Pravastatin	1.0E+03	MDH
Prednisolone	6.0E+01	MDH
Prednisone	6.0E+01	MDH
Pregabalin	2.0E+05	MDH
Primidone	1.0E+04	WRRF-15-01[i]
Promethazine	2.0E+03	MDH
Propoxyphene	4.0E+04	MDH
Propyphenazone	3.0E+02	German Environment Agency
p-Toluolsulfonic acidamid (4-Methylbenzosulfonamide)	3.0E+02	German Environment Agency

Quetiapine	2.0E+03	MDH
Rosuvastatin	2.0E+02	MDH
Sertraline	3.0E+03	MDH
Sildenafil	4.0E+03	MDH
Sitagliptin	4.0E+03	MDH
Sucralose	1.5E+08	WE&RF (2016)
Sulfadiazine	7.0E+04	MDH
Sulfamethizole	1.0E+04	MDH
Tadalafil	3.0E+03	MDH
Tamsulosin	5.0E+01	MDH
Tramadol	7.0E+04	MDH
Trazodone	5.0E+03	MDH
Triamterene	4.0E+04	MDH
Triclocarban	1.4E+05	MDH[j]
Trifluoroacetate	1.0E+03	German Environment Agency
Valsartan	9.0E+04	MDH
Valsartan acid	3.0E+02	German Environment Agency
Verapamil	6.0E+04	MDH
Zolpidem	6.0E+02	MDH

Notes:

na = not available; an ADI or RfD is not available for this chemical
ng/L= nanograms per liter

a. German Environment Agency (2016)

b. CEC added based on Science Panel judgement.

c. MTL derived using FDA ADI of 32,800 ug/kg/day and 2010 Science Panel Report exposure assumptions.
d. From Pharmaceuticals Screening Water Values 2015 and Supporting Information Excel file, "All Data and Values" tab. Pharmaceutical Water Screening Values Report. Minnesota Department of Health. August 2015.
e. Concentrations shown in this column are 10 times higher than those shown in the original MDH tables. The screening values in the original MDH tables are based on infant exposure assumptions that assume daily water ingestion is about 10 times greater for infants than adults on a kilogram bodyweight basis.

f. The Science Panel was unable to locate a reliable ADI prior to the publication of this table.

g. MTL set equal to the November 2017 USEPA tapwater RSL.
h. MTL derived using ADI of 20 ug/kg/day (as cited in Hayes Handbook of Pesticide Toxicology) and 2010 Science Panel Report exposure assumptions.

i. WE&RF (2016), WRRF-15-01 Final report.
j. MTL derived using RfD of 24 ug/kg/day derived from the Minnesota Department of Health (2015) and 2010 Science Panel Report exposure assumptions

k. MTL derived using FDA ADI of 300 ug/kg/day and 2010 Science Panel Report exposure assumptions.

l. MTL derived using FDA ADI of 50,000 ug/kg/day and 2010 Science Panel Report exposure assumptions.

m. CEC added based on listing by German Environment Agency, MTL set equal to 2017 USEPA tapwater RSL.

Table D.2. CEC removed from the CEC master list during the 2018 Panel's review process.

CEC	MTL (ng/L)	Reference
1,1,1-Trichloroethane	2.0E+05	Cotruvo et al. 2010[a]
1,1-Dichloroethene	3.0E+04	Australia (2008)[b]
1,2-Dibromo-3-chloropropane	2.0E+02	Cotruvo et al. 2010
1,4-Dichlorobenzene	7.5E+04	Cotruvo et al. 2010
2,3,7,8-Tetrachlorodibenzo-1,4-dioxin	6.0E-03	Cotruvo et al. 2010
2,4-D (2,4-Dichlorophenoxyacetic acid)	3.0E+04	Schriks et al. 2009[c]
Alachlor OA	4.0E+02	CCL[d]
Aldicarb sulfone	6.0E+03	Cotruvo et al. 2010
Aldicarb sulfoxide	6.0E+03	Cotruvo et al. 2010
Benzene	3.0E+03	Cotruvo et al. 2010
Benzo(a)pyrene	1.0E+01	Australia (2008)
Bis(2-ethylhexyl)phthalate	2.4E+04	Cotruvo et al. 2010
Bromodichloromethane	6.0E+03	Australia (2008)
Bromoform	1.0E+05	Australia (2008)
Carbon tetrachloride	4.2E+03	Cotruvo et al. 2010
Chloroform	6.0E+04	Cotruvo et al. 2010
Glyphosate	9.0E+05	Schriks et al. 2009
Heptachlor	4.0E+02	Cotruvo et al. 2010
Hexachlorobenzene	4.8E+03	Cotruvo et al. 2010
Methylene chloride (dichloromethane)	4.0E+03	Australia (2008)
o-Dichlorobenzene	9.0E+04	Cotruvo et al. 2010
p-Dichlorobenzene	7.5E+04	Cotruvo et al. 2010
Pentachlorophenol	1.0E+04	Australia (2008)
Simazine	2.0E+03	Schriks et al. 2009
Tetrachloroethylene	5.0E+03	Cotruvo et al. 2010
Trichloroacetic acid	6.0E+04	Cotruvo et al. 2010
Trichloroethene	5.0E+03	Cotruvo et al. 2010

Notes:

ng/L= nanograms per liter

a. From Table 3.2 in Cotruvo et al. 2010. Identifying Health Effects Concerns of the Water Reuse Industry and Prioritizing Research Needs for Nomination of Chemicals for Research to Appropriate National and International Agencies
b. From Tables 4.4, A1, A2, A8a, and A8b in Environment Protection and Heritage Council et al. 2008. Australian Guidelines for Water Recycling. Augmentation of Drinking Water Supplies. May 2008.
c. From Table 2 in Schriks et al. 2009. Toxicological Relevance of Emerging Contaminants for Drinking Water Quality. Water Research, doi: 10.1016/j.wateres.2009.08.023.
d. From USEPA CCL 3 and CA PCC Dossier of Chemicals

Table D.3 Master list of CECs considered by the 2018 Panel

CEC	Summary of Drinking Water Benchmarks for Constituents of Emerging Concern (CECs)																		
	CA Depth Public Health (2007)[a]	USEPA CCL 3 List/PCCL[b]		USEPA (2017)[c]		Schwab et al. (2005)[d]		Australia (2008)[e]		AwwaRF (2008)[f]		Schriks et al. (2009)[g]		Cotruvo et al. (2010)[h]	Minnesota Dept Health (2015)[i]		WE&RF (2016)[j]	Science Panel[k]	German Environ. Agency[l]
	Drinking Water Notifi-cation Levels (ng/L)	ADI or RfD (µg/kg /day)	PNEC (ng/L)	ADI (µg/kg /day)	Tap-water RSL (ng/L)	ADI (µg/kg /day)	$PNEC_{dw}$ (ng/L)	ADI (µg/kg/ day)	DWG (ng/L)	ADI (µg/kg day)	DWEL (ng/L)	TDI, ADI, or RfD (µg/kg/ day)	PGV (ng/L)	Lowest Guideline Value (ng/L)	ADI (µg/kg/ day)	Screen. Water Value[i] (ng/L)	PHC (ng/L)	MTL (ng/L)	HAV (ng/L)
1,1,1,2-Tetrachloroethane		30	1.0E+03	30	5.7E+02														
1,1-Dichloroethane		200	6.1E+03	200	2.8E+03														
1,2,3,4,6,7,8-Heptachlorodibenzo-1,4-dioxin														3.0E+00					
1,2,3,4,6,7,8-Heptachlorodibenzofuran														3.0E+00					
1,2,3,4,7,8,9-Heptachlorodibenzofuran														3.0E+00					
1,2,3,6,7,8-Hexachlorodibenzo-1,4-dioxin														3.0E+00					
1,2,3,7,8-Pentachlorodibenzofuran														6.0E-02					
1,2,3-Trichloropropane (1,2,3-TCP)	5.0E+00	6	5.0E+00	4	7.5E-01														
1,2,4-Trimethylbenzene	3.3E+05	50	3.5E+05	10	5.6E+04														
1,3,5-Trimethylbenzene	3.3E+05			10	6.0E+04														
1,3-Butadiene		na	1.0E+01	na	1.8E+01														
1,3-Dinitrobenzene		0.1	7.0E+02	0.1	2.0E+03														
1,4-Dioxane	1.0E+03	na	3.0E+03	30	4.6E+02							na	3.0E+04	3.0E+04					
1,7-Dimethylxanthine (Paraxanthine)								na	7.0E+02										
10,11-Dihydroxy-carbamazepine																			3.0E+02
17 α-ethinyl estradiol		na	2.8E+02					0.000043	1.5E+00	0.0001	3.5E+00						5.0E+03		
17α-estradiol		0.05	3.5E+02					na	1.8E+02										
17β-estradiol		0.05	9.0E-01					0.05	1.8E+02	0.05	1.8E+03						5.0E+03		
1-Butanol		100	7.0E+05																
2,3,4,7,8-Pentachlorodibenzofuran														6.0E-03					
2,3,7,8-Tetrachlorodibenzofuran														3.0E-01					
2,4,5-Trichlorophenol				100	1.2E+06									1.8E+04					
2,4,6-Trichlorophenol				1	4.1E+03			na	2.0E+04					1.8E+04					
2,4,6-Trinitor-1,3-dimethyl-5-tert-butylbenzene (musk xylene)								100	3.5E+05										
2,4,6-Trinitrotoluene (TNT)	1.0E+03																		
2,4-Dichlorophenol				3	4.6E+04			na	2.0E+05					1.8E+04					

Table D-3. Continued

CEC	CA Depth Public Health (2007)[a]	USEPA CCL 3 List/PCCL[b]		USEPA (2017)[c]		Schwab et al. (2005)[d]		Australia (2008)[e]		AwwaRF (2008)[f]		Schriks et al. (2009)[g]		Cotruvo et al. (2010)[h]	Minnesota Dept Health (2015)[i]		WE&RF (2016)[j]	Science Panel[k]	German Environ. Agency[l]
	Drinking Water Notifi-cation Levels (ng/L)	ADI or RfD (µg/kg /day)	PNEC (ng/L)	ADI (µg/kg /day)	Tap-water RSL (ng/L)	ADI (µg/kg /day)	$PNEC_{dw}$ (ng/L)	ADI (µg/kg/ day)	DWG (ng/L)	ADI (µg/kg day)	DWEL (ng/L)	TDI, ADI, or RfD (µg/kg/ day)	PGV (ng/L)	Lowest Guideline Value (ng/L)	ADI (µg/kg/ day)	Screen. Water Value[i] (ng/L)	PHC (ng/L)	MTL (ng/L)	HAV (ng/L)
2,4-Dimethylphenol				20	3.6E+05									1.0E+05					
2,5-Dihydroxybenzoic acid								na	7.0E+03										
2,6-Dichlorobenzamide (BAM)												15	5.3E+04						
2,6-Dichlorophenol								3	1.0E+04										
2,6-Dinitrotoluene				0.3	4.9E+01									6.0E+03					
2,4-Di-tert.-butylphenol																			3.0E+03
2,6-di-tert-butyl-1,4-benzoquinone (2,6-bis(1,1-dimethylethyl)-2,5-Cyclohexadiene-1,4-dione)								na	1.4E+01										
2,6-di-tert-butylphenol (2,6-bis(1,1-dimethylethyl)phenol)								na	2.0E+03										
2,7-Dichlorodibenzo-p-dioxin (DCDD)								0.02	1.6E-02										
2-Butanone														3.6E+06					
2-Butoxyethanol														3.0E+06					
2-Chloroethanol				20	4.0E+05														1.0E+02
2-Chloronaphthalene														4.8E+05					
2-Chlorotoluene	1.4E+05	20	1.4E+05																
2-Methoxyethanol		3	2.1E+04																
2-Phenylphenol				na	3.0E+04			na	1.0E+03										
2-Propen-1-ol		5	3.5E+04																
3-Hydroxycarbofuran		0.06	4.2E+02																
4,4'-DDE				na	4.6E+01			na	2.0E+04										
4,4'-DDT				0.5	2.3E+02			na	2.0E+04										
4,4-Methylenedianiline		na	2.2E+01	na	4.8E+02														
4-Acctyl-6-t-butyl-1,1-dimethylindan								na	7.0E+03										
4-Chloro-3-methylphenol														7.0E+05					
4-Chlorophenol								3	1.0E+04										
4-Chlorotoluene	1.4E+05	20	1.4E+05	20	2.5E+05														
4-Cumylphenol								na	3.5E+02										
4-Isopropyltoluene														3.0E+03					
4-Methyl-2-Pentanone														7.0E+06					
4-Methylbenzenesulfonamide												750	2.6E+06						
4-Methylphenol (p-cresol)								170	6.0E+05					3.0E+05					

Table D.3. Continued

CEC	CA Depth Public Health (2007)[a] Drinking Water Notifi-cation Levels (ng/L)	USEPA CCL 3 List/PCCL[b] ADI or RfD (µg/kg /day)	USEPA CCL 3 List/PCCL[b] PNEC (ng/L)	USEPA (2017)[c] ADI (µg/kg /day)	USEPA (2017)[c] Tap-water RSL (ng/L)	Schwab et al. (2005)[d] ADI (µg/kg /day)	Schwab et al. (2005)[d] $PNEC_{dw}$ (ng/L)	Australia (2008)[e] ADI (µg/kg/ day)	Australia (2008)[e] DWG (ng/L)	AwwaRF (2008)[f] ADI (µg/kg day)	AwwaRF (2008)[f] DWEL (ng/L)	Schriks et al. (2009)[g] TDI, ADI, or RfD (µg/kg/ day)	Schriks et al. (2009)[g] PGV (ng/L)	Cotruvo et al. (2010)[h] Lowest Guideline Value (ng/L)	Minnesota Dept Health (2015)[i] ADI (µg/kg/ day)	Minnesota Dept Health (2015)[i] Screen. Water Value[i] (ng/L)	WE&RF (2016)[j] PHC (ng/L)	Science Panel[k] MTL (ng/L)	German Environ. Agency[l] HAV (ng/L)
4-Nitrophenol								8	3.0E+04										
4-Nonylphenol (4NP)		na	1.1E+05					150	5.0E+05	50	1.8E+06								
4-tert octylphenol								15	5.0E+04										
5-methyl-1H-benzotriazole								na	7.0E+00										
6-Acetyl-1,1,2,4,4,7-hexamethyltetraline								na	4.0E+03										
Acephate		1.2	4.0E+03	1.2	2.4E+04														
Acesulfame																		2.0E+08	
Acetaldehyde		10000	2.3E+04	na	2.6E+03									1.0E+04					
Acetamide		na	5.0E+02																
Acetaminophen		50	3.5E+05			340	5.0E+06												
Acetochlor		20	1.4E+05	20	3.5E+05														
Acetochlor ethane sulfonic acid (ESA)		na	1.6E+05																
Acetochlor oxanilic acid (OA)		na	1.6E+05																
Acetone				900	1.4E+07									5.4E+06					
Acetophenone				100	1.9E+06			100	4.0E+05										
Acrolein		0.5	3.5E+03	0.5	4.2E+01									3.0E+03					
Acyclovir																			3.0E+02
Alachlor (Lasso)								na	2.0E+03										
Alachlor ethanesulfonic acid (ESA)		na	1.1E+06																
Albuterol						2.8	4.1E+04								0.75	2.0E+04			
Alendronate															0.21	6.0E+03			
Allopurinol															25	7.0E+05			
Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)												300	9.0E+05						
Alprazolam								0.0071	2.5E+02						0.0094	3.0E+02			
Aluminum				1000	2.0E+07			na	2.0E+05										
Amidotrizoic acid (diatrizoic acid)												na	2.5E+08						1.0E+03
Amitriptyline															0.074	2.0E+03			
Amlodipine															0.37	1.0E+04			
Amoxycillin								0.43	1.5E+03						13	4.0E+05			
Amphetamine															0.013	4.0E+02			
Ampicillin															4.2	1.0E+05			

Table D.3. Continued

CEC	CA Depth Public Health (2007)[a] Drinking Water Notifi-cation Levels (ng/L)	USEPA CCL 3 List/PCCL[b] ADI or RfD (µg/kg /day)	USEPA CCL 3 List/PCCL[b] PNEC (ng/L)	USEPA (2017)[c] ADI (µg/kg /day)	USEPA (2017)[c] Tap-water RSL (ng/L)	Schwab et al. (2005)[d] ADI (µg/kg /day)	Schwab et al. (2005)[d] $PNEC_{dw}$ (ng/L)	Australia (2008)[e] ADI (µg/kg/ day)	Australia (2008)[e] DWG (ng/L)	AwwaRF (2008)[f] ADI (µg/kg day)	AwwaRF (2008)[f] DWEL (ng/L)	Schriks et al. (2009)[g] TDI, ADI, or RfD (µg/kg/ day)	Schriks et al. (2009)[g] PGV (ng/L)	Cotruvo et al. (2010)[h] Lowest Guideline Value (ng/L)	Minnesota Dept Health (2015)[i] ADI (µg/kg/ day)	Minnesota Dept Health (2015)[i] Screen. Water Value[i] (ng/L)	WE&RF (2016)[j] PHC (ng/L)	Science Panel[k] MTL (ng/L)	German Environ. Agency[l] HAV (ng/L)
Anatoxin-a		**0.5**	**3.5E+03**																
Androsterone								**na**	**1.4E+04**										
Androstenedione																			
Anhydro-erthromycin A								5	1.8E+04										
Aniline		7	6.0E+03	7	1.3E+04									4.2E+04					
Anthracene				300	1.8E+06			na	1.5E+05										
Antipyrine								28.4	1.0E+06										
Aspartame																		3.0E+08	
Aspirin								8.3	2.9E+04			7	2.5E+04						
Atenolol										2	7.0E+04				0.63	2.0E+04	4.0E+03		
Atorvastatin								0.14	5.0E+03	0.54	1.9E+04				0.043	1.0E+03			
Atrazine				35	3.0E+02			na	4.0E+04	0.1	3.0E+03			2.0E+03					
Azinphos-methyl				3	5.6E+04			na	3.0E+03										
Azithromycin								11	3.9E+03						1	3.0E+04			3.0E+02
Azobenzene				na	1.2E+02									3.0E+03					
Bensulide		5	3.5E+04																
Bentazone												100	3.0E+05						
Benzatropine															0.0043	1.0E+02			
Benzoic acid				4000	7.5E+07									2.4E+07					
Benzothiozole												26	9.0E+04						
Benzotriazole (1H-benzotriazole)												295	1.0E+06						3.0E+03
Benzyl alcohol				100	2.0E+05									3.0E+06					
Benzyl chloride		na	2.0E+02					na	2.0E+02										
Betaxolol								0.28	1.0E+04						0.13	4.0E+03			
Bezafibrate(Benzafibrate)								8.6	3.0E+05										
Bis(2-ethylhexyl)adipate														2.4E+06					
Bis(chloroisopropyl)ether (BCIPE)												40	1.4E+05						
Bisoprolol								0.018	6.3E+02						0.6	2.0E+04			
Bisphenol A		50	3.5E+05	50	7.7E+05			50	2.0E+05	50	1.8E+06			3.0E+05					
Bisphenol A diglycidyl ether														1.0E+06					
Boron	1.0E+06			200	4.0E+06			na	4.0E+06										
Bromide								1000	7.0E+06										
Bromine								1000	7.0E+06										
Bromoacetic acid								na	3.5E+02										

Table D-3. Continued

CEC	CA Depth Public Health (2007)[a]	USEPA CCL 3 List/PCCL[b]		USEPA (2017)[c]		Schwab et al. (2005)[d]		Australia (2008)[e]		AwwaRF (2008)[f]		Schriks et al. (2009)[g]		Cotruvo et al. (2010)[h]	Minnesota Dept Health (2015)[i]		WE&RF (2016)[j]	Science Panel[k]	German Environ. Agency[l]
	Drinking Water Notifi-cation Levels (ng/L)	ADI or RfD (µg/kg /day)	PNEC (ng/L)	ADI (µg/kg /day)	Tap-water RSL (ng/L)	ADI (µg/kg /day)	$PNEC_{dw}$ (ng/L)	ADI (µg/kg/ day)	DWG (ng/L)	ADI (µg/kg day)	DWEL (ng/L)	TDI, ADI, or RfD (µg/kg/ day)	PGV (ng/L)	Lowest Guideline Value (ng/L)	ADI (µg/kg/ day)	Screen. Water Value[i] (ng/L)	PHC (ng/L)	MTL (ng/L)	HAV (ng/L)
Bromochloroacetonitrile								**na**	**7.0E+02**										
Bromochloromethane		**10**	**7.0E+04**	**na**	**8.3E+04**			**10**	**4.0E+04**					**9.0E+04**					
Bromomethane		1.4	9.8E+03	1.4	7.5E+03									6.0E+03					
Bromophos-ethyl								na	1.0E+04										
Butylated hydroxyanisole (3-tert-butyl-4-hydroxy anisole) (BHA)		na	5.8E+02	na	1.5E+05			500	1.8E+06										
Butylated hydroxytoluene (2,6-Di-tert-Butyl-p-Cresol)				300	3.4E+03			300	1.0E+06										
Butylbenzyl phthalate				200	1.6E+04					100	3.5E+06			1.2E+06					
Caffeine								na	3.5E+02										
Candesartan																			3.0E+02
Captan		130	1.5E+04	130	3.1E+04														
Carazolol								0.01	3.5E+02										
Carbamazepine								2.8	1.0E+05	0.34	1.2E+04	0.34	1.0E+03				1.0E+04		
Carbendazim								na	1.0E+05			30	1.1E+05						
Carbon disulfide	1.6E+05	100	7.0E+05	100	8.1E+05									6.0E+05					
[(Carboxymethyl)imino bis(ethylenenitrilo)] tetra acetic acid								na	5.0E+03										
Carisoprodol															9.4	3.0E+05			
Carvedilol															1	3.0E+04			
Cefaclor								7.1	2.5E+05										
Celecoxib															2.5	7.0E+04			
Cephalexin								10	3.5E+04						13	4.0E+05			
CFC-12		200	1.4E+06																
Chloral hydrate				100	2.0E+06									6.0E+05					
Chloramphenicol								5	1.8E+05										
Chlorate	8.0E+05	30	2.1E+05											4.2E+06					
Chlordane (gamma-chlordane)				0.5	2.0E+01			na	1.0E+03										
Chlorfenvinphos				0.7	1.1E+04									4.2E+03					
Chloridazon (pyrazon)												54	1.9E+05						
Chloromethane		4	2.7E+03	na	1.9E+05									2.4E+04					
Chlorophene								na	3.5E+02										
Chlorotetracycline								30	1.1E+05										

Table D.3. Continued

CEC	CA Depth Public Health (2007)[a] Drinking Water Notifi-cation Levels (ng/L)	USEPA CCL 3 List/PCCL[b] ADI or RfD (µg/kg /day)	USEPA CCL 3 List/PCCL[b] PNEC (ng/L)	USEPA (2017)[c] ADI (µg/kg /day)	USEPA (2017)[c] Tap-water RSL (ng/L)	Schwab et al. (2005)[d] ADI (µg/kg /day)	Schwab et al. (2005)[d] $PNEC_{dw}$ (ng/L)	Australia (2008)[e] ADI (µg/kg/ day)	Australia (2008)[e] DWG (ng/L)	AwwaRF (2008)[f] ADI (µg/kg day)	AwwaRF (2008)[f] DWEL (ng/L)	Schriks et al. (2009)[g] TDI, ADI, or RfD (µg/kg/ day)	Schriks et al. (2009)[g] PGV (ng/L)	Cotruvo et al. (2010)[h] Lowest Guideline Value (ng/L)	Minnesota Dept Health (2015)[i] ADI (µg/kg/ day)	Minnesota Dept Health (2015)[i] Screen. Water Value[i] (ng/L)	WE&RF (2016)[j] PHC (ng/L)	Science Panel[k] MTL (ng/L)	German Environ. Agency[l] HAV (ng/L)
Chlorpropham				50	7.0E+07									1.2E+06					
Chlorpyrifos				1	8.4E+03			na	1.0E+04										
Chlorpyrifos-methyl				10	1.2E+05			na	1.0E+04										
Cholesterol								na	7.0E+03										
Cimetidine						29	4.2E+05	5.7	2.0E+05						10	3.0E+05			
Ciprofloxacin						1.6	2.3E+04	7.1	2.5E+05						2.1	6.0E+04			
Clarithromycin								7.1	2.5E+05						2.1	6.0E+04			
Clavulante															3.1	9.0E+04			
Clenbuterol								4.2	1.5E+04										
Clethodim		10	7.0E+04																
Clindamycin								8.6	3.0E+05						2.5	7.0E+04			
Clofibric acid (clofibrate)								21.4	7.5E+05			10	3.0E+04						3.0E+03
Clonazepam															0.0043	1.0E+02			
Clonidine															0.0083	2.0E+02			
Clopidogrel															3.1	9.0E+04			
Cobalt		10	7.0E+04	0.3	6.0E+03														
Codeine						2	2.9E+04	1.4	5.0E+04						0.19	5.0E+03			
Copastanol								na	7.0E+02										
Cotinine								0.28	1.0E+04								1.0E+03		
Coumarin								na	5.0E+02										
Cumene hydroperoxide		na	7.6E+04																
Cyclobenzaprine															0.019	5.0E+02			
Cylcophosphamide								0.1	3.5E+03										
Cylindrospermopsin		0.03	2.1E+02																
Cypermethrin				60	1.2E+06			na	5.0E+02										
Dalapon				30	6.0E+05									1.8E+05					
Dehydronifedipine						100	1.5E+06	0.57	2.0E+04										
Demeclocycline								8.6	3.0E+05						0.23	6.0E+03			
Demeton-S				0.04	4.2E+02			0.04	1.5E+02										
Diatrizoate sodium								na	3.5E+02										
Diatrizoic acid								na	3.5E+02										
Diazepam (Valium)								0.071	2.5E+03	1	3.5E+04				0.15	4.0E+03			
Diazinon	1.2E+03	0.2	1.4E+03	0.7	1.0E+04			na	3.0E+03					1.2E+03					

Table D.3. Continued

CEC	CA Depth Public Health (2007)[a]	USEPA CCL 3 List/PCCL[b]		USEPA (2017)[c]		Schwab et al. (2005)[d]		Australia (2008)[e]		AwwaRF (2008)[f]		Schriks et al. (2009)[g]		Cotruvo et al. (2010)[h]	Minnesota Dept Health (2015)[i]		WE&RF (2016)[j]	Science Panel[k]	German Environ. Agency[l]
	Drinking Water Notifi-cation Levels (ng/L)	ADI or RfD (µg/kg /day)	PNEC (ng/L)	ADI (µg/kg /day)	Tap-water RSL (ng/L)	ADI (µg/kg /day)	$PNEC_{dw}$ (ng/L)	ADI (µg/kg/ day)	DWG (ng/L)	ADI (µg/kg day)	DWEL (ng/L)	TDI, ADI, or RfD (µg/kg/ day)	PGV (ng/L)	Lowest Guideline Value (ng/L)	ADI (µg/kg/ day)	Screen. Water Value[i] (ng/L)	PHC (ng/L)	MTL (ng/L)	HAV (ng/L)
Dibromoacetonitrile														7.0E+04					
Dibromochloromethane				20	8.7E+02			na	1.0E+05					8.0E+04					
Dibutyl phthalate				100	9.0E+05									3.1E+05					
Dibutyltin (DBT)				0.3	6.0E+03			0.25	2.0E+03										
Dichloroacetic acid				4	1.5E+03			na	1.0E+05					7.0E+03					
Dichloroacetonitrile								na	2.0E+03					2.0E+04					
Dichlorodifluoromethane (Freon 12)	1.0E+06			200	2.0E+05														
Dichlorodiphenyldicloroethane (DDD)				na	3.2E+01									1.0E+03					
Dichlorvos				0.5	2.6E+02			na	1.0E+03										
Diclofenac								0.5	1.8E+03	67	2.3E+06				4.2	1.0E+05			3.0E+02
Dicrotophos		0.07	4.9E+02	0.07	1.4E+03														
Dieldrin														3.0E+01					
Diethylstilbestrol																		5.1E-02	
Diethyl glycol dimethyl ether												50	1.8E+05						
Diethyl phthalate												800	2.8E+06	8.0E+05					
Diethylamine (DEA)												2140	7.5E+05						
Diethylene triamine penta acetic acid												100	3.5E+05						
Diethylhexyl phthalate				20	5.6E+03					12	4.2E+05								
Digoxigenin						0.07	1.0E+03												
Digoxin						0.07	1.0E+03								0.00016	4.0E+00			
Diltiazem						14	2.0E+05	1.7	6.0E+04						1.5	4.0E+04			
Dimethenamid												70	2.5E+05						
Dimethipin		21.8	1.5E+05																
Dimethoate		2.2	1.5E+04					na	5.0E+04										
Dimethyl phthalate														3.0E+03					
Dimethylamine (DMA)												540	1.9E+05						
Di-n-butyl phthalate				100	9.0E+05			10	3.5E+04										
Dipyrone								150	5.3E+05										
Disulfoton		0.13	9.1E+02	0.04	5.0E+02														
Diuron		3	1.8E+03	2	3.6E+04			na	3.0E+04			2	7.0E+03	1.8E+04					
Dodecylguanidine acetate														2.4E+04					
Doxepin															0.32	9.0E+03			

Table D.3. Continued

CEC	CA Depth Public Health (2007)[a]	USEPA CCL 3 List/PCCL[b]		USEPA (2017)[c]		Schwab et al. (2005)[d]		Australia (2008)[e]		AwwaRF (2008)[f]		Schriks et al. (2009)[g]		Cotruvo et al. (2010)[h]	Minnesota Dept Health (2015)[i]		WE&RF (2016)[j]	Science Panel[k]	German Environ. Agency[l]
	Drinking Water Notifi-cation Levels (ng/L)	ADI or RfD (µg/kg /day)	PNEC (ng/L)	ADI (µg/kg /day)	Tap-water RSL (ng/L)	ADI (µg/kg /day)	$PNEC_{dw}$ (ng/L)	ADI (µg/kg/ day)	DWG (ng/L)	ADI (µg/kg day)	DWEL (ng/L)	TDI, ADI, or RfD (µg/kg/ day)	PGV (ng/L)	Lowest Guideline Value (ng/L)	ADI (µg/kg/ day)	Screen. Water Value[i] (ng/L)	PHC (ng/L)	MTL (ng/L)	HAV (ng/L)
Doxycycline						30	4.4E+05	3	1.1E+04						0.03	8.0E+02			
Drospirenone															0.0038	1.0E+02			
Duloxetine															0.5	1.0E+04			
Enalaprilat (enalapril)						70	1.0E+06	0.036	1.3E+03	0.23	8.1E+03				0.21	6.0E+03			
Endosulfan				6	1.0E+05									3.6E+04					
Endosulfan sulfate								na	3.0E+04										
Endrin				0.3	2.3E+03									1.8E+03					
Enrofloxacin								6.2	2.2E+04										
Equilenin		0.05	3.5E+02					0.00086	3.0E+01										
Equilin		0.05	3.5E+02					0.00086	3.0E+01										
Erythromycin-H_2O		0.7	4.9E+03			40	5.8E+05	5	1.8E+04						13	4.0E+05			
Escitalopram															0.043	1.0E+03			
Estriol		0.05	3.5E+02					0.0014	5.0E+01										
Estrone		0.05	3.5E+02					0.00086	3.0E+01	0.013	4.6E+02						3.2E+02		
Ethion								na	3.0E+03										
Ethoprop		0.1	7.0E+02																
Ethoprophos (Mocap)								na	1.0E+03										
Ethyl N,N-diphynylcarbamate																			3.0E+02
Ethyl tert-butyl ether (ETBE)												150	5.3E+05						
Ethylene glycol	1.4E+07	2000	1.4E+07	2000	4.0E+07														
Ethylene oxide		na	1.1E+02	na	6.7E-01														
Ethylene thiourea		0.2	6.0E+01	0.08	1.6E+03														
Ethylenediaminetetraacetic acid (EDTA)								na	2.5E+05			1900	6.0E+05						
Ezetimibe															0.42	1.0E+04			
Fenamiphos		0.1	7.0E+02																
Fenofibrate															0.2	6.0E+03			
Fenoprofen								12.9	4.5E+05						0.83	2.0E+04			
Fenthion (fenthion-methyl)								na	5.0E+02										
Fluconazole															0.13	4.0E+03			
Fluorene				40	2.9E+05									2.4E+05					
Fluoxetine (Prozac)						2.9	4.2E+04	0.28	1.0E+04	0.97	3.4E+04				0.083	2.0E+03			
Formaldehyde	1.0E+05	200	1.4E+06	200	4.3E+02									1.2E+06					

Table D.3 Continued

CEC	CA Depth Public Health (2007)[a]	USEPA CCL 3 List/PCCL[b]		USEPA (2017)[c]		Schwab et al. (2005)[d]		Australia (2008)[e]		AwwaRF (2008)[f]		Schriks et al. (2009)[g]		Cotruvo et al. (2010)[h]	Minnesota Dept Health (2015)[i]		WE&RF (2016)[j]	Science Panel[k]	German Environ. Agency[l]
	Drinking Water Notifi-cation Levels (ng/L)	ADI or RfD (µg/kg /day)	PNEC (ng/L)	ADI (µg/kg /day)	Tap-water RSL (ng/L)	ADI (µg/kg /day)	$PNEC_{dw}$ (ng/L)	ADI (µg/kg/ day)	DWG (ng/L)	ADI (µg/kg day)	DWEL (ng/L)	TDI, ADI, or RfD (µg/kg/ day)	PGV (ng/L)	Lowest Guideline Value (ng/L)	ADI (µg/kg/ day)	Screen. Water Value[i] (ng/L)	PHC (ng/L)	MTL (ng/L)	HAV (ng/L)
Furosemide															0.83	2.0E+03			
Fyrol FR 2 (tri(dichlorisopropyl phosphate)				20	3.6E+05			na	1.0E+06										
Gabapentin															110	3.0E+06			1.0E+03
Gabapentin lactam																			1.0E+03
Galaxolide								500	1.8E+06										
Gemfibrozil						55	8.0E+05	17	6.0E+05	1.3	4.5E+04				5	1.0E+05			
Germanium		na	7.4E+02																
Glipizide															0.019	5.0E+02			
Glyburide															0.0016	4.0E+01			
Glyoxal		200	1.4E+06																
HCFC-22		na	3.2E+04																
Hexane		60	4.2E+05	na	1.5E+06														
HMX	3.5E+05																		
Hydrazine		na	1.0E+01	na	1.1E+00														
Hydrochlorothiazide															0.016	4.0E+02			
Hydrocodone															0.025	7.0E+02			
Hydrocortisone															0.0083	2.0E+02			
Ibuprofen						110	1.6E+06	11.4	4.0E+05						6.7	5.0E+04			
Imidacloprid												60	2.1E+05						
Imipramine															0.037	1.0E+03			
Indomethacin								0.71	2.5E+04						2.1	6.0E+04			
Iodide								17	1.0E+05										
Iohexol								20.6	7.2E+05				3.8E+08						
Iomeprol (iomeron)												1900	6.7E+06						
Iopamidol								11.4	4.0E+05				4.2E+08						1.0E+03
Iopromide								21.4	7.5E+05				2.5E+08						
Isophorone				200	7.8E+04									4.0E+05					
Isophosphamide								0.1	3.5E+03										
Isopropylbenzene	7.7E+05													6.0E+05					
Isoproturon												3	9.0E+03						
Ketoprofen								1	3.5E+03						0.94	3.0E+04			
Lamotrigine															9.4	3.0E+05			3.0E+02

Table D.3 Continued

CEC	CA Depth Public Health (2007)[a]	USEPA CCL 3 List/PCCL[b]		USEPA (2017)[c]		Schwab et al. (2005)[d]		Australia (2008)[e]		AwwaRF (2008)[f]		Schriks et al. (2009)[g]		Cotruvo et al. (2010)[h]	Minnesota Dept Health (2015)[i]		WE&RF (2016)[j]	Science Panel[k]	German Environ. Agency[l]
	Drinking Water Notifi-cation Levels (ng/L)	ADI or RfD (µg/kg /day)	PNEC (ng/L)	ADI (µg/kg /day)	Tap-water RSL (ng/L)	ADI (µg/kg /day)	$PNEC_{dw}$ (ng/L)	ADI (µg/kg/ day)	DWG (ng/L)	ADI (µg/kg day)	DWEL (ng/L)	TDI, ADI, or RfD (µg/kg/ day)	PGV (ng/L)	Lowest Guideline Value (ng/L)	ADI (µg/kg/ day)	Screen. Water Value[i] (ng/L)	PHC (ng/L)	MTL (ng/L)	HAV (ng/L)
Levothyroxine															0.0043	1.0E+02			
Lincomycin						25	3.7E+05	1000	3.5E+06										
Lindane (gamma-BHC)								na	2.0E+04	0.56	2.0E+04			2.0E+02					
Linuron		2	5.6E+04	7.7	1.3E+05					2	7.0E+04								
Lisdexamfetamine															0.13	4.0E+03			
Lisinopril															0.021	6.0E+02			
Lomefloxacin															6.7	2.0E+05			
Lorazepam															0.0083	2.0E+02			
Losartan															2.1	6.0E+04			
Lovastatin															0.013	4.0E+02			
Malathion				20	3.9E+05			na	9.0E+05										
Manganese	5.0E+05	47	3.0E+05					na	5.0E+05										
m-Dichlorobenzene														5.4E+05					
Mefenamic acid															4.2	1.0E+05			
Meloxicam															0.094	3.0E+03			
Memantine															0.25	7.0E+03			
Meprobramate										7.5	2.6E+05				4	1.0E+05	2.0E+05		
								0.00007											
Mestranol		na	2.8E+02					1	2.5E+00										
Metformin						62	9.1E+05	7.1	2.5E+05						1.5	4.0E+04			1.0E+03
Methamidophos		0.3	2.1E+03	0.05	1.0E+03														
Methanol		500	3.5E+06	2000	2.0E+07														
Methomyl				25	5.0E+05									1.5E+05					
Methoxychlor				5	3.7E+04					0.02	7.0E+02			2.0E+04					
Methylisothiocyanate																		1.2E+05	
Methyl isobutyl ketone (MIBK)	1.2E+05			na	6.3E+06														
Methyl tert-butyl ether (MTBE)		na	1.9E+04	na	1.4E+04							300	9.4E+06	6.0E+04					
Methyl-oxirane		1	2.3E+02																
Methylphenidate															0.25	7.0E+03			
Methylprednisolone															0.0017	5.0E+01			
Metolachlor		100	7.0E+05	150	2.7E+06			na	3.0E+05										
Metolachlor (ESA)		na	7.0E+06																
Metolachlor (OA)		na	7.0E+06																
Metoprolol								0.71	2.5E+04			14	5.0E+04		1	3.0E+04			
Microcystin-LR		0.003	2.1E+01																
Minocycline															0.083	2.0E+03			
Mirex				0.2	8.8E-01									4.8E+03					

Table D.3 Continued

CEC	CA Depth Public Health (2007)[a] Drinking Water Notifi-cation Levels (ng/L)	USEPA CCL 3 List/PCCL[b] ADI or RfD (µg/kg /day)	USEPA CCL 3 List/PCCL[b] PNEC (ng/L)	USEPA (2017)[c] ADI (µg/kg /day)	USEPA (2017)[c] Tap-water RSL (ng/L)	Schwab et al. (2005)[d] ADI (µg/kg /day)	Schwab et al. (2005)[d] $PNEC_{dw}$ (ng/L)	Australia (2008)[e] ADI (µg/kg/ day)	Australia (2008)[e] DWG (ng/L)	AwwaRF (2008)[f] ADI (µg/kg day)	AwwaRF (2008)[f] DWEL (ng/L)	Schriks et al. (2009)[g] TDI, ADI, or RfD (µg/kg/ day)	Schriks et al. (2009)[g] PGV (ng/L)	Cotruvo et al. (2010)[h] Lowest Guideline Value (ng/L)	Minnesota Dept Health (2015)[i] ADI (µg/kg/ day)	Minnesota Dept Health (2015)[i] Screen. Water Value[i] (ng/L)	WE&RF (2016)[j] PHC (ng/L)	Science Panel[k] MTL (ng/L)	German Environ. Agency[l] HAV (ng/L)
Molinate		2	1.4E+04	2	3.0E+04														
Molybdenum		5	3.5E+04	5	1.0E+05			na	5.0E+04										
Monensin								10	3.5E+04										
Monobutyltin (MBT)								na	7.0E+02										
Monochloroacetic acid														6.0E+04					
Montelukast															0.81	2.0E+04			
Musk ketone								100	3.5E+05										
Musk tibetene								na	3.5E+02										
N,N-diethyltoluamide (NN-diethyl-3-methylbenzamide (DEET)								750	2.5E+03			1800	6.3E+06				2.0E+05		
Nadolol								0.57	2.0E+04										
Naladixic Acid								28.4	1.0E+06										
Naphthalene	1.7E+04			20	1.7E+02			na	7.0E+04					1.2E+05					
Naproxen								6.3	2.2E+05	570	2.0E+07				6.3	2.0E+05			
n-Butylbenzene	2.6E+05			50	1.0E+06														
n-Butylbenzenesulphonamide												83	2.9E+05						
Nebivolol															0.031	9.0E+02			
Neotame																		1.8E+06	
Nicosulfuron												200	7.0E+05						
Nifedipine															0.38	1.0E+04			
Nitrilotriacetic acid (NTA)								na	2.0E+05					2.0E+05					
Nitrobenzene		2	1.4E+04	2	1.4E+02									1.2E+04					
N-methyl-2-pyrrolidone		600	4.2E+06																
N-nitrosodiethylamine (NDEA)	1.0E+01	na	2.0E-01	na	1.7E-01			na	1.0E+01										
N-nitrosodimethylamine (NDMA)	1.0E+01	0.008	6.9E-01	0.008	1.1E-01			na	1.0E+01			na	1.0E+02						
N-nitrosodi-n-propylamine (NDPA)	1.0E+01	na	5.0E+00	na	1.1E+01														
N-nitrosomorpholine (NMOR)				na	1.2E+01			na	1.0E+00										
N-nitrosopyrrolidine (NPYR)		na	2.0E+01	na	3.7E+01														
N-Octadecane														3.0E+03					
Norethindrone		16.7	4.0E+01					0.0071	2.5E+02										
Norfloxacin						190	2.8E+06	11.4	4.0E+05						3.3	1.0E+05			
Norfluoxetine										0.97	3.4E+04								
n-Propylbenzene	2.6E+05	na	5.8E+03	100	6.6E+05														
Octachlorodibenzo-4-dioxin														3.0E+02					
Octachlorodibenzo-p-dioxin (OCDD)								0.02	1.6E-02										

Table D.3 Continued

CEC	CA Depth Public Health (2007)[a]	USEPA CCL 3 List/PCCL[b]		USEPA (2017)[c]		Schwab et al. (2005)[d]		Australia (2008)[e]		AwwaRF (2008)[f]		Schriks et al. (2009)[g]		Cotruvo et al. (2010)[h]	Minnesota Dept Health (2015)[i]		WE&RF (2016)[j]	Science Panel[k]	German Environ. Agency[l]
	Drinking Water Notifi-cation Levels (ng/L)	ADI or RfD (µg/kg /day)	PNEC (ng/L)	ADI (µg/kg /day)	Tap-water RSL (ng/L)	ADI (µg/kg /day)	$PNEC_{dw}$ (ng/L)	ADI (µg/kg/ day)	DWG (ng/L)	ADI (µg/kg day)	DWEL (ng/L)	TDI, ADI, or RfD (µg/kg/ day)	PGV (ng/L)	Lowest Guideline Value (ng/L)	ADI (µg/kg/ day)	Screen. Water Value[i] (ng/L)	PHC (ng/L)	MTL (ng/L)	HAV (ng/L)
Octylphenol										150	5.3E+06								
Ofloxacin															1.7	5.0E+04			
Olanzapine															0.0037	1.0E+02			
Olmesartan																			3.0E+02
Olmesartan medoxomil															0.83	2.0E+04			
o-Toluidine		na	1.9E+02	na	4.7E+03														
Oxamyl				25	5.0E+05									6.0E+03					
Oxycodone															0.0083	2.0E+02			
Oxydemeton-methyl		0.13	9.1E+02																
Oxyfluorfen		3	4.8E+02	30	5.4E+02														
Oxypurinol																			3.0E+02
Oxytetracycline						30	4.4E+05	30	1.1E+05						0.21	6.0E+03			
p-Chlorobenzene sulfonic acid				100	2.0E+03														
p,p'-Sulfonyldiphenol												17	6.0E+04						
Paracetamol								50	1.8E+05										
Parathion (ethyl parathion)				6	8.6E+04			na	1.0E+04										
Parathion-methyl (methyl parathion)				0.25	4.5E+03			na	1.0E+05										
Paroxetine metabolite						2.9	4.2E+04												
PCB 105				0.023	4.0E+00			0.02	1.6E-02										
PCB 118				0.023	4.0E+00			0.02	1.6E-02										
PCB 156				0.023	4.0E+00			0.02	1.6E-02										
PCB 167				0.023	4.0E+00			0.02	1.6E-02										
PCB 169				2.3E-05	4.0E-03			0.02	1.6E-02										
PCB 77				0.007	6.0E+00			0.02	1.6E-02										
Penicillin G								0.43	1.5E+03										
Penicillin V								0.43	1.5E+03						3.1	9.0E+04			
Pentamethyl-4,6-dinitroindane								na	3.5E+02										
Pentoxyifylline															17	5.0E+05			
Perchlorate		0.7	4.9E+03	0.7	1.4E+04														
Perfenofos		0.05	3.5E+02																
Perfluoroctane sulfonate (PFOS)	7.0E+01	na	2.0E+02									0.15	5.0E+02						
Perfluorooctanoic acid (PFOA)	7.0E+01	na	1.1E+03									1.5	5.3E+03						

Table D.3 Continued

CEC	CA Depth Public Health (2007)[a] Drinking Water Notifi-cation Levels (ng/L)	USEPA CCL 3 List/PCCL[b] ADI or RfD (µg/kg /day)	USEPA CCL 3 List/PCCL[b] PNEC (ng/L)	USEPA (2017)[c] ADI (µg/kg /day)	USEPA (2017)[c] Tap-water RSL (ng/L)	Schwab et al. (2005)[d] ADI (µg/kg /day)	Schwab et al. (2005)[d] $PNEC_{dw}$ (ng/L)	Australia (2008)[e] ADI (µg/kg/ day)	Australia (2008)[e] DWG (ng/L)	AwwaRF (2008)[f] ADI (µg/kg day)	AwwaRF (2008)[f] DWEL (ng/L)	Schriks et al. (2009)[g] TDI, ADI, or RfD (µg/kg/ day)	Schriks et al. (2009)[g] PGV (ng/L)	Cotruvo et al. (2010)[h] Lowest Guideline Value (ng/L)	Minnesota Dept Health (2015)[i] ADI (µg/kg/ day)	Minnesota Dept Health (2015)[i] Screen. Water Value[i] (ng/L)	WE&RF (2016)[j] PHC (ng/L)	Science Panel[k] MTL (ng/L)	German Environ. Agency[l] HAV (ng/L)
Permethrin		50	3.7E+03	50	1.0E+06														
Phenanthrene								na	1.5E+05					2.4E+05					
Phenazone												36	1.3E+05						3.0E+02
Phenobarbital																			3.0E+02
Phenol				300	5.8E+06			40	1.5E+05					2.4E+05					
Phenytoin (Dilantin)		na	1.2E+04							0.19	6.8E+03						2.0E+03		
Phthalic anhydride								2000	7.0E+06										
Pioglitazone															0.019	5.0E+02			
Pravastatin															0.035	1.0E+03			
Prednisolone															0.002	6.0E+01			
Prednisone															0.0021	6.0E+01			
Pregabalin															6.3	2.0E+05			
Primidone															3.1	9.0E+04	1.0E+04		3.0E+03
Progesterone								30	1.1E+05						0.083	2.0E+03			
Promethazine															0.077	2.0E+03			
Prometon				15	2.5E+05									9.0E+04					
Propachlor	9.0E+04																		
Propoxyphene															1.6	4.0E+04			
Propranolol								1.14	4.0E+04						0.13	4.0E+03			
Propylenedinitrilotetraacetic acid (PDTA)								na	7.0E+02										
Propyphenazone																			3.0E+02
p-Toluolsulfonic acidamid (4-Methylbenzosulfonamide)																			3.0E+02
Pyrene				30	1.2E+05			na	1.5E+05										
Pyridine				1	2.0E+04									6.0E+03					
Quetiapine															0.063	2.0E+03			
Quinoline		na	1.0E+01	na	2.4E+01														
Ranitidine						11	1.6E+05								20	6.0E+05			
RDX	3.0E+02	3	3.0E+02																
Risperidone										0.014	4.9E+02				0.0025	7.0E+01			
Rosuvastatin															0.0063	2.0E+02			
Roxithromycin								4.3	1.5E+05										
Salbutamol								0.086	3.0E+03										

Table D.3 Continued

CEC	CA Depth Public Health (2007)[a] Drinking Water Notifi-cation Levels (ng/L)	USEPA CCL 3 List/PCCL[b] ADI or RfD (µg/kg /day)	USEPA CCL 3 List/PCCL[b] PNEC (ng/L)	USEPA (2017)[c] ADI (µg/kg /day)	USEPA (2017)[c] Tap-water RSL (ng/L)	Schwab et al. (2005)[d] ADI (µg/kg /day)	Schwab et al. (2005)[d] $PNEC_{dw}$ (ng/L)	Australia (2008)[e] ADI (µg/kg/ day)	Australia (2008)[e] DWG (ng/L)	AwwaRF (2008)[f] ADI (µg/kg day)	AwwaRF (2008)[f] DWEL (ng/L)	Schriks et al. (2009)[g] TDI, ADI, or RfD (µg/kg/ day)	Schriks et al. (2009)[g] PGV (ng/L)	Cotruvo et al. (2010)[h] Lowest Guideline Value (ng/L)	Minnesota Dept Health (2015)[i] ADI (µg/kg/ day)	Minnesota Dept Health (2015)[i] Screen. Water Value[i] (ng/L)	WE&RF (2016)[j] PHC (ng/L)	Science Panel[k] MTL (ng/L)	German Environ. Agency[l] HAV (ng/L)
Salicylic acid								**na**	**1.1E+05**										
sec-Butylbenzene	**2.6E+05**	**na**	**1.0E+04**	**100**	**2.0E+06**									**3.0E+03**					
Sertraline															**0.1**	**3.0E+03**			
Sildenafil															**0.13**	**4.0E+03**			
Silver				**5**	**9.4E+04**			**na**	**1.0E+05**										
Simvastatin										**0.54**	**1.9E+04**				**0.0063**	**2.0E+02**			
Sitagliptin															0.13	4.0E+03			
Stigmastanol								28.4	1.0E+06										
Strontium		600	4.2E+06	600	1.2E+07														
Sucralose																	1.5E+08		
Sulfadiazine															2.5	7.0E+04			
Sulfadimethoxine								10	3.5E+04										
Sulfamethazine								10	3.5E+04										
Sulfamethiazole								10	3.5E+04										
Sulfamethizole															0.42	1.0E+04			
Sulfamethoxazole						130	1.9E+06	10	3.5E+04	510	1.8E+07	130	4.4E+05						
Sulfasalazine								14.2	5.0E+05										
Sulfate								na	5.0E+08										
Sulfathiazole						50	7.3E+05												
Tadalafil															0.1	3.0E+03			
Tamsulosin															0.0017	5.0E+01			
Tebuconazole		29	2.1E+05																
Tebufenozide		18	1.3E+05																
Tellurium		na	1.8E+05																
Temazepam								0.14	5.0E+03						0.03	8.0E+02			
Terbufos		0.05	3.5E+02																
Terbufos sulfone		0.05	3.5E+02																
Terbutaline								0.13	4.5E+03										
tert-Butylbenzene	2.6E+05	na	1.0E+04	100	6.9E+05														
Tertiary butyl alcohol (TBA)	1.2E+04	na	6.3E+05											6.0E+06					
Testosterone								2	7.0E+03										
Tetracycline						30	4.4E+05	30	1.1E+05						0.63	2.0E+04			
Thiodicarb		30	1.9E+03																
Thiophanate								na	5.0E+03										

Table D.3 Continued

CEC	CA Depth Public Health (2007)[a] Drinking Water Notifi-cation Levels (ng/L)	USEPA CCL 3 List/PCCL[b] ADI or RfD (µg/kg /day)	USEPA CCL 3 List/PCCL[b] PNEC (ng/L)	USEPA (2017)[c] ADI (µg/kg /day)	USEPA (2017)[c] Tap-water RSL (ng/L)	Schwab et al. (2005)[d] ADI (µg/kg /day)	Schwab et al. (2005)[d] $PNEC_{dw}$ (ng/L)	Australia (2008)[e] ADI (µg/kg/ day)	Australia (2008)[e] DWG (ng/L)	AwwaRF (2008)[f] ADI (µg/kg day)	AwwaRF (2008)[f] DWEL (ng/L)	Schriks et al. (2009)[g] TDI, ADI, or RfD (µg/kg/ day)	Schriks et al. (2009)[g] PGV (ng/L)	Cotruvo et al. (2010)[h] Lowest Guideline Value (ng/L)	Minnesota Dept Health (2015)[i] ADI (µg/kg/ day)	Minnesota Dept Health (2015)[i] Screen. Water Value[i] (ng/L)	WE&RF (2016)[j] PHC (ng/L)	Science Panel[k] MTL (ng/L)	German Environ. Agency[l] HAV (ng/L)
Thiophanate-methyl		80	3.0E+03	27	6.7E+03														
Timolol								0.28	1.0E+04										
Tolfenamic acid								5	1.8E+04										
Toluene				80	1.1E+05									4.8E+05					
Toluene diisocyanate		na	9.0E+02	na	1.7E+01														
Tolyltriazole												250	8.8E+05						
Tramadol															2.5	7.0E+04			
Trazodone															0.19	5.0E+03			
Triamterene															1.6	4.0E+04			
Tri(butyl cellosolve) phosphate (ethanol,2-butoxy-phosphate)								15	5.0E+04										
Tribufos		1	7.0E+03																
Tributyl phosphate				10	5.2E+03			na	5.0E+02										
Tributyltin (TBT)				0.3	6.0E+03			na	1.0E+03										
Tributyltin Oxide				0.3	5.7E+03									9.0E+00					
Triclocarban															24	1.4E+05			
Triclosan								na	3.5E+02	75	2.6E+06			5.0E+05			2.1E+06		
Triethylamine		na	2.3E+03	na	1.5E+04														
Triethylphosphate (TEP)												560	2.0E+06						
Trifluoroacetate																			1.0E+03
Trifluralin				7.5	2.6E+03			na	5.0E+04										
Trihalomethanes (total)														8.0E+04					
Trimethoprim						4.2	6.1E+04	20	7.0E+04	190	6.7E+06				1.5	4.0E+04			
Triphenyl phosphate								na	1.0E+03										
Triphenylphosphine oxide (TPPO)				20	3.6E+05							8	2.8E+04						
Triphenyltin hydroxide (TPTH)		0.3	1.9E+00																
Tris(2-chloroethyl)phosphate (TCEP)		300	2.5E+03	7	3.8E+03			na	1.0E+03			22	7.7E+04				5.0E+03		
Tylosin								300	1.1E+06										
Urethane		na	3.5E+01	na	2.5E+01														
Urotropine												150	5.0E+05						
Valsartan															3.3	9.0E+04			3.0E+02
Valsartan acid																			3.0E+02

Table D.3 Continued

CEC	CA Depth Public Health (2007)[a] Drinking Water Notifi-cation Levels (ng/L)	USEPA CCL 3 List/PCCL[b] ADI or RfD (µg/kg /day)	PNEC (ng/L)	USEPA (2017)[c] ADI (µg/kg /day)	Tap-water RSL (ng/L)	Schwab et al. (2005)[d] ADI (µg/kg /day)	$PNEC_{dw}$ (ng/L)	Australia (2008)[e] ADI (µg/kg/ day)	DWG (ng/L)	AwwaRF (2008)[f] ADI (µg/kg day)	DWEL (ng/L)	Schriks et al. (2009)[g] TDI, ADI, or RfD (µg/kg/ day)	PGV (ng/L)	Cotruvo et al. (2010)[h] Lowest Guideline Value (ng/L)	Minnesota Dept Health (2015)[i] ADI (µg/kg/ day)	Screen. Water Value[i] (ng/L)	WE&RF (2016)[j] PHC (ng/L)	Science Panel[k] MTL (ng/L)	German Environ. Agency[l] HAV (ng/L)
Vanadium	5.0E+04	3	2.1E+04	5	8.6E+04														
Verapamil															2.3	6.0E+04			
Vinclozolin		25	5.5E+02	1.2	2.0E+04					12	4.2E+05								
Warfarin				0.3	5.6E+03	0.16	2.3E+03								0.025	7.0E+02			
Xylenes (total)				200	1.9E+05									5.0E+05					
Ziram		16	5.7E+02																
Zolpidem															0.021	6.0E+02			
α-BHC								na	2.0E+04										
α-Hexachlorocyclohexane		na	6.0E+00	8	7.2E+00														
β-BHC								na	2.0E+04										

Notes:
na = not available
ADI = acceptable daily intake
PNECdw = predicted no effect concentration in drinking water
DWG = drinking water guideline
DWEL = drinking water equivalent level
HAV = Health assessment value
PHC = Public health criteria
PGV = provisional guideline value
RfD = reference dose
TDI = tolerable daily intake
µg/kg/day = micrograms per kilogram per day
ng/L= nanograms per liter

Pink highlighted cells denote the MTL. The sequence of selecting the MTL is: CA NL if available > the lower of either the EPA CCL PNEC or tap water RSL > lowest value from the remaining sources, excluding the German EA. German EA value used as MTL when no other value is available for a given chemical.
a. From CA Dept of Public Health (2007). Drinking Water Notification Levels and Response Levels: An Overview. Drinking Water Program.
b. From USEPA CCL 3 and CA PCC Dossier of Chemicals. For CECs considered to potentially cause cancer by USEPA, the tapwater RSLs are based on the cancer endpoint and not the ADI
c. From USEPA (2017) tapwater RSL
d. From Table 6 in Schwab et al. (2005). Human pharmaceuticals in US surface waters: a human health risk assessment. Regulatory Toxicology and Pharmacology 42: 296-312.
e. From Tables 4.4, A1, A2, A8a, and A8b in Environment Protection and Heritage Council et al. 2008. Australian Guidelines for Water Recycling. Augmentation of Drinking Water Supplies. May 2008.
f. From Tables 9.1 and 9.2 in Snyder et al. (2008). Toxicological Relevance of EDCs and Pharmaceuticals in Drinking Water. Awwa Research Foundation. 484 pp.
g. From Table 2 in Schriks et al., 2009. Toxicological Relevance of Emerging Contaminants for Drinking Water Quality. Water Research, doi: 10.1016/j.wateres.2009.08.023.
h. From Table 3.2 in Cotruvo et al., 2010. Identifying Health Effects Concerns of the Water Reuse Industry and Prioritizing Research Needs for Nomination of Chemicals for Research to Appropriate National and International Agencies

i. From Pharmaceuticals Screening Water Values 2015 and Supporting Information Excel file, "All Data and Values" tab. Pharmaceutical Water Screening Values Report. Minnesota Department of Health. August 2015. Concentrations are 10 times higher than those shown in the original MDH tables. MDH original screening values are based on infant exposure and assumptions that daily water ingestion is about 10 times greater for infants than adults on a kilogram bodyweight basis.

j. From WE&RF (2016). WRRF-15-01 final report, DPR Public Health Criteria

k. MTL derived based on published ADI (see table D.1, Apendix D) and 2010 Science Panel Report exposure assumptions.

l. Health assessment valued from German Environment Agency (2016). Ableitung gesundheitlicher Orientierungswerte (GOW). Umweltbundesamt, Berlin, Germany (in German).

Appendix E – Importance of Antibiotic Resistance in Water Recycling

Table E.1. Reported antibiotic resistance genes (ARGs) removal by wastewater treatment processes from DPR Final report (Olivieri et al., 2016).

Treatment Process	ARG[a]	Reported Concentrations (copies/100 mL)[b]	Log_{10} Reduction[c]	References
Raw wastewater	*mecA*	10^2-10^4	NA	Borjesson et al., 2009
	tet	10^8-10^{11}	NA	Auerbach et al., 2007; Chen and Zhang, 2013; Zhang et al., 2009; Negreanu et al., 2012
	sul	10^7-10^{11}	NA	Czekalski et al., 2012; Chen et al, 2013; Munir et al., 2011; Negreanu et al., 2012
	bla	10^7-10^8	NA	Lachmayr et al., 2009; Uyaguari et al, 2011
	erm	10^9-10^{10}	NA	Negreanu et al., 2012
Activated sludge	*mecA*	10^4-10^5	<1	Borjesson et al., 2009
	tet	10^6-10^{11}	<1-3	Auerbach et al., 2007; Zhang et al., 2009; Negreanu et al., 2012
	sul	10^7-10^8	2-3	Negreanu et al., 2012; NRC, 2012
	bla	10^7	<1-1	Lachmayr et al., 2009; Uyaguari et al., 2011
	erm	10^6-10^7	2-3	Negreanu et al., 2012
Secondary effluent	*mecA*	10^2-10^3	1-2	Borjesson et al., 2009
	tet	10^4-10^8	1-3	Chen and Zhang, 2013; Bockelmann et al., 2009; Auerbach et al., 2007; Zhang et al., 2009; Fahrenfeld et al., 2013
	sul	10^6-10^8	1-2	Czekalski et al., 2012; Chen and Zhang, 2013; Fahrenfeld et al., 2013
	bla	ND-10^5	<1-2	Bockelmann et al., 2009; Lachmayr et al., 2009
	erm	ND-10^5	NR	Bockelmann et al., 2009
Tertiary effluent[d]	*mecA*	ND	ND	Bockelmann et al., 2009
	tet	10^1-10^6	<1-5	Munir et al., 2011; Yuan et al., 2015; Bockelmann et al., 2009; Fahrenfeld et al., 2013
	sul	10^3-10^8	<1-3	Chen and Zhang, 2013; Munir et al., 2011; Fahrenfeld et al., 2013
	bla	ND	ND	Bockelmann et al., 2009
	erm	ND-10^6	<1-4	Yuan et al., 2015; Bockelmann et al., 2009

[a] Each gene category includes data for all ARG variants described in the accompanying references.

[b] The values represent the concentration range for all variants in each gene category coalesced from the published reports listed. ND: Not detected. mL = Milliliter.

[c] The values represent the ARG log_{10} reduction range between two successive treatment stages (i.e., raw to activated sludge, activated sludge to secondary effluent, and secondary effluent to final effluent) calculated from the given references. Calculations were based on the concentrations given in each publication. When antibiotic resistance concentrations were reported in graphical form, the concentrations were estimated from the appropriate graph. When multiple samples were reported for the same effluent in the same publication, the values were averaged. Log_{10} reductions were rounded to the nearest whole number. NR: Not reported; ND: Not detected; NA: Not applicable.

[d] Tertiary treatment refers to processes to improve water quality that occur after secondary biological treatment stages. The processes described in the accompanying references include one or more of the following: media filtration, lagooning, ultrafiltration, reverse osmosis, ultraviolet disinfection, chlorine disinfection, and biological aerated filter processes.

Table E.2. Reported antibiotic resistant bacteria (ARB) removal by wastewater treatment processes from DPR Final report (Olivieri et al., 2016).

Treatment Process	Antibiotic Class[a]	Reported Concentrations (CFU/100 mL)[b]	Log_{10} Removal[c]	References
Raw wastewater	Tetracyclines	FC:10^5-10^7 HP: 10^6-10^7 Ent:10^5-10^7	NA	Rijal et al., 2009; Novo and Manaia, 2010; Munir et al., 2011; Łuczkiewicz et al., 2010; Ferreira da Silva et al., 2006
	β-lactams	FC:10^5-10^8 HP: 10^7-10^8 Ent:ND-10^7	NA	Rijal, 2009; Novo, 2010; Łuczkiewicz, 2010; Ferreira da Silva et al., 2006
	Macrolides	Ent: 10^6	NA	Ferreira da Silva et al., 2006
	Vancomycin	Ent: 10^3-10^4	NA	Rosenberg Goldstein et al., 2014
	Quinolones	FC:10^5-10^7 HP: 10^6-10^7 Ent:10^4-10^5	NA	Novo and Manaia, 2010; Łuczkiewicz et al., 2010; Ferreira da Silva et al., 2006
	Aminoglycosides	FC:ND-10^4	NA	Łuczkiewicz, 2010; Ferreira da Silva et al., 2006
	Sulfonamides	HP:10^7-10^8 Ent:ND	NA	Munir et al., 2011; Ferreira da Silva et al., 2006
Activated sludge	Tetracyclines	FC: 10^5[d]	1	Galvin et al., 2010
	β-lactams	FC: 10^5[d]	1	Galvin et al., 2010
	Vancomycin	Ent: 10^2-10^5	<1-2	Rosenberg Goldstein et al., 2014
	Quinolones	FC: 10^4[d]	1	Galvin et al., 2010
	Aminoglycosides	FC: 10^5[d]	1	Galvin et al., 2010
	Sulfonamides	FC: 10^5[d]	<1	Galvin et al., 2010
Secondary effluent	Tetracyclines	FC: ND-10^5 HP: 10^4-10^6 Ent: 10^2-10^5	FC:1-4 HP: 1-2 Ent1-3	Novo and Manaia, 2010; Ferreira da Silva et al., 2006; Łuczkiewicz et al., 2010; Rijal et al., 2009
	β-lactams	FC: ND-10^7 HP: 10^5-10^7 Ent:ND-10^3	FC: 1-5 HP: <1-2 Ent:<1-1	Novo and Manaia, 2010; Ferreira da Silva et al., 2006; Łuczkiewicz et al., 2010; Rijal et al., 2009
	Macrolides	Ent: 10^4	Ent:1	Ferreira da Silva et al., 2006
	Vancomycin	Ent:ND-10^3	Ent:1-3	Rosenberg Goldstein et al., 2014
	Quinolones	FC: 10^3-10^7 HP: 10^4-10^6 Ent:ND-10^4	FC: 1-4 HP: 1-2 Ent: 1->2	Novo and Manaia, 2010; Łuczkiewicz et al., 2010
	Aminoglycosides	FC: ND-10^3 Ent: 10^4	FC:1-4 Ent: 1	Galvin et al., 2010; Ferreira da Silva et al., 2006; Łuczkiewicz et al., 2010; Rijal et al., 2009
	Sulfonamides	FC: 10^4[d]	FC: 1	Galvin et al., 2010
Tertiary effluent[e]	Tetracyclines	HP:10^3-10^4	HP: 2-4	Munir et al., 2011
	Vancomycin	Ent: ND	Ent: >3	Rosenberg Goldstein et al., 2014
	Sulfonamides	HP:10^4-10^5	HP: 3-4	Munir et al., 2011

[a] Each category includes data for all drug class variants described in the accompanying references.

[b] The values represent the ARB concentration ranges coalesced from the listed publications rounded to the nearest power of 10. The ARB data refer to indicator organisms that typically do not contain extensive numbers of pathogens. ND: Not detected; HP: Heterotrophic bacteria; FC: Fecal coliforms; Ent: Enterococci. CFU = Colony forming unit. mL = Milliliter.

[c] The values represent the log_{10} reduction range between the raw wastewater and each treatment stage for the accompanying references. Calculations were based on the concentrations given in each publication. When antibiotic resistance concentrations were reported in graphical form, the concentrations were estimated from the appropriate graph. When multiple samples were reported for the same effluent in the same publication, the values were averaged. Log_{10} reductions were rounded to the nearest whole number. NA: Not applicable.

[d] Reported values in Most Probable Number (MPN) per 100 milliliters.

[e] Tertiary processes described in the accompanying references include one or more of the following: media filtration, lagooning, ultraviolet disinfection, and chlorine disinfection.

Table E.3. Log reduction of antibiotic resistant bacteria and antibiotic resistance genes (ARB and ARGs) in water by disinfection and barrier processes from DPR Final report (Olivieri et al., 2016).

Process	Application	Concentration Range	ARB Log_{10} Reduction[a]	ARG Log_{10} Reduction[b]	References
Chlorine disinfection	Drinking water	15-200 mg × min./L	2-4 logs	NR	EPA, 1999; Dodd, 2012; Armstrong et al., 1982[c]
	WWTP disinfection (typical)	30-300 mg × min./L	3-5 logs	<1	Huang et al., 2011; Yuan et al., 2015
	WWTP disinfection (CA Title 22)	450 mg × min./L	2->4 logs	1-2	Macauley et al., 2006; Zhang et al., 2015; Yuan et al., 2015
Ultraviolet disinfection	WWTP disinfection	10-200 mJ/cm^2	4-5 logs	<1-4	McKinney and Pruden, 2012; Zhang et al., 2015; Zhuang et al., 2015
Ozone	WWTP disinfection	0.1-200 mg × min./L	2-4 logs	1-3	Dodd, 2012; Lüddeke et al., 2015; Oh et al., 2014; Zhuang et al., 2015
Ultrafiltration[d]	WWTP disinfection	NA	NR	4->5.9	Breazeal et al., 2013
Reverse osmosis	WWTP disinfection	NA	NR	NR	--

[a] The values represent the log_{10} reduction range for ARB corresponding to each type of treatment derived from laboratory-based disinfection experiments. Calculations were based on the concentrations given in each publication. When antibiotic resistance concentrations were reported in graphical form, the concentrations were estimated from the appropriate graph. The ARB data refer to indicator organisms that typically do not contain extensive numbers of pathogens. When multiple samples were reported for the same effluent in the same publication, the values were averaged. Log_{10} reductions were rounded to the nearest whole number. NA = Not applicable. NR = Not reported.

[b] The values represent the log_{10} reduction range for ARG corresponding to each type of treatment derived from laboratory-based disinfection experiments. Calculations were based on the concentrations given in each publication. When antibiotic resistance concentrations were reported in graphical form, the concentrations were estimated from the appropriate graph. When multiple samples were reported for the same effluent in the same publication, the values were averaged. Log_{10} reductions were rounded to the nearest whole number. NA = Not applicable. NR = Not reported.

[c] Data from Armstrong (1982) represent reductions of ARB in a full-scale drinking water treatment facility occurring after the flash mix treatment.

[d] Ultrafiltration data refers to membranes with molecular weight cutoffs of 10,000 and 1,000 Daltons.

WWTP = Wastewater treatment plant.

mg × min./L =Milligrams multiplied by minute per liter.

mJ/cm^2 = Millijoules per centimeters squared.

Appendix F – Decision Tree for Bioanalytical Measurements

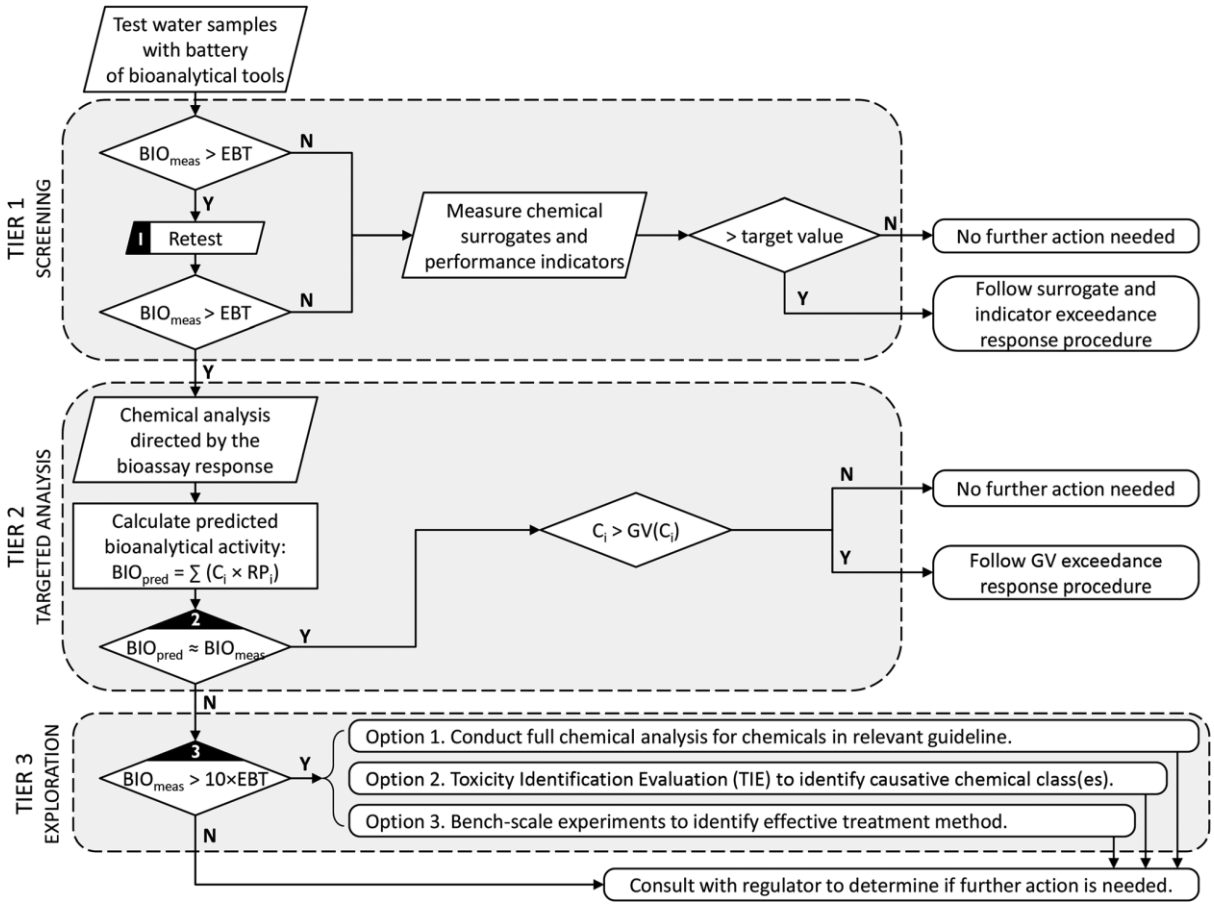

Figure F.1. Decision tree for bioanalytical measurements (from Leusch and Snyder, 2015). Abbreviations: BIOmeas = bioassay response; BIOpred = predicted bioassay response from detected chemicals; Ci = concentration of chemical i; EBT = Effect Based Trigger level; GVIJCi) = Guideline Value for chemical i; RPi = Relative Potency of chemical i in the specific bioassay.